Report of the Expert Committee (EC-II) on Bt Brinjal Event EE-1 Developed by:

- M/s Maharashtra Hybrid Seeds Company Ltd. (Mahyco), Mumbai
- University of Agricultural Sciences (UAS), Dharwad and
- Tamil Nadu Agricultural University (TNAU), Coimbatore

Submitted to:

Genetic Engineering Approval Committee,
Ministry of Environment and Forests,
Government of India
New Delhi

October 8, 2009

Contents

REPORT OF THE EXPERT COMMITTEE CONSTITUTED ON 29/5/2009, TO REVIEW THE FINDINGS OF THE LARGE SCALE TRIALS AND OTHER RELATED BIOSAFETY STUDIES ON Bt BRINJAL EXPRESSING EVENT EE-1 DEVELOPED BY M/S MAHYCO

BACKGROUND

M/s Maharashtra Hybrid Seeds Company Limited (Mahyco) has developed transgenic brinjal plants expressing *cry1Ac* gene isolated from *Bacillus thuringiensis* tolerant to the fruit and shoot borer (herein after referred to as Bt brinjal), one of the major pests which attacks the brinjal crop throughout its life cycle. The transformation was carried out using genetic engineering techniques viz. *Agrobacterium tumefaciens* mediated method. Bt brinjal contains three genes namely:

1. The *cry1Ac* gene derived from *Bacillus thuringiensis* (Bt) to produce an insecticidal protein. The *cry1Ac* gene is driven by a viral promoter, the cauliflower mosaic virus (*CaMV) 35S* promoter.
2. The *nptII* gene for an antibiotic resistance marker, neomycin phosphotransferase.
3. The *aad* gene for another marker O-aminoglycoside adenyl transferase.

M/s Mahyco has applied to the Genetic Engineering Approval Committee (GEAC) for environmental release of Bt brinjal. The development of Bt brinjal was initiated in 2000 in accordance with the regulatory procedures prescribed under ''Rules for the Manufacture, Use, Import, and Export and Storage of Hazardous Micro Organisms / Genetically Engineered Organisms or Cells, 1989', hereafter referred as 'Rules 1989' issued by the Ministry of Environment and Forests (MoEF) under the provisions of the Environment (Protection) Act (EPA), 1986. The chronology of Bt brinjal development is discussed in Section I of this report.

Bt brinjal has been evaluated for its efficacy and safety as per the protocols and procedures prescribed under the Rules 1989 and relevant biosafety guidelines. The Review Committee on Genetic Manipulation (RCGM) in its 40th meeting held on April 25, 2006 considered in detail the data generated by M/s Mahyco to establish the efficacy and safety of the inserted gene. The RCGM concluded that Bt brinjal is effective in controlling target pests, safe to the environment, non toxic, non-allergenic and has potential to benefit the farmers. RCGM recommended that GEAC may consider granting approval for conduct of large scale field trials on Bt brinjal as per the protocols submitted by M/s Mahyco.

The request of M/s Mahyco and recommendations of the RCGM for conduct of large scale field trials and seed production were considered by the GEAC in its 68th meeting held on June 1, 2006. In accordance with the decision taken in the above meeting, summary of biosafety data was posted on the GEAC website (http://envfor.nic.in) for inviting public comments. Subsequently, the GEAC constituted an "Expert Committee on Bt Brinjal" under the chairmanship of Dr. Deepak Pental, Vice Chancellor, Delhi University (herein after referred as EC-I). The EC-I reviewed the biosafety data submitted by M/s Mahyco as well as the

submissions received from several stakeholders. The EC-I submitted its recommendations to the GEAC in July 2007. Recommendations of the EC-I are discussed in Section II of this report. Further, in accordance with the Hon'ble Supreme Court directions dated May 8, 2007, the biosafety dossier on Bt brinjal was posted on the GEAC website.

Based on the recommendations of the EC-I, the GEAC in its 79th meeting held on August 8, 2007, permitted the conduct of large scale trials (LST) of Bt brinjal for two seasons under the direct supervision of Director, Indian Institute of Vegetable Research (IIVR), Varanasi and to conduct some additional biosafety related studies by M/s Mahyco. The field trials were subject to compliance of the following conditions:

1. Maintaining an isolation distance of 300 metres.
2. Submission of validated event specific test protocol at limit of detection (LOD) of at least 0.01% to detect and confirm that there has been no contamination.
3. Designating a lead scientist who would be responsible for all aspects of the trials including regulatory requirements.

The GEAC has received the final reports from IIVR and M/s Mahyco. The GEAC has also received several representations on concerns to human health and environment from Bt brinjal. Issues raised by the NGOs and other stakeholders are discussed in Section V of this report.

To review the findings of the LST and other studies on Bt brinjal as well as address the concerns expressed in various representations, the GEAC has constituted an 'Expert Committee' in accordance with the decision taken in the 91st GEAC meeting held on January 14, 2009 (hereinafter referred to as EC-II). The composition of the EC-II is as follows:

1.	Prof. Arjula R. Reddy, Vice Chancellor, Yogi Vemana University, Hyderabad and Co-chairman, GEAC	-	Chairman
2.	Dr. Vasantha Muthuswamy, Former Chief (Basic Medical Sciences), Indian Council of Medical Research (ICMR), New Delhi	-	Member
3.	Dr. B. Sesikeran, Director, National Institute of Nutrition, Hyderabad	-	Member
4.	Dr. Lalitha R. Gowda, Senior Scientist, Central Food Technological Research Institute (CFTRI), Mysore	-	Member
5.	Dr. N. Madhusudan Rao, Deputy Director, Centre for Cellular and Molecular Biology (CCMB), Hyderabad	-	Member
6.	Dr. C. M. Gupta, Former Director, Central Drug Research Institute (CDRI), Lucknow	-	Member

7.	Dr. Dhir Singh, ADG (PFA), Food Safety and Standards Authority of India (FSSAI) [representative of Ministry of Health and Family Welfare (MoH&FW)]	-	Member
8.	Shri S.B. Dongre, Director (Fruit and Vegetable Products), FSSAI (representative of MoH&FW)	-	Member
9.	Dr. K. Satyanarayan, Scientist G, Indian Council of Medical Research (ICMR), New Delhi	-	Member
10.	Dr. Dharmeshwar Das, Director, Indian Veterinary Research Institute (IVRI), Izatnagar	-	Member
11.	Dr. A. K. Srivastava, Director, National Diary Research Institute (NDRI), Karnal	-	Member
12.	Dr. Dilip Kumar, Director, Central Institute of Fisheries Education, Fisheries (CIFE), University Road, Versova, Mumbai	-	Member
13.	Dr. Mathura Rai, Director, Indian Institute of Vegetable Research (IIVR), Varanasi	-	Member
14.	Dr. P. Ananda Kumar, Project Director, National Research Centre on Plant Biotechnology (NRCPB), IARI, New Delhi	-	Member
15.	Dr. K. K. Tripathi, Adviser, Department of Biotechnology (DBT), New Delhi	-	Member
16.	Dr. Ranjini Warrier, Director, MoEF and Member Secretary, GEAC	-	Convener

The terms of reference of the EC-II are:

- to review the findings of the data generated during the large scale trials;
- to review the biosafety data of Bt brinjal in light of the available scientific evidence, reports from international/national experts and representations from NGOs and other stakeholders;
- to make appropriate recommendations for consideration of the GEAC based on the above review.

The following documents were made available to the members to facilitate the above:

1. Note on Development of Bt brinjal.
2. Summary of biosafety assessments of Bt brinjal.
3. Biosafety dossier comprising of reports of all the studies submitted to the regulatory agencies.
4. Recommendations of the EC-I on Bt brinjal constituted in 2006 (including response to comments received from stakeholders).

5. Conditions stipulated by the GEAC while permitting the conduct of LST and other related studies.
6. Report on status of compliance of LST conditions submitted by M/s Mahyco.
7. Report on "Large Scale Trials on Bt brinjal Hybrids" conducted by IIVR, Varanasi during 2007-2009.
8. Report on "Pollen flow studies in Bt brinjal" conducted by IIVR, Varanasi during 2007-2009.
9. Report on "Assessment of Crossability of Bt Brinjal (*Solanum melongena*) with *Solanum incanum* conducted by IIVR, Varanasi during 2007-2009.
10. Report on "Effects on Health and Environment of Transgenic (or GM) Bt Brinjal" by Prof. G. Seralini, University of Caen, France.
11. Report on "Biological effects of transgenic maize NK603xMON810 fed in long term reproduction studies in mice" by A. Velimirov *et al.* – Austrian report.
12. Comments on Possible Consequences of Gene Flow from Bt Brinjal to Brinjal Wild Relatives in India, and the Inadequacy of the Current Risk Assessment by Dr. Doug Gurian-Sherman, 2009.
13. Paper on "Genetically Engineered Plants and Foods: A Scientist's Analysis of the Issues (Part I) by Dr. Peggy G. Lemaux, Department of Plant and Microbial Biology, University of California.
14. Paper on "Genetically Engineered Plants and Foods: A Scientist's Analysis of the Issues (Part II) by Dr. Peggy G. Lemaux, Department of Plant and Microbial Biology, University of California.
15. Biology document on brinjal.
16. List of studies recommended by Dr. P. M. Bhargava before release of a GMO into the environment.
17. Comments by Prof. Jack A. Heinemann, University of Canterbury, New Zealand.
18. A review of Mahyco's GM Brinjal food safety studies by Dr Judy Carman, Australia.
19. Letter from Ms Kavitha Kurgunti to members of the EC-II constituted on May 29, 2009, to review the biosafety studies and large scale trials of Bt Brinjal.
20. Appeals submitted to PMO.
21. "I AM NO LAB RAT CAMPAIGN"

Two meetings of the EC-II were held on July 30, 2009 and August 31, 2009 under the chairmanship of Prof. Arjula R. Reddy, Co-chairman GEAC. The deliberations of the EC-II are elaborated in the subsequent sections as given under:

I. Development of Bt brinjal in India

II. Review of status of regulatory compliance

i. Approvals taken by M/s Mahyco.

ii. Compliance with the "Revised guidelines for research in transgenic plants & guidelines for toxicity and allergenicity evaluation of transgenic seeds, plants and plant parts, 1998".

iii. Compliance with regulatory conditions stipulated by GEAC in the permit letter for large scale trials, 2007

iv. Compliance with the Guidelines for the Safety Assessment of Foods Derived from Genetically Engineered (GE) Plants, 2008

III. Review of Bt brinjal safety assessment dossier

i. Nature and effect of gene modification

ii. Environmental safety

iii. Food and feed safety

IV. Review of efficacy and agronomic performance

V. Consideration of issues raised by NGOs, national and international groups

VI. Summary and recommendations

*****

SECTION I
DEVELOPMENT OF Bt BRINJAL BY M/S MAHYCO IN INDIA

1.1 BRINJAL AS A CROP

Brinjal, *Solanum melongena* Linn., is one of the principal vegetable crops grown in many geographical parts in India. The area under brinjal cultivation is estimated at 0.512 million ha with production of 8.4 million metric tonnes in 2007 (FAOSTAT, 2007). This accounts for 8.14% of area under vegetable cultivation with a contribution of 9% to total vegetable production. Brinjal is primarily grown by small farmers and is thus an important source of income for them. It is a versatile crop, adapted to different agro-climatic regions and can be grown throughout the year. A number of cultivars are grown in the country, consumer preference being dependent upon fruit colour, size and shape. The fruits are consumed as cooked vegetables in various ways, and dried stem are used as fuel in rural areas.

1.2 RATIONALE FOR THE DEVELOPMENT OF Bt BRINJAL

1.2.1 Economic loss due to pest damage

Damage by insect pests is a major problem in brinjal production. *Leucinodes orbonalis* (Guen.) commonly referred to as fruit and shoot borer (FSB) is regarded as one of the major insect pests, which attacks throughout the life cycle of brinjal crop. It is estimated that FSB causes yield losses of 60-70% even after repeated insecticide sprays. The biology and nature of damage of FSB is elaborated in Box 1.1.

Box 1.1: Fruit and shoot borer

Fruit and shoot borer (FSB) was first described as *Leucinodes orbonalis* by Guenée in 1854. This insect belongs to family Pyralidae of the insect order Lepidoptera. The biology of FSB is explained as under:

Egg: Adult females lay eggs on the foliage. The number of eggs laid by an average female varies from 80 to 253. Oviposition takes place during the night and eggs are laid singly on the lower surface of the young leaves, green stems, flower buds, or calyces of the fruits. Eggs hatch in 3 to 6 days.

Larva: Soon after hatching from eggs, young caterpillars search for and bore into tender shoots near the growing point, into flower buds, or into the fruits. Caterpillars prefer fruits over other plant parts. Larvae go through at least five instars (Atwal, 1976) and there are reports of the existence of six larval instars. Larval period lasts 12 to 15 days in the summer and up to 22 days in the winter. Sandanayake and Edirisinghe (1992) studied the larval distribution on mature brinjal in Sri Lanka. They found first instars in flower buds and flowers, second instars in all susceptible plant parts, third and fourth instars in shoots and fruits, and fifth instars mostly in fruits. Larval feeding in fruit and shoot is responsible for the damage to brinjal crop. A full-grown larva measures 18 to 23 mm in length.

Pupa: Mature larvae come out of their feeding tunnels and pupate in tough silken cocoons among the fallen leaves and other plant debris on the soil surface near the base of brinjal plants. The color and texture of the cocoon matches the surroundings making it difficult to detect. Some studies indicate the presence of cocoons at soil depths of 1 to 3 cm. The pupal period lasts 6 to 17 days depending upon temperature. Adult Moths come out of pupal cocoons at night. Young adults are

generally found on the lower leaf surfaces following emergence. Longevity of adults was 1.5 to 2.4 days for males and 2.0 to 3.9 days for females.

Nature of damage: Within one hour after hatching, FSB larva bores into the nearest tender shoot, flower, or fruit. Soon after boring into shoots or fruits, they plug the entrance hole with excreta. In young plants, caterpillars are reported to bore inside petioles and midribs of large leaves. As a result, the affected leaves may drop off (Butani and Jotwani, 1984). Larval feeding inside shoots result in wilting of the young shoot. Presence of wilted shoots in a brinjal field is the surest sign of damage by this pest. The damaged shoots ultimately wither and drop off. This reduces plant growth, which in turn, reduces fruit number and size. New shoots can arise but this delays crop maturity and the newly formed shoots are also subject to larval damage. Larval feeding in flowers - a relatively rare occurrence - results in failure to form fruit from damaged flowers. Larval feeding inside the fruit results in destruction of fruit tissue. This makes even slightly damaged fruit unfit for marketing. The yield loss varies from season to season and from location to location. Damage to the fruits in India, particularly in autumn, is very severe and the whole crop can be destroyed (Atwal, 1976). FSB is active throughout the year at places having moderate climate but its activity is adversely affected by severe cold. FSB is practically monophagous, feeding principally on brinjal; however, other plants belonging to family Solanaceae are reported to be hosts of this pest. They include tomato (*Lycopersicon esculentum*), potato (*Solanum tuberosum*), selected nightshades (*S. nigrum and S. indicum*), and turkey berry (*S. torvum*).

1.2.2 Extensive use of chemical insecticides

In recent years, an increasing amount of pesticides have been used in cultivating brinjal due to increasing damage by arthropod pests. In fact brinjal is infested by a plethora of insect pests throughout Asia. A survey of vegetable pests conducted by Asian Vegetable Research Development Centre (AVRDC)-The World Vegetable Center indicated that FSB is the most destructive pest in most major brinjal producing countries of South Asia. Currently farmers rely exclusively on the application of pesticides to control FSB and to produce blemish-free brinjal fruit. Pesticide use is very intensive for killing the larvae before they bore inside shoots or fruits; once in the shoots or fruits, larvae are inaccessible to the killing action of surface applied chemicals. Since neonate larvae can enter fruits or shoots within only a few hours of hatching from eggs, pesticides have to be applied frequently in order to have sufficient toxic residues on the plant surface adequate enough to kill the crawling larvae. Surveys conducted in Bangladesh indicated that farmers spray insecticides up to 84 times during a 6-7month cropping season (BARI, 1995). The research and development activities to combat FSB have largely been confined to screening pesticides to select the most effective chemical and determining the frequency of their use. The approach of pesticide spray schedules that involved calendar spraying whether the pest was present or not has led to increased dependence on pesticides and consequent adverse effects of higher costs of production, environmental pollution, destruction of natural enemies, and development of pesticide resistance in FSB (Atwal, 1976; Srivastava and Butani, 1998).

An indicative list of various insecticides approved for use for controlling FSB in brinjal is presented in Table 1.1.

Table 1.1: Insecticides recommended for fruit and shoot borer in brinjal

S. No.	*Name of insecticide*	*Group*	*Mode of action*	*Maximum residue levels (MRL) (mg/kg)*	*Waiting period (days)****
1.	Carbaryl 50 %WDP	Carbamate	Contact and stomach	5.00	7
2.	Quinalphos 25 EC	Organophosphate	Contact and stomach	0.25	4-5
3.	Endosulfan 35 EC	Cyclodiene	Contact and stomach	2.00	7
4.	Chlopyriphos 20 EC	Organophosphate	Contact and stomach	0.20	5
5.	Deltamethrin 2.8 EC	Synthetic pyrethroid	Contact and stomach	0.20 (EU std.)	3
6.	Triazophos 40 EC	Organophosphate	Contact and stomach	0.10	5
7.	Fenvalerate 20 EC	Synthetic pyrethroid	Contact and stomach	0.20	5
9.	Methyl parathion 50 EC	Organophosphate	Contact and stomach	1.00	5
10.	Cypermethrin 10 EC	Synthetic pyrethroid	Contact and stomach	0.50	3
11.	Thiodicarb 75% WP	Organophosphate	Contact and stomach	0.10	6
12.	Profenophos 50 EC	Organophosphate	Contact and stomach, Translaminar action	0.05 (EU std.)	----
13.	Lamda Cyhalothrin 5 EC	Synthetic pyrethroid	Contact and stomach	0.20 (EU std.)	4
14.	Cypermethrin 3% + Quinalphos 20% EC		Contact and stomach	----	7
15.	Acephate 75% SP	Organophosphate	Systemic, Contact and stomach	0.02 (EU std.)	----

Sources: CIBRC, 2008; PMFAI, 2008; Sharma, 2007; NCIPM, 2006; Reddy and Rao, 2000; Agarwal,2009; www.ikisan.com ; http://www.tribuneindia.com/2001/20010611/agro.htm

******"Waiting period signifies the minimum period for which the farmers have to wait after spray of pesticides before selling their produce in the market."***

As per the LST report by IIVR, on an average an amount Rs. 5,952 per acre was spent to control FSB in a cropping season. This in addition to cost of insecticides used to control other pests including epilachna beetle (hadda), red spider mite, whiteflies and jassids.

1.2.3 Effectiveness of chemical pesticides

In spite of the extensive use of chemical pesticides, FSB is difficult to control by the application of pesticides as the larvae are often hidden in the fruit and do not come in contact with the insecticides. Further the application of pesticides has to be critically timed by farmers in such a way so as to kill the larvae before they bore into shoots and fruits. If they are not controlled at this early stage, FSB larvae remain and feed within the shoots and fruits. Infested shoots retard vegetative growth and affect yield while damaged fruits are responsible for direct losses in marketable yield.

1.2.4 Impact of chemical pesticides

The extensive use of chemical pesticides has also led to several problems like resurgence of secondary pests, resistance in pests against pesticides, health hazards and pesticide residues in edible fruit (Kabir *et. al.* 1996).

Health problems: Application of frequent insecticide sprays results in a high pesticide exposure for farmers and sometimes this can be associated with recurring health problems (Kaur, 2008). There are also negative implications in relation to pesticide residues in fruits. As an example the pesticide residue levels in brinjal as reported by various researchers are summarised in Table 1.2.

Table 1.2: Pesticide residue levels in brinjal

Insecticide (active ingredient)	MRL (ppm)	Residue level	Reference
Organochlorines	0.25	Above Below	Reddy *et al*. 1998 Rajeswaran *et al*. 2004
Carbosulfan Chlorpyriphos	0.20	Above	Beena-Kumari *et al*. 2004 , Patel *et al*. 1999, Arora 2008, Goswami-Giri 2007
Cypermethrin	0.20	Above Below	Beena-Kumari *et al*. 2004, Arora 2008 Duara *et al*. 2003
Endosulfan	2.00	Above	Chandrasekaran et al. 1997, Nisha Kumari *et al*. 2005
Fenvalerate	0.20	Below Above	Duara *et al*. 2003 Chandrasekaran *et al*. 1997
Monocrotophos	0.20	Above	Beena-Kumari *et al*. 2004, Ahuja *et al*. 1998, Singh and Mukherjee 1992, Srinivas *et al*. 1996, Goswami-Giri 2007
Quinalphos	0.25	Above	Beena-Kumari *et al*. 2004, Kale *et al*. 1997, Goswami-Giri 2007
Triazophos	0.10	Above Below	Goswami *et al*. 2002 Raj *et al*. 1999

It may be noted that use of monocrotophos has not been permitted for vegetables by the Central Insecticide Board as per the circular F.No.16-2/99-CIR-11 dated 13-06-2005. The presence of its residues in brinjal clearly demonstrates the indiscriminate use of chemical pesticides in brinjal.

Human exposure to pesticides usually occurs through ingestion of pesticide residues in food, water, dermal and inhalation exposure and occupational exposure among the farmers. Pesticide exposure causes ill effects (including neurotoxic, birth and reproductive) such as headache, dizziness, nausea, vomiting, lack of coordination, tremor, mental confusion, seizures, coma etc. (Chitra et al. 2006, Mancini et al. 2005, Singh et al. 2004, Singh and Sharma 2000, Rupa et al. 1991, Lorenz 2009). Some pesticides have long residual effect and are reported as probable/possible carcinogens (Source: US EPA's List of Chemicals Evaluated for Carcinogenic Potential 2004)

1.2.5 Contamination of soil and ground water

It is well established fact that the presence and bio-availability of pesticides in soil can adversely impact human and animal health, beneficial organisms and soil organisms. Pesticides can move off-site contaminating surface and groundwater and possibly causing adverse impacts on aquatic ecosystems.

1.2.6 Non target organisms

In addition to the failure to control FSB resulting in heavy financials losses for farmers, broad spectrum insecticides suppress parasitoid and predator species. Several parasitoids, especially the ichneumonid *Trathala flavoorbitalis*, with a parasitisation rate ranging up to 28.1% (Tewari and Sardana, 1987) are capable of producing significant combined mortality of FSB.

1.2.7 Genetic improvement by conventional techniques

Genetic improvement by conventional plant breeding has not been successful due to the lack of resistance to FSB in brinjal germplasm. Some wild *Solanum* species showed high levels of resistance, but it has proved to be impossible to incorporate the genes for resistance from wild species into commercial cultivars due to breeding incompatibilities (Dhankhar *et al*., 1982).

1.2.8 Alternate strategies

It is evident from the above that the current practices of using extensive pesticides is not only harmful to the health and environment but also non-sustainable in future for control of FSB in brinjal crop. In view of the above, there is an urgent need for developing alternative control strategies.

Adoption of transgenic crops engineered primarily using the cry proteins to prevent damage caused by insect pests has given excellent results in cotton

and maize worldwide resulting in significant economic benefits. A similar approach in brinjal is expected to provide substantial benefits to farmers.

1.3 DEVELOPMENT OF Bt BRINJAL BY M/S MAHYCO

As an alternate strategy to control FSB, M/s Mahyco has produced Bt brinjal plants with the *cry1Ac* gene from *Bacillus thuringiensis* subsp. *kurstaki* (*B.t. k.*) tolerant to the fruit and shoot borer, one of the major pests which attack the brinjal crop throughout its life cycle. Bt brinjal has following three genes inserted via genetic engineering techniques:-

i. The cry1Ac gene derived from *Bacillus thuringiensis* (Bt) to produce an insecticidal protein. The *cry1Ac* gene is driven by a viral promoter, the cauliflower mosaic virus *CaMV* 35S promoter.

ii. The *nptII* gene for an antibiotic resistance marker, neomycin phosphotransferase.

iii. The *aad* gene for another marker O-aminoglycoside adenlyl transferase.

It has been indicated that the expression of the *cry1Ac* genes would provide an effective built-in control in brinjal for FSB to reduce pests-linked damages as well as protect the environment from adverse effects of pesticides. This is also expected to bring down the cultivation costs of brinjal, as the contribution of chemical pesticides to brinjal cultivation is sizable. The *cry1Ac* protein produced in Bt brinjal is similar in structure and activity to that found in nature and in commercial *B.t.k.* microbial formulations. *Bacillus thuringiensis* and *B.t.k.* microbial formulations have been shown to be very specific to target insect pests, and do not have deleterious effects on non-target organisms such as beneficial insects, birds, fish and mammals including humans.

As Bt brinjal plants have an inbuilt mechanism of protection against targeted pests, the protein produced by the plants does not get washed away nor is destroyed by sunlight unlike externally applied pesticides. The plant is thus protected from the FSB round the clock and throughout its life. The advantages of Bt brinjal with the *cry1Ac* gene integrated in the plant are as follows:

- Active protein provides effective control of FSB.
- Active protein is expressed in all plant parts.
- Active protein expressed throughout the season, hence timing of insecticide applications in relation to an infestation is not an issue.
- Wash off of insecticide during rain, and degradation in sunlight are not issues as they are with spray formulations.
- Less farmer exposure to insecticide.
- Labour saving technology, due to elimination or reduction of insecticide sprays

- Decreases production risks
- Contributes to, and provides the foundation for an integrated pest management (IPM) strategy.

The development of Bt Brinjal was initiated in 2000. The chronology of Bt brinjal development is as follows:

2000 - Brinjal transformation started.

2000-01 - Greenhouse evaluation.

2002
- Pollen flow studies- 2 Locations.
- Backcrossing program initiated.
- Germination and weediness studies.
- Aggressiveness studies.
- Molecular characterization and event ID.

2003
- Acute oral toxicity studies in rats (Intox, Pune).
- Germination and weediness studies.
- Aggressiveness studies.

2004
- Mucous membrane irritation test in female rabbit (Intox, Pune).
- Primary skin irritation test in rabbit (Intox, Pune).
- RCGM multilocation field trials-11 Locations, five hybrids (MHB-4, 9, 10, 80 and 99).
- Effects on non-target and beneficial insects.
- ICAR first year trials with five hybrids (MHB-4, 9, 10, 80 and 99) under AICRP (VC).
- Soil micro-biota studies (two years).
- Baseline susceptibility studies
- Substantial equivalence studies.
- Protein expression studies.

2005
- Sub chronic oral toxicity study in Sprague Dawley rats (Intox, Pune).
- Assessment of allergenicity of protein extract using Brown Norway Rats (Rallis, Bangalore).
- Responses, as a dietary feed ingredient to common carp (*Cyprinus carpio)* growth performances (Central Institute of Fisheries Education, Mumbai).
- IRM workshop and recommendations.
- RCGM trials for three new hybrids (MHB-11, 39, 112).
- ICAR second year trials for five hybrids (MHB-4, 9, 10, 80 and 99).
- Effects on non-target and beneficial insects.
- ICAR first year trials for three new hybrids (MHB-11, 39, 112). -
- Baseline susceptibility studies
- Protein expression studies.
- Food cooking and protein estimation in cooked fruits.

2006	-	Chemical fingerprinting of Bt and non-Bt brinjal (including alkaloids) (Indian Institute of Chemical Technology, Hyderabad).
	-	Sub-chronic (90 days) feeding studies using New Zealand rabbit (Advinus Therapeutics, Bangalore).
	-	Effect on performance and health of broiler chickens (Central Avian Research Institute, Izatnagar).
	-	Sub-chronic (90 days) feeding studies in goats (Advinus Therapeutics, Bangalore).
	-	Feeding studies in lactating crossbred dairy cows (G. B. Pant University of Agriculture and Technology, Pantnagar).
	-	Socio-economic and risk assessment (three external reports)
	-	Protein expression studies.
2007-08	-	Large Scale Trials for seven hybrids.
	-	Pollen flow studies at two locations.
	-	Soil microflora studies.
	-	Crossability studies
	-	Baseline susceptibility studies
	-	Protein expression studies.
2008-09	-	Second year Large Scale Trials for seven hybrids.
	-	Pollen flow studies at two locations.
	-	Soil microflora studies.
	-	Crossability studies
	-	Baseline susceptibility studies
	-	Detailed compositional analysis (Vimta Labs, Hyderabad)

In addition to the above studies, M/s Mahyco has also submitted reports of the following studies conducted by the technology provider and accepted by the regulatory authorities in various countries.

- Assessment of the *in vitro* digestive fate of *B.t.k.* HD73 *cry1Ac* protein
- *B.t.k.* HD-73 protein: A Dietary Toxicity Study with parasitic hymenoptera (*Nasonia Vitripennis*)
- Evaluation of the dietary effect(s) of purified *B.t.k.* endotoxin proteins on honey bee larvae
- Evaluation of the dietary effect(s) of purified *B.t.k.* endotoxin proteins on honey bee adults
- *B.t.k.* HD-73 protein: A dietary toxicity study with ladybird beetles (*Hippodamia convergens*)
- *B.t.k.* HD-73 protein: A dietary toxicity study with green lacewing larvae (*Chrysopa Carnea*)
- Effect of *Bacillus thuringiensis* insecticidal protein *cry1Ab*, *cry2A*, cry3A on *Folsomia candida* and *Xenylla grisea* (Insecta: Collembola)
- Acute oral toxicity of *Bacillus Thuringiensis* var. kurstaki *cry1Ac* HD-73 protein in albino mice

- Assessment of degradation of neomycin phosphotransferase II (nptII) protein in *in vitro* mammalian digestion models
- Acute oral toxicity study of nptII protein in albino mice

As per the directions of the Hon'ble Supreme Court, M/s Mahyco submitted an event specific detection method with limit of detection of 0.01% validated by M/s Advinus Therapeutics, Bangalore .

Bt brinjal event EE-1 has been backcrossed by M/s Mahyco into brinjal lines that are best adapted to the different market segments of brinjal. M/s Mahyco has also transferred the technology to public sector institutions under a public-private partnership (PPP) programme with facilitation and support provided by the Agricultural Biotechnology Support Program II (ABSP-II). This PPP is a joint effort between Mahyco and Tamil Nadu Agricultural University (TNAU), Coimbatore, University of Agricultural Sciences (UAS), Dharwad, Indian Institute of Vegetable Research (IIVR) Varanasi, Bangladesh Agricultural Research Institute (BARI), Lal Teer Seeds, Bangladesh, and University of Philippines, Los Baños (UPLB), Philippines. TNAU, UAS Dharwad, UPLB and BARI have conducted confined field trials of Bt brinjal event EE-1 in 2007 and 2008.

The Bt brinjal hybrids/varieties that have been field tested in India are MHB 4 Bt, MHB 9 Bt, MHB 10 Bt, MHB 80 Bt, MHB 99 Bt, MHB 11Bt, MHB 39 Bt, MHB 112 Bt developed by M/s Mayhco, Malapur local (S) Bt, Manjarigota Bt, Rabkavi local Bt, Kudachi local Bt, Udupigulla Bt, GO112 Bt by UAS, Dharwad and Co2-Bt, MDU1-Bt, KKM1-Bt, PLR1-Bt by TNAU, Coimbatore.

*****

SECTION II
REVIEW OF STATUS OF REGULATORY COMPLIANCE

Bt brinjal Event EE-1 being a genetically engineered crop requires environmental clearance under the Rules, 1989 notified by MoEF. As per existing guidelines, the applicant is required to establish safety and efficacy of the product before it is approved for environmental release. It is necessary for the applicant to demonstrate that Bt brinjal is equivalent to currently grown brinjal varieties in composition and agronomic performance and that it has no adverse effect on environment and human health through a series of studies undertaken in line with guidelines prescribed by the regulatory authorities.

The EC-II in its meetings held on July 30, 2009 and August 31, 2009, reviewed the status of regulatory compliance with applicable guidelines as indicated below:

1. Approvals taken by M/s Mahyco.
2. Compliance with the "Revised guidelines for research in transgenic plants & guidelines for toxicity and allergenicity evaluation of transgenic seeds, plants and plant parts, 1998".
3. Compliance with regulatory conditions stipulated by GEAC in the permit letter for large scale trials, 2007
4. Compliance with the Guidelines for the Safety Assessment of Foods Derived from Genetically Engineered (GE) Plants, 2008

2.1 APPROVALS TAKEN BY M/S MAHYCO

The EC-II noted that M/s Mahyco has undertaken various studies after due approval from RCGM and GEAC. The list of approvals is as under (Table 2.1):

Table 2.1: List of regulatory approvals

S. No.	Purpose	Via letter No.	Letter dated
1	Import of the plasmid pMON10518 containing *cry1Ac* gene used in the development of Bt brinjal.	BT/17/ 02/94-PID/IBMAHYCO	March 16, 2000
2	To generate efficacy data (including LD 95 values) of the Bt brinjal grown in contained greenhouse, against target insects.	BT/BS/10/8/98-PID-Vol. III	February 1, 2001
3	To carry out pollen flow studies of Bt Brinjal at two locations (Jalna, Maharashtra and Ranebennur, Karnataka) during Kharif 2002.	BT/17/02/94-PID/MS6/IBMAHYCO-Vol. IV	July 11, 2002
4	To conduct toxicity and allergenicity studies (acute oral toxicity, primary skin irritation, mucous membrane irritation, sub-chronic oral toxicity, allergenicity) of Bt brinjal.	BT/BS/17/02/94-PID-Vol.VIII	September 30, 2003
5	To conduct multilocation (11 locations) contained limited field trials of Bt brinjal for assessing environmental safety and agronomic performance of Bt brinjal hybrids.	BT/17/02/94-PID	June 11, 2004.

6	To include Mahyco Bt brinjal hybrids in AICRP (VC) multilocation (12 centers) ICAR trials.	BT/BS/17/02/94-PID and	June 11, 2004
		IIVR/Dir./1775	July 26, 2004
7	To conduct feeding studies on Bt brinjal containing *cry1Ac* gene in lactating crossbred dairy cows, chicken and catfish.	BT/BS/17/02/94-PID-Vol. VIII	April 8, 2005
8	To include Mahyco Bt brinjal hybrids in second year AICRP (VC) multilocation (11 centers) ICAR trials.	IIVR/SS/Bt Hyb/324	May 12, 2005
9	To cultivate Bt brinjal hybrid containing *cry1Ac* gene at G.B.Pant University of Agr. & Tech., Pantnagar for generating brinjal fruit for feeding of crossbred lactating cows in feeding studies of Bt brinjal.	BT/BS/17/02/94-PID	May 17, 2005
10	To conduct multilocation (6 locations) contained limited field trails of new Bt Brinjal hybrids for assessing environmental safety and agronomic performance of Bt brinjal hybrids.	BT/17/02/94-PID	May 24, 2005
11	To include Mahyco new Bt brinjal hybrids in first year AICRP (VC) multilocation (11 centers) ICAR trials.	IIVR/SS/Bt Hyb/529	May 31, 2005
12	To conduct sub-chronic oral (90 days) toxicity studies in rabbits and goats.	BT/BS/17/02/94-PID	August 08, 2005
13	To cultivate Bt brinjal hybrid containing *cry1Ac* gene during 2005 for generating Bt brinjal fruits for conducting 90 days toxicity studies on rabbits.	BT/BS/17/02/94-PID	August 08, 2005
14.	To cultivate Bt brinjal hybrid containing *cry1Ac* gene during 2005 for generating Bt brinjal fruits for conducting 90 days toxicity studies on goats.	BT/BS/17/02/94-PID	August 08, 2005
15	To conduct the studies on estimation of the alkaloid contents in Bt brinjal in comparison with its non-Bt counterparts.	BT/BS/17/02/94-PID	December 01, 2005
16	To conduct large scale field trials and seed production of Bt brinjal expressing *cry1Ac* gene	12/ 81/ 2006-CS-II	August 30, 2007
17	Letter from IIVR, Varanasi to GEAC with respect to studies mentioned in large scale field trial permit.	IIVR/Dir/BS/Bt Brinjal/2264	Sept. 13, 2007
18	To conduct pollen flow study and experimental seed production of Bt brinjal	12/ 81/ 2006-CS-II	January 31, 2008
19	Recommendation of RCGM with respect to 90 days goat feeding study with Bt brinjal leaf expressing *cry1Ac* gene.	12/ 81/ 2006-CS-II	Feb 06, 2008
20	To conduct experimental seed production of Bt brinjal at the Company's research farm at Jalna	12/ 81/ 2006-CS-II	June 10, 2008
21	To conduct compositional analysis study to test transgenic brinjal and non-transgenic brinjal.	BT/BS/17/12/99-PID	June 10, 2009

2.2 COMPLIANCE WITH THE "REVISED GUIDELINES FOR RESEARCH IN TRANSGENIC PLANTS & GUIDELINES FOR TOXICITY AND ALLERGENICITY EVALUATION OF TRANSGENIC SEEDS, PLANTS AND PLANT PARTS, 1998"

M/s Mahyco has conducted following biosafety studies as per the "Revised guidelines for research in transgenic plants & guidelines for toxicity and allergenicity evaluation of transgenic seeds, plants and plant parts, 1998":

- Acute oral toxicity test of Bt brinjal expressing *cry1Ac* gene in rats.
- Sub chronic (90 days) oral toxicity test of Bt brinjal expressing *cry1Ac* gene in rats.
- Primary skin irritation test of Bt brinjal expressing *cry1Ac* gene in rabbit
- Mucus membrane irritation test of Bt brinjal expressing *cry1Ac* gene in female rabbit
- Sub chronic (90 days) oral feeding study of Bt brinjal expressing *cry1Ac* gene in rabbits and goats
- Feeding studies in chicken, fish and lactating cow.

The protocols used by M/s Mahyco for these studies were approved by RCGM. The studies were conducted at various public sector institutions and accredited contract private laboratories. RCGM reviewed the biosafety data generated by the applicant in its 40th meeting held on April 25, 2006. The RCGM concluded that the target pests are effectively controlled by Bt brinjal and biosafety studies conducted till date show no significant differences between Bt and non Bt brinjal and thus Bt brinjal is safe. RCGM further noted that M/s Mahyco has generated sufficient biosafety data on Bt brinjal expressing *cry1Ac* gene. Based on the information submitted and presented by M/s Mahyco, RCGM observed that Bt brinjal is effective in controlling target pest, safe to environment, non-toxic in animal feeding tests, non-allergenic and has potential to benefit the farmers. Based on the above finding the RCGM had recommended that the GEAC may consider granting approval for conduct of large scale field trials of Bt brinjal as per the protocols submitted by M/s Mahyco.

2.3 COMPLIANCE WITH REGULATORY CONDITIONS STIPULATED BY GEAC IN THE PERMIT LETTER FOR LARGE SCALE TRIALS, 2007

The recommendations of the RCGM and the biosafety data was considered by the GEAC in its 68th meeting held on June 1, 2006. In accordance with the decision taken therein, the summary of biosafety data was posted on the GEAC website. Subsequently, the GEAC constituted EC-I under the chairmanship of Prof. Deepak Pental, Vice Chancellor, Delhi University. The EC-I reviewed the biosafety data submitted by M/s Mahyco as well as submissions received from several stakeholders.

The EC-I concluded that the biosafety data generated by M/s Mahyco is in accordance with the protocol and procedures stipulated by the regulatory agency. However, Bt brinjal being the first GM food crop to be released in India and the first to be released globally, the EC-I was of the view that a cautious step by step approach needs to be taken. It was also indicated that while the data generated by M/s Mahyco demonstrated that Bt brinjal is safe and equivalent to its non Bt counterpart, more studies may be required to re-affirm the findings made in the earlier studies. The EC-I further opined that the short term data generated on the environmental safety and socio economic aspects needs to be further substantiated with additional trials / tests to explicitly conclude the benefits from Bt brinjal and superiority of the technology with respect to existing technologies especially the available methods for pest management and pesticide reduction.

The EC-I was of the opinion that the large scale field trials may be allowed subject to certain conditions. In accordance with the recommendations of the EC-I, the GEAC permitted the conduct of large scale trials at 10 to 11 locations within the institutional research farms of IIVR/state agricultural universities (SAUs)/Indian Council of Agricultural Research (ICAR) under the direct supervision of Director, IIVR for generating additional biosafety data.

The EC-II reviewed the status of compliance of conditions stipulated by the GEAC for large-scale field trials and seed production of Bt brinjal expressing the *cry1Ac* gene (Ref. No. 12/ 81/ 2006-CS-II dated 30.08.2007). The observations of EC-II as deliberated in the meetings held on July 30, 2009 and August 31, 2009 are indicated in Table 2.2:

Table 2.2: Status of Compliance to the conditions in the permit letter issued by GEAC

S.No.	Conditions stipulated by GEAC	Objective of the additional studies prescribed by GEAC	Status of compliance	Remarks
a.	The hybrids shall undergo a minimum of 2 seasons of LST/ICAR (2007 and 2008) prior to its further consideration for commercial release, if any.	To have an independent assessment by Indian Institute of Vegetable Research (IIVR), an ICAR institution.	Seven Bt brinjal event EE-1 hybrids (MHB-4 Bt, MHB-9 Bt, MHB-10 Bt, MHB-11 Bt, MHB-39 Bt, MHB-80 Bt and MHBJ-99 Bt) were evaluated for LST/ ICAR trials for two seasons i.e. Kharif 2007 and Kharif 2008 in various agro-climatic zones under the direct supervision of Director, IIVR, Varanasi.	Reports of the studies have been received and the prescribed condition has been complied with.
b.	The LST/ICAR trials for assessing the environmental safety and agronomic advantage of Bt brinjal shall be carried out at minimum 11 locations within the institutional/research farms of IIVR/ICAR/SAU as per the protocol prescribed by the Director IIVR, Varanasi.	Independent assessment by IIVR to reconfirm the results regarding environmental safety assessment and agronomic advantage of Bt brinjal.	The data on shoot infestation, fruit infestation (peak and cumulative), gross and marketable yield, relative infestation of disease and non-target pests and beneficial arthropods has been generated in the LST/ICAR trials conducted by IIVR at the locations mentioned above. The tests related to impact on soil microflora in the rhizosphere were also conducted.	No deviation from the prescribed conditions. The trials were conducted by the IIVR/SAU scientists as per the protocols approved by Director, IIVR for generating the relevant parameters during 2007 and 2008. The results confirmed that Bt brinjal event EE-1 is effective in controlling FSB and is environmentally safe.
c.	The pollen flow shall be recorded during the field trials every 10 m up to 300 m in one trial plot at a minimum 6 locations representing different agro climactic zones for a period of two seasons. The pollen flow study should be conducted with a minimum of around 100 standing plants, planted	To reconfirm the pollen flow over two seasons as per the revised design.	Pollen flow studies were conducted during 2007-08 and 2008-09 at two locations, Jalna (Maharashtra) and Nizamabad (Andhra Pradesh) as per the approved protocol and under the direct supervision of Director, IIVR. A letter was issued by MoEF whereby Mahyco was directed to provide the required land for conducting pollen flow	There has been a deviation in the prescribed condition as pollen flow study has been undertaken at two locations instead of six. This deviation has the approval of GEAC on the following grounds: The total land required for conducting pollen flow including isolation distance at six locations was 552

	at an interval of 75x50 cm spacing.		studies at two locations as per the approved protocol and under the direct supervision of Director, IIVR, Varanasi. .	acres (92x6 acres), which was not available with IIVR and SAUs Sufficient information is available on biology of brinjal. The design of the protocol is as per the conditions stipulated by GEAC and has the approval of Director, IIVR. The results of these studies were in conformity with earlier information submitted by the applicant and available literature.
d.	The field trials shall include a minimum of one location (at IIVR, Varanasi) to assess the extent of crossability of any one hybrid of Bt brinjal (*Solanum melongena*) with *S. incanum*. The trial should also record the findings with respect to weediness and invasiveness of Bt *S. melongena*.	To address the issue related to pollen mediated crossability of *S. melongena* and *S. incanum*.	Crossability studies were carried out by IIVR for two seasons. The studies showed that pollen mediated crossability from *S. incanum* and *S. melongena* is possible to a limited extent. However pollen flow from *S. incanum* to *S. melongena* is easy as compared to *S. melongena* to *S. incanum*. The plots were also examined for aggressiveness as well as germination tests.	There has been no deviation in the prescribed condition. The results of crossability studies conducted by IIVR are in conformity with the available literature. *S. melongena* and *S. incanum* coexist in nature since ages and there are no reports of natural crossing. Thus, even though they are crossable under artificial conditions, their diversity in nature has in no way been affected and even now there are hundreds of different landraces/farmer varieties of the above species available in pure form.

				Further, it has been established through aggressiveness and germination tests, that Bt trait does not impart any aggressiveness or weediness.
e.	The baseline susceptibility data for at least three pests – Fruit and Shoot borer (*Leucinodes orbonalis*), Gram caterpillar/fruit borer (*Helicoverpa armigera*) and Stem borer (*Euzophera perticella*) shall be conducted during the two season field trials.	To generate baseline susceptibility data on all the three target pests.	The baseline susceptibility for the target insect pests viz. Fruit and Shoot borer (*Leucinodes orbonalis*), Gram caterpillar/fruit borer (*Helicoverpa armigera*) and Stem borer (*Euzophera perticella*) was established during two seasons of field trials.	There has been no deviation from the stipulated conditions. The data generated indicates that the three target insect pests are sensitive and highly susceptible to the Bt protein deployed in Bt brinjal event EE-1.
f.	Cry1Ac protein expression levels in plant parts shall be assessed every 15 days as prescribed by the RCGM throughout the crop cycle.	Cry1Ac protein expression levels were assessed every 30 days, instead of 15 days as prescribed by RCGM during MLRT for two season (2004 and 2005). The applicant was asked to undertake assessment of expression levels every 15 days during the LST.	Quantitation of Cry1Ac protein in various tissues of seven Mahyco Bt brinjal hybrids was carried out during 2007-08, across 11 locations. And during 2008-09, across 10 locations. The concentrations of in-planta expressed Bt insecticidal protein, Cry1Ac, in various tissues (leaf, shoot, stem, flower, fruit and root) were quantified using a quantitative enzyme-linked immunosorbent assay (ELISA).	The data generated is in compliance with stipulated conditions. It reconfirmed the consistency in expression of Cry1Ac protein throughout the crop cycle.
g.	Soil impact assessment study should include tests on the total microbial counts related to Rhizosphere on the soil of Bt and normal	To repeat soil impact assessment study at different depths upto 1 metre for reconfirmation of the results obtained during MLRT.	Soil impact assessment study was conducted on soil samples collected from Bt brinjal large-scale trial conducted at Parbhani (Maharashtra) during 2007-08 and 2008-09 as per protocol approved by	The study is in compliance with stipulated conditions.

	plots and for the presence/absence of Cry1Ac protein at different depths (maximum up to one metre) in the soil at one location. The changes in soil fertility and impact on the next crop may also be recorded, as per protocol devised by the Director, IIVR Varanasi. The study shall also assess carry-over effects of residues of Bt brinjal.		IIVR . The study included tests on the total microbial counts related to rhizosphere. The counts included bacterial population, fungal population, earthworm and Collembola. The samples were drawn prior to sowing as well as post harvest upto 180 days after transplanting. No Cry1Ac protein was detected in any of the soil samples. The Cry1Ac protein was estimated in the samples grown from both root and non root zones at different depths i.e. 30 cm, 60 cm and 1 metre.	
h.	Bt brinjal being a food crop, a flavour analysis of Bt and non-Bt fruits shall be undertaken at Central Food Technology Research Institute (CFTRI), Mysore/ any other NABL accredited laboratory.	Suggested by Dr. V. Prakash, Director, CFTRI in the GEAC meeting.	CFTRI, Mysore was approached for flavour study. However, they expressed their inability to conduct study on transgenic crop product at this stage.	There is a deviation as the institution refused to conduct the study. However, as per the recently adopted "Guidelines for safety assessments of food derived from GE plants, 2008", such kind of studies do not form part of safety assessment. EC-II is of the view that such studies are not required as per the internationally prescribed Codex guidelines and national guidelines prescribed by the GEAC. Therefore, studies of such nature need not be a prerequisite for consideration for environmental release.

i.	The food/feed safety assessment should include any possible foliage/shoot toxicity study in goats.	This condition was stipulated in view of the apprehensions that there were sheep deaths in Andhra Pradesh due to grazing on Bt cotton fields.	GEAC decided to dispense with this requirement on the following ground: i. The reports of sheep deaths due to Bt cotton were unsubstantiated. ii. RCGM indicated that large mammals like goats are not used for toxicity studies using whole foods, anywhere in the world and there are no scientific references on validation of goat as a model for studying sub-chronic feeding studies. iii. Brinjal leaves are not part of natural diet of goats and thus feeding protocol cannot be scientifically validated.	There is no deviation as the requirements for goat study was dispensed by GEAC. Regulatory agencies have adopted the "Guidelines for the safety assessment of foods derived from GE plants, 2008", which is in line with the Codex requirement. The new guidelines do not require any food and feed safety assessment using goats as the model.
j.	The skin sensitization test of transgenic material in guinea pigs as laid down in the DBT guidelines shall be conducted.	The study was recommended as it was part of the DBT guidelines, 1998.	RCGM was of the view that such skin sensitized tests on plants has no relevance especially when Bt brinjal has found to be safe in the feeding studies and even the purified Bt gene has been extensively studied for toxicity and allergenicity. The guidelines since have been revised and the study is not required as per the "Guidelines for safety assessment of foods derived from GE plants, 2008".	The requirement to dispense with the skin sensitization study is as per the guidelines accepted by GEAC.
k.	Additional toxicity/ allergenicity/ compositional/ nutritional studies, if any, as recommended by Director, National Institute of Nutrition (NIN), Hyderabad shall be conducted.	Director, NIN was requested to examine raw data and clarify any variations/anomalies in the toxicity and allergenicity and also indicate the need to repeat additional food safety studies keeping in view the Codex guidelines.	Raw data has been examined by Director, NIN and found to be satisfactory. No additional studies were recommended by Director, NIN regarding toxicity and allergenicity except the need for detailed compositional analysis. The same has been initiated by the applicant after the protocols was approved by RCGM in its 77th meeting held 02.05.2009.	There is no deviation from the stipulated conditions. The toxicity and allergenicity studies conducted by the applicant are in conformity with "Guidelines for the Safety Assessment of Foods Derived from Genetically Engineered Plants,2008".

				The detailed compositional analysis has been completed.
I.	Socio-economic study as prescribed by a three member sub-committee comprising of Dr. S. Parasuraman, Director, TISS, Mumbai, Dr MN Murthy, Director, IEG and Dr Mathura Rai, Director, IIVR Varanasi shall be conducted.	To assess the economic benefits of Bt brinjal	GEAC in its meeting held on 2nd May 2008 decided that the socio-economic studies may be assigned to the National Centre for Agricultural Economics and Policy Research (NCAP).	The *ex ante* assessment of socio-economic benefits of Bt brinjal has been initiated by NCAP with the financial support of MoEF.

2.4 COMPLIANCE WITH THE GUIDELINES FOR THE SAFETY ASSESSMENT OF FOODS DERIVED FROM GE PLANTS, 2008

The Indian Council of Medical Research (ICMR) has formulated guidelines to establish the safety assessment procedures for foods derived from GE plants taking into consideration the international *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants, 2003 by Codex Alimentarius Commission.* These guidelines were adopted by RCGM and GEAC in July 2008.

The EC-II also reviewed the status of compliance of biosafety data generated by M/s Mahyco with the newly adopted "Guidelines for the safety assessment of foods derived from genetically engineered (GE) plants, 2008". The observations of EC-II as deliberated in the meetings held on July 30, 2009 and August 31, 2009 are indicated in Table 2.3:

Table 2.3: Status of compliance with "Guidelines for safety assessment of foods derived from GE plants, 2008"

Checklist	Components	Status
Checklist 1: Description of the GE Plant	Identification of the crop Name of the transformation event(s) Pedigree map for each transformation event Purpose of the modification, sufficient to aid in understanding the nature of the food being submitted for safety assessment	Completed
Checklist 2: Description of the Non-Transgenic Host Plant and its Use as Food	Common or usual name; botanical name; and, taxonomic classification; History of cultivation and development through breeding, in particular identifying traits that may adversely impact on human health; Information on the host plant's genotype and phenotype relevant to its safety, including any known toxicity or allergenicity; and History of safe use for consumption as food.	Completed
Checklist 3: History of Safe Use and Dietary Exposure	Information on how the plant is typically cultivated, transported and stored Information on special processing required to make the plant safe to eat The plant's normal role in the diet Part of the plant is used as a food source Is consumption of the plant important in particular subgroups of the population What important macro- or micro-nutrients does the food contribute to the diet	Completed

Checklist 4: Description of the Donor Organisms (To be provided for each donor organism)	▪ Common name ▪ Scientific name ▪ Taxonomic classification ▪ Information about the natural history of the organism as concerns human health ▪ Information on naturally occurring toxins, anti-nutrients and allergens ▪ For donor microorganisms, additional information on human pathogenicity and the relationship to known human pathogens ▪ Information on the past and present use, if any, in the food supply and exposure route(s) other than intended food use (e.g. possible presence as contaminants).	Completed
Checklist 5: Description of the Genetic Modification(s)	▪ Information on the specific method used for the modification ▪ Description and characterization of all genetic material used to modify the plant, including the source (*e.g.*, plant, microbial, viral, synthetic), identity and expected function in the plant ▪ Details of modifications to introduced, intermediate and recipient genetic material (*e.g.*, changes in amino acid sequence that may affect expression of the expressed protein) Provide a summary diagram of all genetic components of the vector, including coding regions, and non-coding sequences of known function and for each genetic component include: ▪ A citation where these functional sequences are characterized. ▪ Indicate the portion and size of the sequence inserted. ▪ Indicate the location, order, and orientation in the vector. ▪ Indicate the function in the plant. ▪ Indicate the source (common and scientific and/or trade name, of the donor organism). ▪ Indicate if the genetic component is responsible for disease or injury to plants or other organisms and is a known toxicant, allergen, pathogenicity factor, or irritant. ▪ Indicate if the donor organism is responsible for any disease or injury to plants or other organisms, produces toxicants, allergens or irritants or whether closely related to organisms that do. ▪ Indicate if there is a history of safe use of the donor organism or components thereof, if available.	Completed

Checklist 6: Characterization of the Genetic Modification(s)	Information about the DNA insertion(s) into the plant genome is required, including: - Characterization and description of the inserted genetic material. - Number of insertion sites. - Organisation of the inserted genetic material at each insertion site including copy number and data to demonstrate if complete or partial copies were inserted, and if the arrangement of the genetic material was conserved or if significant rearrangements have occurred upon integration. - Sequence data of the inserted material and of the flanking regions bordering the site of insertion**.** - Identification of any open reading frames within the inserted DNA or created by the insertions with contiguous plant genomic DNA including those that could result in fusion proteins.	Completed
	For any expressed substances in the GE plant provide: - The gene product(s) (e.g. a protein or an untranslated RNA); - The gene product(s)' function; - The phenotypic description of the new trait(s); - The level and site of expression of the expressed gene product(s) in the plant, and the levels of its metabolites in the edible portions; and	Completed
	The amount of the target gene product(s), where possible, if the function of the expressed sequence(s)/gene(s) is to alter the accumulation of a specific endogenous mRNA or protein. Information is required to demonstrate each of the following : - Deliberate modifications made to the amino acid sequence of the expressed protein result in changes in its post-translational modification or affect sites critical for its structure or function. - The intended effect of the modification has been achieved and that all expressed traits are expressed and inherited in a manner that is stable through several generations consistent with laws of inheritance. - The newly expressed trait(s) are expressed as expected in the appropriate tissues in a manner and at levels that are consistent with the associated regulatory sequences driving the expression of the corresponding gene. - Evidence to suggest that one or several genes in the host plant has been affected by the transformation process. - Confirm the identity and expression pattern of any new fusion proteins.	**Not applicable for Bt brinjal as the expressed sequence is not to alter the accumulation of any specific mRNA or protein.**

Checklist 7: Assessment of Possible Toxicity	▪ Indicate if the donor organism(s) is a known source of toxins. ▪ Amino acid sequence homology comparison of the newly expressed protein and known protein toxins and anti-nutrients. ▪ Demonstrate the susceptibility of each newly expressed protein to pepsin digestion. ▪ Where a host other than the transgenic plant is used to produce sufficient quantities of the newly expressed protein for toxicological analyses, demonstrate the structural, functional and biochemical equivalence of the non-plant expressed protein with the plant expressed protein. ▪ Acute oral toxicity study completed for newly expressed proteins.	Completed
Checklist 8: Assessment of Possible Allergenicity (Proteins)	▪ Indicate if the donor organism(s) is a known source of allergens (defined as those organisms for which reasonable evidence of IgE mediated oral, respiratory or contact allergy is available). ▪ Amino acid sequence homology comparison of the newly expressed protein and known allergens. ▪ Demonstrate the susceptibility of each newly expressed protein to pepsin digestion. ▪ Where a host other than the transgenic plant is used to produce sufficient quantities of the newly expressed protein for toxicological analyses, demonstrate the structural, functional and biochemical equivalence of the non-plant expressed protein with the plant expressed protein. ▪ For those proteins that originate from a source known to be allergenic, or have sequence homology with a known allergen, testing in immunological assays is to be performed where sera are available.	Completed **Not applicable as the Bt protein is neither known to be allergenic nor has sequence homology with any known allergen**
Checklist 9: Compositional Analyses of Key Components	For all parts of the GE plant and its conventional counterpart that may be used as food in India, provide the following: ▪ Proximate composition e.g., ash, moisture content, crude protein, crude fat, crude carbohydrate; ▪ Content of true protein, non-protein nitrogenous material (e.g., nucleic acids and aminoglycosides), amino acid profile [unusual amino acids should be determined if their presence is suspected (e.g., d-amino acids from bacterial proteins)]; ▪ Quantitative and qualitative composition of total lipids, i.e., saponifiable and nonsaponifiable components, complete fatty acid profile, phospholipids, sterols, cyclic fatty acids and known toxic fatty acids; ▪ Composition of the carbohydrate fraction e.g., sugars, starches, chitin, tannins, nonstarch	Completed

polysaccharides and lignin;
- Qualitative and quantitative composition of micronutrients, i.e., significant vitamin and mineral analysis;
- Presence of naturally occurring or adventitious anti-nutritional factors e.g., phytates, trypsin inhibitors, etc.;
- Predictable secondary metabolites, physiologically active (bioactive) substances, other detected substances.

CONCLUSIONS

In light of above stated facts, the EC-II in its deliberations held on August 31, 2009 concluded that the development and safety assessment of Bt brinjal event EE-1 is in accordance with the prevailing biosafety guidelines and is fully compliant with the conditions stipulated by GEAC, while according approval for large scale trials. The EC-II also noted that the data requirements for safety assessment of GM crops in India are comparable to the internationally accepted norms in different countries and by international agencies and therefore no additional studies need to be prescribed for safety assessment.

*****

SECTION III
REVIEW OF Bt BRINJAL SAFETY ASSESSMENT DOSSIER

The EC-II considered information from the primary data provided by M/s Mahyco, studies conducted by IIVR and other public sector institutions, biology of the host plant (Brinjal) and information from the scientific literature to assess the environmental and food and feed safety of the Bt brinjal event EE-1. Review of the data, as per the deliberations of the EC-II in its two meetings held on July 30, 2009 and August 31, 2009 is divided into three parts *viz*. nature and effect of genetic modification, environmental safety and food and feed safety.

3.1 NATURE AND EFFECT OF GENETIC MODIFICATION

3.1.1 Host plant/recipient organism:

The EC-II noted that M/s Mahyco has used seeds of a proprietary line of cultivated brinjal as the source material for the genetic transformation. As mentioned in the previous section, brinjal is extensively grown in India as an agricultural crop. It belongs to the family Solanaceae and its botanical name is *Solanum melongena* L. There are three main botanical varieties under the species *melongena*. The round or egg-shaped cultivars are grouped under var. *esculentum*. The long, slender types are included under var. *serpentinum* and the dwarf brinjal plants are put under var. *depressum*. The common brinjal, to which the large fruited forms belong, is known under the name *S. melongena* var. *esculentum*.

3.1.2 Transformation system:

Standard *Agrobacterium*-mediated transformation methods were used for developing Bt brinjal event EE-1, utilising established protocols to obtain single-copy insertion plants. *Agrobacterium*-mediated transformation has been used for the development of numerous biotech crops grown around the world for the past two decades, and has a proven track from a biosafety standpoint. *Agrobacterium tumefaciens* strain LBA4404 incorporating the vector pMON 10518 (which bears *cry1Ac*, *nptII* and *aad* genes) was utilised. The *cry1Ac* gene is under the transcriptional control of the enhanced *CaMV35S* promoter (P-E35S). Young cotyledons of brinjal were the explants of choice, and plants carrying the *cry1Ac* and *nptII* gene cassettes were regenerated on kanamycin-containing media. The transformation method was a modified method developed at Mahyco, based on that of Fari *et. al*., 1995.

3.1.3 DNA sequences inserted into Bt brinjal event EE-1

M/s Mahyco has provided data to support that Bt brinjal event EE1 contains a single integration consisting of three genes *viz*. *cry1Ac*, *nptII* and *aad*. The sources of these genes and their regulatory sequences are as follows:

- ***cry1Ac* gene:** The *cry1Ac* gene encodes for an insecticidal protein and has been derived from the common soil bacterium *Bacillus thuringiensis* sub-species kurstaki. *B. thuringiensis* strains has been used for decades

in agriculture as the basis for microbial pesticide formulation i.e. bacteria are grown in laboratories to prepare suspensions that can be applied to plant surfaces to deter plant eating insects.

The Cry (crystalline) 1Ac protein (also called Bt protein or Bt toxin) encoded by *cry1Ac* gene belongs to a diverse family of insecticidal proteins with specific toxicity to certain groups of insects. This Bt protein encoded by *cry1Ac* gene is highly specific to lepidopteran insects including fruit and shoot borer (FSB), which is a major pest of cultivated brinjal.

It has been well established that the Cry proteins diffuse through the midgut membrane of feeding lepidopteran insects and bind to specific high affinity receptors on the midgut epithelium surface (Hofmann *et al*. 1988; Karim *et al*. 2000' Van Rie *et al*. 1990). Non target insects, mammals, birds and fish do not possess these receptors and therefore are not susceptible to the toxic effects of these insecticidal proteins.

For the Cry proteins to be effective, it requires alkaline conditions (as provided in the larval insect gut) to dissolve the crystals, partial digestion by specific proteases to release the active core toxin, and binding to specific receptors found on the insect midgut epithelium surface. Binding leads to formation of pores in the cell membrane which leads to leakage of intracellular contents into the gut lumen and water into the cell, resulting in cell death, gut paralysis and starvation. It is these steps that provide the high degree of target specificity of each protein (English & Slatin 1992; Hofmann *et al*. 1988; Knowles & Dow 1993; Van Rie *et al*. 1989).

Expression of the *cry1Ac* gene in Bt brinjal event EE-1 is controlled by an enhanced 35S promoter from *CaMV*, which is a retrovirus that can infect cruciferous plants (Kay *et al*. 1987; Odell *et al*. 1985). The termination sequence is derived from the alpha subunit of the *beta-conglycinin* gene of soybean (Schuler *et al*. 1982) for the *cry1Ac* gene.

Both regulatory sequences introduced into the Bt brinjal event EE-1 are not capable of causing any disease.

- ***nptII* gene:** The *nptII* gene has been inserted as the selectable marker, which allowed modified brinjal plant cells to grow in the presence of the kanamycin, and therefore be selected, while inhibiting the growth of non-modified cells. It has no pesticidal properties.

The *nptII* gene was isolated from the *E. coli* Tn5 transposon (Beck *et al*. 1982). It encodes the enzyme neomycin phosphotransferase type II (NPTII), which confers resistance to the antibiotics kanamycin and neomycin. NPTII uses ATP to phosphorylate kanamycin and neomycin, thereby inactivating the antibiotic and preventing it from killing the NPTII-producing cell.

Expression of the *nptII* gene in the Bt brinjal event EE-1 is controlled by the *CaMV35S* promoter (Kay *et al*. 1987; Odell *et al*. 1985) and the termination sequence of this gene is the 3' non-translated region of the *nos* gene from *A. tumefaciens* (Rogers *et al*. 1985). Although these regulatory sequences are derived from plant pathogens they are not capable of causing disease.

The EC-II also noted that United States Food and Drug Administration (FDA) had approved the use of *nptII* as a processing aid by amending the food additive regulations way back in 1994. In addition, the US Environmental Protection Agency (EPA) has exempted the NPTII protein and the genetic material necessary for the production of the protein from the requirement of a tolerance in or on all agricultural commodities when used as a plant-pesticide inert ingredient in 1994. Subsequently, the safety of this marker gene have been approved for use in crops grown for human and animal use in other countries viz. Canada, Australia, EU, Japan, Philippines etc. including India in Bt cotton.

- ***aad* gene:** The *aad* gene encodes for the bacterial selectable marker enzyme 3"(9)-O- aminoglycoside adenyl transferase (AAD) which confers resistance to the antibiotics streptomycin and spectinomycin. The *aad* gene is also used as the selectable marker and allowed for the selection of bacteria containing the pMON 10518 plasmid.

  The *aad* gene is not expressed in the Bt brinjal plants because it is under the control of a bacterial regulatory sequence, the Tn7 promoter, which is not active in plants.

3.1.4 Molecular characterization

The EC-II noted the molecular analyses of Bt brinjal lines shows that there is a single insert of T-DNA at single location in brinjal genomic DNA. The DNA from the transgenic Bt brinjal and non-transgenic control plants were extracted, digested using an enzyme *Hind III,* separated on 1.0 % agarose gel and hybridized to DIG-labeled *Bt* probe. There is a single restriction site of *Hind III* in the T-DNA. In the transgenic Bt brinjal lines a single band hybridized to the *cry1Ac* probe in the Southern blot analysis, which indicates that the plants have only a single transgene insert.

The EC-II noted that method has been developed to identify insect resistant transgenic brinjal lines comprising specific event EE-1 expressing Cry1Ac, which describes a protocol for detection of this specific Bt brinjal event EE-1. The specific location of the insertion of the heterologous gene was analyzed by molecular methods. This involves cloning of the genomic region flanking the borders of the T-DNA into suitable vectors and analysis of this flanking region by sequencing. PCR reactions, using the brinjal genomic sequence primer in conjunction with a primer within the T-DNA, allow for specific amplification from the Bt brinjal event EE-1 deployed in the Mahyco brinjal hybrids.

3.1.5 Expression of Cry1Ac protein and its quantification

The expression of Cry1Ac protein has been studied in various tissues (leaf, shoot, stem, flower, fruit and root) using a quantitative enzyme-linked immunosorbent assay (ELISA). The levels of Cry1Ac protein were found to vary between 5 to 47 ppm in shoots and fruits. Mean molt inhibitory concentration (MIC_{95} for *Leucinoides orbonalis* has been calculated to be 0.059 ppm for Cry1Ac.

3.1.6 Stability

The *cry1Ac* gene in Bt brinjal has been demonstrated to be stably integrated into the chromosome based on molecular analyses, data on Cry1Ac protein expression and inheritance patterns as indicated below:

- Southern blot analyses of Bt brinjal performed on the original line and in advanced generation plants have provided an identical Southern blot pattern;
- Analyses of seed obtained from multi-site trials over five years using ELISA tests showed similar levels of the Cry1Ac protein;
- The presence of Cry1Ac protein and its bioefficacy has been confirmed over multiple years and growing seasons across 60 field trials, for evaluating different Bt brinjal hybrids under different environmental conditions;
- Inheritance of the *cry1Ac* gene was confirmed as a single dominant Mendelian factor, by both DNA and protein methods. This confirmed stable inheritance of the gene as seen during backcrossing program to develop seven brinjal hybrids;
- The data from the backcrosses to other brinjal hybrids demonstrates the stability of the transfer of the functional insert from generation to generation.

CONCLUSIONS

In light of the above stated facts and taking into consideration the fact that the *cry1Ac* gene as well as the selectable markers used by M/s Mahyco have been extensively studied by research scientists and evaluated and approved by regulatory agencies worldwide, the EC-II concluded that the nature and effect of gene modifications in Bt brinjal event EE-1have been properly defined and all the inserted genes and regulatory sequences have a history of safe use in view of their inherent characteristics/properties. The EC-II noted that the expression of *cry1Ac* gene is consistent and the levels of Cry1Ac protein concentrations are sufficient for effective control of fruit and shoot borer (FSB) at all locations and the entire life of the crop. Further, the EC-II opined that the insect resistance trait is stably integrated in the brinjal genome and there is no evidence or likelihood of genetic instability.

3.2 ENVIRONMENTAL SAFETY ASSESSMENT

The objective of environmental risk assessment of transgenic crops is to identify and evaluate the risks associated with the release and cultivation of these plants in comparison with a conventional counterpart that typically has a history of safe use. As part of the regulatory requirements, M/s Mahyco has conducted studies to assess the potential of gene transfer, relative weediness, impact on non target organisms including beneficial organisms and soil microflora, accumulation or persistence of Bt protein in the soil and variations in pests and disease susceptibility**.**

3.2.1 Potential of gene transfer to related species

i. Gene transfer to wild relatives: The genus *Solanum*, to which brinjal belongs is a very large genus consisting of both tuberous and non-tuberous species. The chromosome number of many species under non-tuberous group is fairly stable as 2x = n=24. There are 22 Indian species, out of which, there is a group of five related ones namely *S. melongena* L., *S. incanum* L., (often considered synonymous to *S. coagulans* L.*), S.xanthocarpum, S. indicum* L. and *S. maccani*. (Choudhury, 1976). From various reports available in the literature, it appears that *S. melongena* is more closely related to *S. incanum* than to any other species.

As per the recommendations of EC-I and conditions stipulated by GEAC in 2007, IIVR conducted the crossability studies of *S. melongena* with other *Solanum* species to confirm the above in 2008. It was concluded from the results of this study that *S. melongena* does not appear to be compatible with most of its wild relatives tested in this study except for *Solanum incanum*. Other wild species *viz*. *S. indicum, S. sisymbrifolium, S. nigrum* and *S. torvum* were found to be non-compatible with the cultivated *S. melongena* lines. Crosses of the cultivated *S. melongena* (Punjab Sadabahar) with *S. torvum* produced a few seeds, but later these seeds failed to germinate. The EC-II noted that these findings are in line with the results of studies conducted earlier (Handique, 1986; Vishwanathan, 1975 and Rao, 1979).

To establish the extent of crossability between *S. incanum* and *S. melongena*, further tests were carried by IIVR with five hybrids of *S. melongena*. It was observed that *the S incanum* was crossable with all the hybrids used in this study. The crossability was very low when *S incanum* was used as female parent and hybrids were taken as male parent, as compared to when hybrids were taken as female parent and *S incanum* was used as male parent. From this study it was concluded that *S incaum* has good crossability with cultivated lines as male parent as compared to female parent.

The EC-II noted that it is known that S. *melongena* and its wild relatives including S. *incanum* co-exist in nature since ages. Even thoug h they are crossable, their diversity in nature has in no way decreased and even now

there are hundreds of different landraces/farmer varieties of the above species available in pure form. Over ages of natural cross-pollination, all the three species are seen in nature and/or farmers fields. No instances of natural interspecific hybridization with wild species have been reported for cultivated brinjal.

ii. **Extent of outcrossing:** Brinjal is a normally highly self-pollinated crop, however the scientific data with regard to cross pollination generated independently by various groups shows a wide range of outcrossing, suggesting its classification as "an often cross pollinated crop". The cone-like formation of anthers favors self pollination; but since the stigma ultimately projects beyond the anthers, there is an opportunity for cross-pollination. The rates of natural cross pollination vary depending on genotype, location/environmental factors, and insect activity. The extent of outcrossing has been reported from 3 to7% in China and from 0 to 8.2% (with a mean of 2.7%) at AVRDC (Chen, 2000); however the Indian researchers have reported 2 to 48% outcrossing in brinjal varieties in India (Agrawal, 1980; Choudhary, 1971; Sambandam, 196 and Choudhary *et. al.* 1970)

The pollen flow studies for Bt brinjal event EE-1 were conducted at 2 locations by Mahyco in 2002. In these experiments, Bt brinjal entry was planted on 20 x 20 metre in the centre surrounded by concentric rings of non Bt brinjal at regular intervals. The bee hives were installed at all the corners of Bt brinjal crop to assist in pollination. The percent outcrossing for Bt trait ranged between 1.46 to 2.7% and the maximum distance traversed by pollen from Bt brinjal plants was determined to be 20m based on the Grow Out Test and ELISA. The majority of cross-pollinations (82-89%) occurred within 6m of the Bt plot. The percentage of outcrossing varied depending on wind direction.

As per the recommendations of EC-II and conditions stipulated by GEAC in its permit letter for LST, two more years of pollen flow study in 2007 and 2008 to reconfirm the above information on pollen flow and outcrossing in brinjal were carried out at two locations each at Jalna, Maharashtra and Nizamabad, Andhra Pradesh under the supervision of the Director, IIVR. The trial design consisted of Bt brinjal core plot in the centre surrounded by equidistant non Bt brinjal blocks upto 300 metres on all sides. The ripened fruits were collected by IIVR scientists. The seeds extracted from these fruits were tested for outcrossing and pollen flow of Bt gene by Grow Out Test and ELISA. The outcrossing percentage during these two years ranged from 0.14% to 0.85% and the Bt pollen traveled upto a maximum of 30 metres distance.

The EC-II noted that the pollen flow studies conducted over a period of four years confirmed that there is limited outcrossing in *S. melongena* even in cases of complete genetic compatibility. The maximum distance that the pollen travelled was only upto 30 metres.

Further, it was observed that since all the studies were conducted with both Bt brinjal hybrids as well as their counterparts non Bt hybrids, it clearly

demonstrated that the introgression of Bt trait has in no way affected the outcrossing potential of *S. melongena*.

The EC-II also discussed the implication of the pollen flow/outcrossing to neighbouring non Bt brinjal fields. The members opined that in view of the relatively short distance that the pollen could travel, it is evident that the isolation distance or differences in planting time can help in minimizing the potential for any unwanted outcrossing of transgenic brinjal to the conventional brinjal varieties, as may be required in cases of seed production (breeders, foundation or certified seeds), organic farming etc.

Further, the EC-II opined that even if there is a very small influx of pollen originating from Bt brinjal varieties, it is not of any consequence, as the Bt protein has been extensively tested for its safety to the environment and food/feed and thus pollen transfer to other cultivated brinjal would not pose any safety risk.

iii. Gene transfer from brinjal to other plants: There are no reports of any gene transfer from brinjal to unrelated plant species. Further it may be noted that such a transfer of any gene is highly improbable because of pre-and post-zygotic genetic incompatibility barriers that are well documented for distantly related plant groups. No evidence for transfer of genes from brinjal to other plant taxa has been identified.

iv. Gene transfer from brinjal to other organisms: Horizontal gene transfer from plants to animals (including humans) or microorganisms is extremely unlikely. No evidence has been identified for any mechanism by which plant genes could be transferred to humans or animals, nor any evidence that such gene transfer has occurred for any plant species during evolutionary history, despite animals and humans eating large quantities of plant DNA. The likelihood of brinjal genes transferring to humans and other animals is therefore effectively zero. Similarly gene transfer from brinjal, or any other plant, to microorganisms is extremely unlikely. Horizontal gene transfer from plants to bacteria has not been demonstrated experimentally under natural conditions (Nielsen *et al*., 1997; Nielsen *et al*., 1998; Syvanen 1999) and deliberate attempts to induce such transfers have so far failed (Schlüter *et al*,. 1995; Coghlan 2000).

3.2.2 Potential for relative weediness of Bt brinjal event EE-1

The EC-II considered whether Bt brinjal event EE-1 hybrids are any more likely to become a weed than their non Bt counterparts. This assessment encompasses a thorough consideration of the basic biology of brinjal and evaluation of parameters such as germination and aggressiveness as indicated below:

i. Weediness characterization of *S. melongena*: No reports of weedy species of cultivated brinjal i.e. *Solanum melongena* are available. Brinjal is not considered to have weedy characteristics, such as seed dormancy, soil

persistence, germination under diverse environmental conditions, rapid vegetative growth, a short life cycle, high seed output and dispersal.

ii. **Germination and vigour:** The rate of germination and vigour of Bt brinjal lines over their non-Bt counterparts was tested by M/s Mahyco. The germination tests were conducted in soil conditions in green house and in the laboratory using standard germination paper/ towel method. The rate of germination and vigour was compared by laboratory test and in soil to the non-transformed counterpart. The EC-II noted that there are no significant differences between Bt and non-Bt brinjal for germination and vigour.

iii. **Aggressiveness:** A field study was conducted by Mahyco to monitor the aggressiveness of Bt brinjal event EE-1 as compared to its non-Bt counterparts. After complete harvesting of the brinjal crop, the area under planting of Bt brinjal at Jalna, Maharashtra was left undisturbed and irrigated on a regular basis to allow for germination of any seeds that might have remained in the ground after harvesting the main crop (plot was observed up to 3 months after final harvesting). The data provides information on germination rates and aggressiveness under field conditions of naturally shed brinjal seeds in the plots where Bt and non-Bt plants had been grown. If any plant growth occurred, the same was checked with ELISA to determine if it was transgenic or not.

There was no brinjal plant observed to grow or germinate in this plot for the period of the study. The data suggest that there is no aggressiveness or weediness demonstrated by Bt brinjal plants and these plants behave in a similar fashion as other conventional brinjal varieties.

iv. **Agronomic observations:** The EC-II noted that the growth and development of Bt brinjal were routinely monitored in all the greenhouse field trials. Bt brinjal does not exhibit any different agronomic or morphological traits compared to non-Bt brinjal/controls that may give it a competitive advantage over other species in the ecosystem in which it is grown.

3.2.3 Impact on non-target organisms

A non-target organism is any plant, animal or microorganism that is unintentionally affected by cultivation of a transgenic plant. The EC-II noted that impact of Bt brinjal event EE-1 on non target organisms has been studied using both lab and field based studies using several indicative species and analyzed the results presented by M/s Mahyco as indicated below:

i. **Specificity of Cry proteins:** As explained above, the Cry1Ac protein is highly specific to lepidopteran pests and is not expected to adversely affect non-target organisms (invertebrates and vertebrates including birds, mammals and human), because they do not have the receptor proteins found in the midgut of target insects.

ii. **Laboratory based ecotoxicology experiments:** The EC-II reviewed data from a series of ecotoxicology experiments submitted by M/s Mahyco. It was

noted that the test substance used in these experiments is the Cry1Ac protein purified from bacterial strain genetically engineered to express Cry1Ac. This was necessary due to extremely low levels of *cry1Ac* produced in the plant and the requirement of large quantity of protein for these studies. It was further noted that M/s Mahyco has confirmed that the bacterially-produced Cry1Ac protein, as purified and prepared for these studies, was similar in its biochemical properties (molecular weight, amino acid sequence, and lack of glycosylation) and in biological activity to warrant use as a test substance comparable to Cry1Ac protein as produced in Bt brinjal event EE-1. The summary of results and conclusions of ecotoxicology experiments with honeybee (adult and larval), parasitic Hymenoptera, Ladybird beetle, Collembola and green lacewing larvae are given Table 3.1.

Table 3.1: Summary of ecotoxicology experiments with Cry1Ac protein

Test Organism	Results*	Conclusions
Adult honey bee	6.54 to 7.87 ppm	The LC_{50} value of Cry1Ac protein is greater than 20 ppm. The NOEL was 20 ppm.
Larval honey bee	9.9 to 26.67 ppm	The LC_{50} value of Cry1Ac protein is greater than 20 ppm. The NOEL was 20 p pm.
Parasitic Hymenoptera	20 ppm	The LC_{50} value of Cry1Ac protein is greater than 20 ppm. The NOEL was 20 ppm.
Ladybird beetle	20 ppm	The LC_{50} value for ladybird beetle exposed to Cry1Ac protein for 30 days was determined to be > 20 ppm. The NOEL was 20ppm.
Collembola	200 ppm	The NOEC (200 ppm) was 100 fold higher than that of the positive control (2.0 ppm – chlorpyriphos)
Green Lacewing Larvae	20 ppm	The LC_{50} value for green lacewing larvae exposed to Cry1Ac protein for 11 days was determined to be > 20 ppm. The NOEL was 20 ppm.

* - Estimated cry1Ac concentration in diet
NOEC- No observed effect concentration
NOEL – No observed effect level

No adverse effects were found at the above levels, which are significantly higher than that would be present in the fields. Effect of Bt protein has also been studied in other organisms such as bumblebees (Box 3.1)

Box 3.1 : Effect of Bt protein on bumblebees

As bumblebees (*Bombus impatiens* Cresson) play an important ecological role, their sensitivity against Bt protein was analysed as well – like that of honeybees and many other non-target-organisms. When bumblebees took up purified Cry1Ac protein with their food, no effect on feeding behaviour, weight, colony size, and amount of brood or the sex ratio of the progeny was observed (Morandin & Winston 2003).

There were no measurable effects on bumble bee colony or individual bee health from exposure to Cry1Ac at concentrations similar to and above the highest residue levels found in pollen, consistent with previously published results for honey bees (Sims 1995, Picard-Nizou et al. 1997). The Cry1Ac protein concentrations that was tested on *B. occidentalis* and *B. impatiens* colonies were chosen to reflect levels present in or higher than pollen of treated or modified commercially grown crops. Results suggest that genetically modified crops expressing field levels of the proteins as tested, will not harm wild bumble bee colonies. The Bt protein Cry1Ac did not cause any lethal or sublethal effects to *B. impatiens* colonies. Access times and foraging rates did not differ from those of control bees, indicating that plants transformed with the c*ry1Ac* gene should be safe for bumble bees in the field.

The EC-II noted that the ecotoxicity of the Cry1Ac protein has also been assessed against a number of reference organisms and no adverse effects were observed at concentrations significantly greater than the predicted environmental concentrations. This is in conformity with published reports (Mendelsohn *et al*., 2003).

iii. **Field based studies to assess impact on non target pests and beneficial insects:** M/s Mahyco conducted a series of field trials at multiple locations during the years 2004-05 and 2005-06. The trials were also conducted by ICAR and SAUs i.e. TNAU, Coimbatore and UAS, Dharwad. The results have been reconfirmed during two year large scale trials by IIVR during 2007 and 2008 The protocol adopted to conduct these trials had specific mention of the assessment of the effect of Bt brinjal on non-target pests (sucking pest and secondary lepidopterans) and beneficial insects of brinjal crop. Non-target insect pests include leaf roller (*Eublemma olivacea*), Epilachna beetle (*Epilachna duodecastigma* (12-spotted), *E. vigintioctopunctata* (28-spotted)), grey weevil (*Myllocerus* sp.), root grub (*Holotrichia* sp.), and sucking pests (Aphids (*Aphis gossypii*), leafhoopers (*Amrasca devastans*), thrips (*Thrips* sp. and *Frankliniella schultzii*), whiteflies (*Bemisia tabaci*), mites (*Tetranychus* sp.).The vast data collected in all these years from field trials conducted at various locations showed that non-target sucking pest counts did not vary significantly among Bt and non-Bt brinjal hybrids.

The incidence of beneficial arthropods such as green lacewing (*Chrysopa* sp.), ladybird beetles, spiders, syrphid flies and praying mantis (*Mantis religiosa*) were recorded and these were also observed to be active in both Bt and non-Bt brinjal crops.

iv. Soil impact studies: Soil impact assessment studies were conducted to analyse the effect of Bt brinjal expressing *cry1Ac* gene event EE-1 on soil microflora, collembola, nematodes, and earthworms, and assessment of Bt protein in soil. The EC-II noted that M/s Mahyco had conducted soil impact assessment studies over two seasons at eight locations covering different agro climatic zones of the country in 2003 and 2004. These studies analyzed root zone and non root zone, as well as pre and post harvest samples. As per the recommendations of the EC-I and conditions stipulated by GEAC, M/s Mahyco and IIVR repeated the above studies to evaluate the effect of Bt brinjal expressing Cry1Ac protein on soil microflora, collembola, nematodes, and earthworms and to determine the levels of Cry1Ac protein, if any, at different depths upto one metre, in soil during and after Bt brinjal cultivation.

The first soil sampling was done before transplanting and subsequent collections were made at 30, 60, 90, 120 and 150 days after transplanting. Finally, soil sampling was also done after harvesting of the crop. Soil samples were analyzed for level of Cry1Ac protein and the populations of culturable bacteria and fungi were measured. Collembola populations were measured using pitfall traps. Earthworms were observed in both Bt and non-Bt plots. Nematodes were extracted from the soil samples and their population was statistically analyzed. In addition the presence of Cry1AC protein, if any in the soil was assayed by ELISA.

The EC-II noted that the findings demonstrate that Bt brinjal event EE-1, expressing Cry1Ac protein, does not have any significant effect on soil microflora (both fungi and bacteria), and soil invertebrates such as earthworm, collembola and nematodes. This is in agreement with numerous published studies which have shown that *B. thuringiensis* and Bt proteins act specifically on the target insect pests and that they do not have any deleterious effect on non-target organisms. No Cry1Ac protein was detected in any of the soil samples from Bt brinjal field plots, which demonstrates that the protein is rapidly degraded.

It was further noted that *cry1Ac* gene has been derived from a common soil bacterium and therefore it is expected that soil microorganisms are already exposed to these proteins within the environment. Extensive scientific literature is available to confirm the above (Box 3.2).

Box 3.2: Effect of Cry proteins on soil microorganisms

Series of research papers have been published to establish that purified B.t.k toxins have no effect on *in vitro* growth of pure or mixed cultures of gram positive bacteria (*Bacillus subtilis*, *B. cereus*, *B. thuringiensis* (subspecies *kurstaki* and *israelensis*), *Arthrobacter globiformis*), gram negative bacteria (*Agrobacterium radiobacter, Pseudomonas aeruginosa, Proteus vulgaris, P. mirabilis, Escherichia coli, Enterobacter aerogenes, E. cloacae, Oscillatoria* sp.), yeast, (*Saccharomyces cerevisiae, Candida albicans*), filamentous fungi (*Rhizopus nigricans, Cunninghamella elegans, Aspergillus niger, Fusarium solani, Penicillium* sp.) algae (*Chlamydomonas* sp., *Oedogonium* sp, *Euglena* sp.) and diatoms (Stotzky 2000c).

The effect of Cry1Ac protein on soil microorganisms was also examined by incubating soil with purified Cry1Ac protein (0.05 ug/g) (Donegan *et al*. 1995a). The numbers and types of protozoans, bacteria and fungi were determined at various time points. Substrate utilisation tests and DNA fingerprinting of eubacterial ribosomal sequences were also used to analyse the composition of bacterial soil community. In these experiments, addition of purified Cry1Ac protein to the soil did not cause any detectable effects on populations of culturable aerobic soil bacteria, fungi or protozoa after exposure for up to 56 days.

The large scale cultivation of Bt cotton event MON 531 containing *cry1Ac* gene since 2002 without evidence of any toxic effects further reconfirms that Cry1Ac protein has no deleterious effect on soil microflora.

3.2.4 Possible accumulation and persistence of Bt protein in soil

The EC-II also evaluated the possibility of accumulation and persistence of Cry1Ac protein in soil where the Bt brinjal crop is likely to be grown repeatedly and plant residues such as roots are ploughed back into soil. It was noted that this important environmental concern has been assessed by measuring the level of Bt protein in soil samples. As mentioned above, the residual Cry1Ac protein is not detectable in any of the soil samples tested during as well as after the harvest of the crop. These results are consistent with the literature reports that the Bt protein is rapidly degraded in the soil and therefore, there is no accumulation of the protein in the soil associated with production of Bt brinjal. The half life of Cry1Ac protein has been reported to be 9.3 to 40 days depending on soil types as depicted in Box 3.3.

Box 3.3: Reports regarding persistence and accumulation of Cry proteins in soil

- **Persistence in soil of transgenic plant produced *Bacillus thuringiensis var. kurstaki* delta-endotoxin (Palm *et al*., 1996):** Experiment demonstrated that the transgenic plant B.t.k. toxin, similar to the commercial microbial Bt formulations, does not persist at high amounts in the soil but that low amounts may persist for several weeks or months. The B.t.k. endotoxin used in these studies was the purified tryptic core protein Cry1Ac isolated from B.t.k. HD-73. Half-lives, calculated from an exponential curve fit to the data points, were 40 days in the Millican soil and 22 days in the Thatuna-Naf soil.
- **No detection of Cry1Ac protein in soil after multiple years of transgenic Bt cotton (Bollgard) use (Head *et al*., 2002):** Here, the soil samples were collected from different sites where bollgard cotton had been planted in each year for the previous 3-6 consecutive years. The quantification of both functional and non-functional Cry1Ac

protein was done, using ELISA and insect susceptible bioassays. No Cry1Ac protein was detected by ELISA in any of the soil samples collected from the sites. The LOD for Cry1Ac protein in soil based on three standard deviations was3.68 ng of extractable protein per gram of soil. *H. virescens*larvae assayed for both non-Bt and Bt soil treatments in this study. The dose-response relationships between the standard Cry1Ac protein concentrations spiked in soil. The results indicate that levels of Cry1Ac protein in the soil from Bt cotton fields are not high enough to be biologically active against even a highly susceptible insect.

- **Insect bioassay for determining soil degradation of *Bacillus thuringiensis subsp. kurstaki* Cry1Ab protein in Corn tissue (Sims. S. R *et al.*, 1996):** Here the fate of Cry1Ab is assessed. This concludes that teheCry1Ab protein in corn plant tissue will be unstable under field conditions. Cry1Ab protein is likely to degrade rapidly under the reduced cultivation practices associated with no-till corn production as well as under conventional cultivation in which corn plant residue is plowed into the soil. Dissipation of transgenic corn Cry1Ab protein not in soil contact was relatively slow up to the 21day sample. This was followed by a rapid loss of remaining bioactivity between 21 and 43 days.
- **Aerobic soil degradation of *Bacillus thuringiensis* var. *kurstaki* HD-73 protein bioactivity (Ream *et al.*, 1994):** it was estimated that at room temperature (24 deg C), the half-life of Cry1Ac protein was 9.3-20.3days when spiked into silt loam soil, and 41 days when added to soil as transgenic Bt cotton plant tissues.

3.2.5 Susceptibility to pests and diseases

The EC-II noted that susceptibility to various pests and diseases has been recorded by M/s Mahyco during more than 50 trials conducted with different Bt brinjal hybrids and reconfirmed during LST conducted under the supervision of Director, IIVR.

The diseases for which the data was collected, includes little leaf disease, *Fusarium wilt*, *Verticilium wilt*, Phomopsis blight and bacterial wilt. Little leaf disease and wilts were observed in these trials and no differences were found in their incidence between Bt hybrids, their non-Bt counterparts and checks during the trials.

Overall no differences have been noticed with respect to susceptibility to various pests and diseases by virtue of presence of *cry1Ac* gene in Bt brinjal event EE-1. This is consistent with published reports.

CONCLUSION

Based on the above information and data generated, the EC-II concluded that introgression of *cry1Ac* gene has in no way affected outcrossing potential and the weediness characteristics of Bt brinjal. Bt brinjal event EE-1 is highly specific in its action on target organisms and has no adverse impact on non target organisms including beneficial organisms and soil microflora. No accumulation and persistence of Bt protein in the soil takes place and no differences with respect to susceptibility to pests and diseases have been noticed.

3.3 FOOD AND FEED SAFETY ASSESSMENT

The food and feed safety analysis of Bt brinjal event EE-1 has been undertaken through toxicity and allergenicity assessment of the purified inserted proteins as well as brinjal fruit, compositional analysis, feeding studies and the impact of cooking.

3.3.1 Toxicity and allergenicity of pure proteins

i. Cry1Ac protein

- **Lack of homology of the Cry1Ac protein to known protein toxins and allergens:** The Cry1Ac protein has been compared to known protein toxins in the PIR, EMBL, SwissProt and GenBank protein databases (Keck and Mitsky, 1994). Results from this search establish that, using the best methods available today, there are no biologically significant homologies between the Cry1Ac protein sequence and the protein sequence of all known toxins in the current protein databases. Searches of protein sequence allergen databases also do not show any significant matches of the Cry1Ac protein to known allergens (Metcalfe *et al*. 1996)

- ***T*oxicity of purified Cry1Ac protein:** Purified Cry1Ac protein, at acute oral doses of up to 4300 mg/kg body weight, produced no adverse effects in mice as per data submitted by M/s Mahyco and also published reports (Naylor 1993a; Naylor 1993b). Multiple studies on the acute oral toxicity of Bt microbial preparations, containing Cry1Ac in mammals such as rats and rabbits have revealed no adverse effects at very high doses (Barbera 1995; Carter & Ligget 1994; McClintock *et al*. 1995; Spencer *et al*. 1996). Two human studies found no observable health effect of an oral dose of 1000 mg of Bt microbial spores per day for 3 or 5 days (Betz *et al*. 2000; McClintock *et al*. 1995).

- **Allergenicity of Cry1Ac protein:** The Cry1Ac protein is approximately 133 kDa in size, which is considerably larger than typical allergenic proteins. It is heat labile and is rapidly degraded (under 30 seconds) under simulated mammalian gastrointestinal conditions (Fuchs *et al*. 1993a). The Cry1Ac does not display characteristics common to known food allergen proteins such as glycosylation; resistance to degradation by heat, acid and proteases of the digestive system; or derivation from a known allergenic source (Astwood *et al*. 1996; Kimber *et al*. 1999; Metcalfe *et al*. 1996; Taylor & Lehrer 1996).

- **Digestive fate studies of Cry1Ac in simulated gastric and intestinal fluids:** The rate of degradation of the Cry1Ac protein was evaluated in simulated gastric (pepsin, pH 1.2) and intestinal (pancreatin, pH 7.5) fluid (Ream, 1994). After approximately 30 seconds incubation in gastric fluid, no intact Cry1Ac protein was detected by western blot analysis.

ii. NPTII protein

- **Lack of homology of the NPTII protein to known protein toxins and allergens:** Protein and DNA sequence comparisons using sequences from four separate databases (Genbank, EMBL, PIR29, Swiss-Prot) indicated that NPTII does not have significant homology to any proteins listed as food toxins in these databases (FDA 1994).

- **Toxicity of NPTII protein:** An acute oral toxicity study in mice, in which the purified NPTII protein was fed at doses up to 5000 mg/kg of body weight (2500 mg/kg administered twice, four hours apart), did not show any adverse effects (Berberich *et al*. 1993). A similar study in mice also reported no adverse effects when fed NPTII protein at 5000 mg/kg of body weight (Fuchs *et al*. 1993c). The NPTII protein produced in GM tomatoes has been fed to rodents and reported to be rapidly inactivated and degraded (Calgene 1990). Furthermore, the product of the *nptII* gene is considered safe and is approved by the FDA as a food additive in GM cotton, canola and tomatoes (21 CFR 173:170)(FDA 1994).

The *nptII* gene introduced into mammalian cell lines had no effects on viability or growth. During gene therapy experiments, mammalian cells expressing the NPTII protein were infused into cancer patients with no adverse effects (Flavell *et al*. 1992).

The safety of the NPTII protein to mammals was assessed by mouse gavage and by examination of the metabolic fate of the protein in simulated gastric and intestinal fluids. Mice were administered a maximum dose of approximately 5000 mg/kg of the purified NPTII protein in one day. There was no mortality, no adverse reactions, no differences attributed to treatment in body weight gain or food consumption in dosed mice compared to untreated mice. No abnormal changes were observed in the tissues of mice necropsies approximately eight days after dosing (Naylor, 1992).

- **Allergenicity of NPTII:** The NPTII protein is approximately 29 kDa in size, which is within the typical size range of allergenic proteins. However, it does not possess glycosylation sites, is not stable in the mammalian digestive system and is heat labile, all of which decreases the probability that it is allergenic (ANZFA 2001a; ANZFA 2001b; FDA 1994; Fuchs *et al*. 1993b; US FDA 1998). Fuchs *et al*. (1993b) reported that no NPTII was detected 10 seconds after the addition of simulated gastric fluid as measured by both western blot and enzymatic activity (Fuchs *et al*. 1993c). Protein sequence comparisons with sequences from protein databases indicated that NPTII does not have significant sequence homology to any known protein food allergens (Fuchs & Astwood 1996).

The FDA has evaluated data submitted for deliberate releases of GMOs expressing the NPTII protein and concluded that NPTII does not have

any of the characteristics associated with allergenic proteins (US FDA 1998)

- **Digestive fate studies of NPTII in simulated gastric and intestinal fluids**" The metabolic fate of the protein was evaluated in simulated gastric (pepsin, pH 1.0) and intestinal (pancreatin, pH 7.5) fluids (Ream, 1993). Both western blot analysis and enzymatic activity assays confirmed that NPTII readily degrades in simulated gastric and intestinal fluids with half-lives of less than 10 seconds and between 2 and 5 minutes, respectively

3.3.2 Toxicity and allergenicity of Bt brinjal

i. **Acute oral toxicity of Bt brinjal:** Acute oral toxicity study of transgenic Bt brinjal was conducted in Sprague Dawley rats to assess the toxic characteristics of transgenic Bt brinjal containing *cry1Ac gene* when administered to rats orally by gavage, within a period of 24 hours (acute exposure). Acute oral administration of transgenic Bt brinjal to Sprague Dawley rats at the limit dose of 5000mg/ kg did not cause any toxicity. Proteins that are non-toxic by the oral route are not expected to be toxic by the dermal or pulmonary route.

ii. **Subchronic oral toxicity (90 days) of Bt brinjal:** Subchronic oral (90 Days) toxicity study of transgenic Bt brinjal in Sprague Dawley Rat was conducted to assess the toxicological profile of Bt brinjal when administered by oral gavage, once daily, five days a week, for 90 days. This study provided information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time. The results of this study provided information on target organs, the possibilities of cumulative effects and can provide an estimate of a no-observed-adverse-effect-level of exposure which can be of use in selecting dose levels for chronic studies and for establishing safety criteria for human exposure. Based on the findings of this study, the no-observed-adverse-effect-level (NOAEL) of Bt brinjal in Sprague Dawley rat, following oral administration for 90 days was found to be more than 1000 mg/kg body weight. This study demonstrates that Bt brinjal is non-toxic to the study animal by oral route.

iii. **Allergenicity assessment of Bt brinjal in brown Norway rats:** Assessment of the allergenicity of protein extract from transgenic Bt brinjal expressing Cry1Ac protein was conducted in Brown Norway rats to assess allergenicity potential of protein present in Bt brinjal as measured by active cutaneous anaphylaxis (ACA). The animals were observed daily for signs of toxicity and pre-terminal deaths, weekly body weights and food consumption. There were no clinical signs of toxicity and pre-terminal deaths (mortalities). No statistically significant intergroup difference in body weights and no significant differences in food consumption between treatment and control groups. No biological difference between the allergenicity responses was observed for Bt brinjal event EE-1 and its non-Bt counterpart.

iv. **Primary skin irritation test in rabbit:** Primary skin irritation test of transgenic Bt brinjal in New Zealand white rabbits was conducted to assess the possible irritation potential of transgenic Bt Brinjal containing *cry1Ac gene* when a single dose is applied on intact skin of rabbit. Bt brinjal applied to intact rabbit skin for 4 hours did not cause any skin reaction throughout the observation period. The irritancy index was also 0.0. The observations and results of this study leads to the conclusion that transgenic Bt brinjal expressing Cry1Ac protein can be classified as non-irritant to skin in rabbit.

v. **Mucous membrane irritation test in female rabbit:** Mucous membrane irritation test of transgenic Bt brinjal in New Zealand white female rabbits was conducted to assess the possible irritation likely to arise from exposure of the mucous membranes to Bt Brinjal. Application of Bt brinjal to the vaginal mucous membrane of the female rabbit did not cause any erythema or edema as observed for 72 hours after application. Based on the average irritation index (0.0), Bt brinjal was classified as non-irritant to mucous membrane in rabbit.

The EC-II noted that the studies related to allergenicity assessment in animals and the irritation tests are not required as per the new guidelines adopted by GEAC. The DBT biosafety guidelines, 1998 were based on OECD protocols for toxicity and allergenicity assessment of chemicals whereas the new guidelines are based on the principles and guidelines adopted by Codex Alimentarius for genetically engineered plants.

3.3.3 Compositional analysis

Brinjal has a history of safe use as a source of food in India. It is a highly productive crop and the fruits are consumed as cooked vegetables in various ways. The EC-II noted that M/s Mahyco has carried out the compositional analysis including estimation of alkaloid content and the effect of cooking to establish the substantial equivalence of Bt brinjal event EE-1 with its non Bt counterpart.

i. **Proximate analysis:** A comparative study for the chemical composition of the tissues of brinjal plants was made using transgenic Bt brinjal and three non-Bt controls. The chemical composition was determined in the fruit, leaf, stem and root tissues of the brinjal plant. The parameters analyzed were protein, carbohydrate, oil, calories, ash, nitrogen, crude fibers and moisture contents. No statistical differences between Bt brinjal and non-Bt brinjal groups were observed in the chemical constituents.

ii. **Alkaloid content:** Isolation and identification of major alkaloid principles in Bt and non-Bt counterpart hybrids was carried out in fruits and roots by the Indian Institute of Chemical Technology, Hyderabad. The residue extraction and separation was carried out by using approved protocol and the same was chromatographed over silica gel and eluted to obtain two alkaloids namely Solamargine (mol wt 867) and Solasonine mol wt 883). The structure of alkaloids was identified

based on extensive 1-D and 2-D NMR and other spectroscopic studies. It appears from the present study that the alkaloid profile from powder samples of fruit and roots of Bt and non-Bt *Solanum melongena* are the same with not much of appreciable variation in their relative abundances.

iii. Detailed compositional analysis:

Detailed compositional analysis has been done to estimate and compare the major nutritional components in tissues collected from brinjal (*Solanum melongena Linn.*) hybrid MHB 80 Bt expressing Cry1Ac protein and MHB–80 non-Bt counterpart hybrid. The study was designed to estimate the levels of nutrients in brinjal fruits, leaf and seed. Samples were analyzed to measure proximate (protein, fat , ash, fibre, carbohydrate, moisture, calories), amino acid, fatty acid, minerals (Calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, selenium and zinc), vitamins (vitamin c, thiamin, riboflavin, niacin, vitamin B6, folic acid, Beta carotene, vitamin A, lycopene, vitamin E and vitamin K) and lipids.
The test substance in the study was lyophilized powder of fruit, leaf and seed collected from insect protected transgenic Bt brinjal plant which express Cry1Ac protein. The control substance was collected from near isogenic, non transgenic brinjal plant.

The study used analytical methods for the analysis of lyophilized powder of fruit, leaf and seed samples of Bt and non-Bt brinjal plants. Compositional methods are validated assays which are currently used to evaluate nutritional parameters in food and vegetable products for commercial purposes. All methods have been validated according to Official Methods of Analysis of AOAC International – Volume I and II, 18th Edition, 2005.

In the study total 58 comparisons in three plant parts of Bt and non-Bt brinjal, namely leaf, fruit and seed were made. The levels of nutrients (protein, fat, ash, fiber, carbohydrates, calories, amino acids and lipids) for Bt brinjal hybrid were comparable to those of the non-Bt control hybrid. These data, together with the safe history of use of the host organism (brinjal) as a common source of food, lead to the conclusion that the fruits, leaves and seeds produced by Bt brinjal containing Cry1Ac protein are compositionally equivalent and nutritious as the fruit, leaves and seeds produced by brinjal variety currently on market.

iv. Feeding studies: In addition to compositional analysis the wholesomeness of feed from Bt brinjal was demonstrated in separate feeding studies with fish, chickens, cows, goats and rabbits.

a. Rabbits: Subchronic (90 days) rabbit feeding study of Bt brinjal was conducted on New Zealand White rabbits to compare the wholesomeness and safety of Bt brinjal containing *cry1Ac* gene with control non-Bt brinjal. Histopathological examination was carried out

on the preserved organs and all the tissues during the study. It was concluded based on the health, growth and physio-pathological parameters analysed during the experiment that there were no significant differences between the groups fed with Bt brinjal containing *cry1Ac* gene and control non-Bt brinjal fruit.

b. Fish: A fish feeding study of Bt brinjal was conducted in Common carp (*Cyprinus carpio)* to evaluate Bt brinjal as a feed ingredient for common carp and to study the comparative growth and survival of fish fed with Bt brinjal and non-Bt brinjal. The study found that fish fed with Bt brinjal showed similar growth patterns to those fed with non-transgenic brinjal. There were no significant differences in terms of food conversion ratio, feed efficiency ratio and protein efficiency ratio among Bt and non-Bt brinjal treatments. Bt brinjal, non-Bt counterpart and checks were found to be statistically similar in terms of fish growth responses, and histopathological alterations in common carp.

c. Chicken: A chicken feeding study of Bt brinjal was conducted in broiler chicken to assess the impact of Bt brinjal expressing *cry1Ac* gene on chickens, in terms of growth performance and nutrient utilization. Results of the present study showed that body weight gain, feed intake and feed conversion ratio did not differ among Bt and non-Bt treatments. Several blood biochemical constituents did not differ statistically due to dietary treatments including Bt and non-Bt brinjal incorporated diets. This study found Bt brinjal to be as safe as non-transgenic brinjal in terms of responses of chickens fed with diet incorporating the both Bt and non-Bt brinjal.

d. Goats: Subchronic (90 days) goat feeding study of Bt brinjal was conducted to compare the wholesomeness and safety of Bt brinjal containing *cry1Ac* gene with control non-Bt brinjal. Histopathological examination was carried out on the preserved organs and all the tissues during the study. It was concluded based on the health, growth and physio-pathological parameters analysed during the experiment that there were no significant differences between the groups fed with Bt brinjal containing *cry1Ac* gene and control non-Bt brinjal fruit.

e. Cows: Cow feeding study was conducted to assess the nutritional value of Bt brinjal fruit in comparison to non-Bt brinjal fruit in lactating crossbred cows in terms of feed intake, milk production and milk composition and to determine if the Bt protein was detectable in milk and blood of lactating crossbred cows fed ration containing Bt brinjal fruits. From the present studies, it was concluded that the nutritional value of both Bt and non-Bt brinjal fruits were similar in terms of feed intake, milk yield and milk constituents without any adverse effect on health of lactating crossbred cows.

v. **Food cooking and protein estimation in cooked fruits:** Cooked brinjal fruits are consumed in various forms in India. Accordingly, M/s Mahyco has conducted studies to determine whether the Bt protein was present after cooking Bt brinjal fruits. The first sampling time-point was 5 min for roasted fruit and 1 min for the other forms of cooking. The data indicates that Bt protein was undetectable in the cooked fruits at the first sampling time-point irrespective of the cooking method used (roasted, shallow-fried, deep-fried or steamed). The results of the above study confirm that the Cry1Ac protein in Bt brinjal fruits is rapidly degraded upon cooking.

CONCLUSIONS

The EC-II concluded that the known properties of the Cry1Ac and NPTII proteins coupled with rapid digestion of the proteins in simulated digestive fluids and lack of toxicity in animal feeding studies confirm that these proteins are neither toxic nor allergenic to human and animals. The proteins that are non-toxic by the oral route are not expected to be toxic by the dermal or inhalation routes. There is no detectable Cry1Ac protein in processed brinjal fruit used for food as the Cry1Ac protein is heat labile and is not expected to be present in any cooked form of the brinjal. The detailed compositional analysis confirms that Bt brinjal is substantially equivalent to its non-Bt counterpart, as no significant differences were observed in any of the components.

*****

SECTION IV
REVIEW OF EFFICACY AND AGRONOMIC PERFORMANCE

In this section the EC-II has reviewed the data provided by the applicant to demonstrate the efficacy of Bt brinjal event EE-1 and relative agronomic performance with non Bt counterpart.

4.1 LAB BASED INSECT BIOASSAYS

Insecticidal activity of the Bt brinjal Event EE1 against brinjal fruit and shoot borer (*Leucinodes orbonalis*) and fruit borer (*Helicoverpa armigera)* was assayed. Bt brinjal was found to be effective against these target pests. Insect mortality of 98% for FSB was observed in the Bt brinjal shoots, whereas in the control shoots, mortality was < 30%. The fruit bioassays results demonstrate that Bt brinjal fruits are resistant to *Leucinodes*, as the mortality rates of the larvae are very high (upto 100%) when compared with non-transgenic control plants. The results of leaf and fruit bioassays against *Helicoverpa armigera* indicates that the Bt brinjal leaves and fruits are highly resistant (99%) to *Helicoverpa*.

4.2 FIELD EVALUATION STUDIES TO ASSESS EFFICACY OF THE INTENDED TRAIT AND AGRONOMIC PERFORMANCE

Bt brinjal event EE-1 has been field tested in more than 50 locations that represent major brinjal growing regions in India during 2004 to 2008 for over five years during MLRTs, ICAR trials and LSTs as per details in Table 4.1. The following parameters were assessed during the field trials:

A. Insect reactions :

(i) Shoot Damage
(ii) Fruit borer larvae
(iii) Fruit Damage
(iv) Stem borer damage
(v) Sucking pest infestation
(vi) Beneficial insects

B. Yield Parameters

(i) Number of healthy (marketable) fruits and those damaged by borers.
(ii) Weight of healthy (marketable) fruits and those damaged by borers.

C. Insecticide usage

(i) Sprays for fruit and shoot borer based on ETL in Bt, non-Bt counterpart and check.

D. Economic benefits

(i) Savings on number of sprays
(ii) Yield benefit due to protection against fruit and shoot borer

The results of the field tests are summarized below:

Table 4.1: Field Trials conducted with Bt brinjal in India

S. No.	Year	Type of Field Trial	Entries	Locations
Multi Location Research Trials of Bt Brinjal hybrids conducted by Mahyco				
1a	2004-2005	MLRT	MHB 4 Bt	Solapur, Ahmednagar
			MHB 9 Bt	Tumkur, Dharmapuri
			MHB 10 Bt	Pune, Dharwad
			MHB 80 Bt	Jalandhar, Bhopal, Alwar, Mirzapur,
			MHB 99 Bt	Kurnool
1b	2005-2006	MLRT	MHB 11Bt	Akola, Coimbatore
			MHB 39 Bt	Dindigal, Kolar
			MHB 112 Bt	Karnal, Jaipur
ICAR trials under All India Coordinated Research Improvement Project (AICRIP-Vegetables) conducted for Mahyco Bt brinjal hybrids				
2a	2004-2005	ICAR (Ist Year)	MHB 4 Bt, MHB 9 Bt, MHB 10 Bt, MHB 80 Bt, MHB 99 Bt	Varanasi, Pantnagar, Ranchi, Kanpur, Raipur, Rahuri, Anand, Hyderabad, Bangalore, Coimbatore and Ludhiana
2b	2005-2006	ICAR (IInd Year)	MHB 4 Bt, MHB 9 Bt, MHB 10 Bt, MHB 80 Bt, MHB 99 Bt	Varanasi, Pantnagar, Ranchi, Kanpur, Raipur, Rahuri, Anand, Hyderabad, Coimbatore, Dharwad and Ludhiana
2c	2005-2006	ICAR (Ist Year)	MHB 11 Bt, MHB 39 Bt, MHB 112 Bt	Varanasi, Pantnagar, Ranchi, Kanpur, Raipur, Rahuri, Anand, Hyderabad, Coimbatore, Dharwad and Ludhiana
2d	2006-2007	ICAR (IInd Year)	MHB 11 Bt, MHB 39 Bt, MHB 112 Bt	Varanasi, Pantnagar, Ranchi, Kanpur, Raipur, Rahuri, Anand, Hyderabad, Dharwad, Coimbatore and Ludhiana
Large scale trials (LST) of Mahyco Bt Brinjal hybrids conducted by IIVR, Varanasi				
3°	2007-2008	LST (Ist Year)	MHB 4 Bt, MHB 9 Bt, MHB 10 Bt, MHB 11 Bt, MHB 39 Bt, MHB 80 Bt, MHBJ 99Bt	Varanasi, Raipur, Coimbatore, Ludhiana, Hisar, Jabalpur, Dharwad, Anand, Parbhani, Rahuri and Ranchi
3b	2008-2009	LST (IInd Year)	MHB 4 Bt, MHB 9 Bt, MHB 10 Bt, MHB 11 Bt, MHB 39 Bt, MHB 80 Bt, MHBJ 99Bt	Varanasi, Raipur, Coimbatore, Ludhiana, Hisar, Jabalpur, Dharwad, Anand, Parbhani, Rahuri
MLRT of Bt brinjal OPV's conducted by technology partners UAS, Dharwad				
4	2007-08	MLRTs	Malapur local (S) Bt, Manjarigota Bt, Rabkavi local Bt, Kudachi local Bt, Udupigulla Bt, GO112 Bt	Udupi, Belgaum, Kolhapur (ZARS), Kolhapur (ARS)
MLRT of Bt brinjal OPV's conducted by technology partners TNAU, Coimbatore				
5	2007-08	MLRTs	Co2-Bt, MDU1-Bt, KKM1-Bt, PLR1-Bt	Coimbatore, Madurai

4.2.1 Efficacy of the intended trait

The EC-II noted that the efficacy of Bt brinjal hybrids against target pest has been well demonstrated by assessment of shoot and fruit damage during various field trials. The results of the field evaluations indicate that the mean shoot damage was significantly lower in Bt brinjal hybrids (1.51%) as compared to their non-Bt counterparts (7.06%), and checks (13.07%) across locations in all three trial models (MLRTs, ICAR trials and LSTs).The cumulative fruit damage during these trials in Bt brinjal hybrids, their non-Bt counterparts and checks was 8.15%, 26.10% and 25.02% respectively. The mean cumulative fruit damage in Bt hybrids ranged from 6.28% to 10.04%, whereas the range for non-Bt hybrids and checks was 23.52% to 30.36%.

4.2.2 Baseline susceptibility studies

The baseline susceptibility data was generated for fruit and shoot borer during 2004 and 2005. Subsequently as pe the stipulated condition, baseline susceptibility data for all the three target pests viz fruit and shoot borer (*L. orbonalis*), fruit borer (*H. armigera*) and stem borer (*E. perticella*) was generated during LST at 11 and 10 locations for two seasons during 2007 and 2008 respectively. It was observed that the variation in susceptibility found in these populations in terms of their mortality or growth inhibition, is natural variability found in insect populations. All the three target insect species demonstrate limited variability in their mortality to the Cry1Ac protein. The data also indicates that all the three target pests: fruit and shoot borer (*L. orbonalis*), fruit borer (*H. armigera*) and stem borer (*E. perticella*) are highly susceptible to the Cry1Ac protein level expressed in Bt brinjal hybrids.

i. **Agronomic performance:** Bt brinjal hybrids yielded significantly higher marketable yield in all the three trial models. Mean marketable yield for Bt hybrids was 404.91 q/ha as compared to 236.84 q/ha in non-Bt counterparts and 205.80 q/ha in checks. The mean increase in marketable yield of Bt hybrids over their non-Bt counterparts and checks was 71% and 97%, respectively. The marketable yield for Bt brinjal hybrids, non-Bt counterparts and checks ranged from 293.45 q/ha (MHB 10 Bt) to 638.02 q/ha (MHBJ 99 Bt), 171.76 q/ha (MHB 10 Non Bt) to 305.83 q/ha (MHB 39 Non Bt) and 189.70 q/ha to 221.90 q/ha, respectively

ii. **Economics of Bt brinjal:** The IIVR estimated economic benefit values during LSTs conducted at 21 locations across 10 states over two years (2007 and 2008). Savings in insecticide costs were derived using economic threshold level (ETL) based sprays. ETLs were assessed at each picking during the entire crop period. The fruit and shoot borer damage crossed ETL 0.94 times in Bt hybrids while it crossed ETL 7.44 and 7.40 times in non-Bt counterparts and check, respectively.

IIVR has reported that the mean cost of sprays (based on ETL) was Rs. 752 per ha in Bt hybrids, whereas Rs. 5,952/- per ha and Rs. 5920 per ha were spent on ETL based sprays in non-Bt counterparts and

check, respectively. Further, the estimated economic benefit due to increased marketable yield in Bt hybrids over non-Bt counterparts and check was Rs. 64800/- per ha and Rs. 80,800/- per ha, respectively. In the light of the above, the net economic gain in Bt hybrids over the non-Bt counterparts has been estimated to be Rs. 69,239/- per ha and Rs. 85,291/- per ha over check hybrid.

CONCLUSIONS

The EC-II concluded from the cumulative results of various trials conducted that *cry1Ac* gene provides effective protection to the brinjal crop from the fruit and shoot borer resulting in enhanced economic benefits accrued from higher marketable yield and lower usage of pesticides sprays.

*****

SECTION V
CONSIDERATION OF ISSUES RAISED BY NGOs, NATIONAL AND INTERNATIONAL GROUPS

The EC-II considered all the issues raised by NGOs, national and international groups regarding the safety of Bt brinjal. It was noted that the concerns raised in the representations to GEAC were related to safety of the protein, environmental safety and food and feed safety, several of which were already addressed by EC-I.

The EC-II in its deliberations held on July 30, 2009 and August 31, 2009 extensively debated on the issues raised in the following reports/representations:

1. Report on "Effects on Health and Environment of Transgenic (or GM) Bt Brinjal" by Prof. G. Seralini, University of Caen, France.
2. Report on "Biological effects of transgenic maize NK603xMON810 fed in long term reproduction studies in mice" by A. Velimirov *et al*. – Austrian report.
3. Comments on Possible Consequences of Gene Flow from Bt Brinjal to Brinjal Wild Relatives in India, and the Inadequacy of the Current Risk Assessment by Dr. Doug Gurian-Sherman, 2009.
4. List of studies recommended by Dr. P. M. Bhargava before release of a GMO into the environment.
5. Comments by Prof. Jack A. Heinemann, University of Canterbury, New Zealand.
6. A review of Mahyco's GM Brinjal food safety studies by Dr Judy Carman, Australia.
7. Letter from Ms Kavitha Kurunganti to members of the EC-II constituted on May 29, 2009, to review the biosafety studies and large scale trials of Bt Brinjal.
8. Appeals submitted to PMO.
9. "I AM NO LAB RAT CAMPAIGN"

The observations of the EC-II on each of the concerns expressed in the above reports/representations are discussed in detail at Annexure-1. The major issues raised in the above reports/representations and considered by EC-II are summarized below:

5.1 SAFETY OF THE EXPRESSED PROTEIN

Issue 1: Difference in protein used (Pure protein vs. protein in Bt Brinjal): Bt brinjal has been modified to produce an unknown chimeric insecticide toxin containing *cry1Ab* and *cry1Ac* modified sequences. In the toxicity tests on target and non-target insects, this chimeric toxin has not been used but instead, an improper *cry1Ac* toxin was used because this control was easier. This could also make these tests not valid.

Observations by EC-II:

- The *cry1Ac* gene inserted in Bt brinjal event EE-1 has been constructed by combining the first 1398 nucleotides of the *cry1Ab* gene (corresponding to amino acids 1 to 466) (Fischhoff *et. al*., 1987) with nucleotides number 1399 to 3534 of the *cry1Ac* gene (corresponding to amino acids 467 to 1178). The resultant protein encoded by this gene is 99.4% identical to native Cry1Ac from *Bacillus thuringiensis* sub sp. *kurstaki*. This difference of 0.6% is attributed to the difference in presence of one amino acid at position 766 i.e. serine in place of leucine.

- The molecular characterization studies (western blot) have confirmed that Cry1Ac protein expressed in EE-1 brinjal is equivalent to native *B.t.k* Cry1Ac as also Bollgard cotton event MON 531 already approved and commercialized in India since 2002.

- The EC-II concluded that the argument that this protein is unknown is incorrect as detailed characterization has been undertaken and is based on wrong presumption that this protein is a combination of two proteins. Therefore all the biosafety studies conducted are valid.

Issue 2: Antibiotic resistance: Two unnecessary antibiotic marker genes, called *nptII* (neomycin phosphotransferase II) and *aad* (coding resistance to streptomycin or spectinomycin) have been used in Bt brinjal. Antibiotic resistance is recognized to be a major health problem and the commercialization of such a food is not advisable.

Observations by EC-II:

- Antibiotic resistance markers have been extensively used in the production of GM plants. The health issues related to antibiotic marker genes have already been addressed by numerous peer reviewed publications and studies as well as reviews by regulatory authorities worldwide such as US FDA, Health Canada, EFSA, FSANZ etc.

- Though, the antibiotic resistance genes produce enzymes that can degrade antibiotics, it has been well researched and proven that the enzymes from these genes are produced at such low levels that is absolutely ineffective on the antibiotic. Numerous studies have also been carried out on the fate of antibiotic resistance marker from GE plants in digestives tracts. It has been well established that the probability of transfer of transgenic from GM plant material to bacteria (including that normally inhabit stomach and intestine) is unlikely because of series of well established barriers. All the above is supported by experimental evidence.

- The EC-II concluded that the two genes used in Bt brinjal Event EE-1 i.e. *nptII* and *aad* genes have already been accepted for use by regulatory authorities around the world, such as USA, EU, Australia, Philippines etc

and that the crops containing the same have a history of safe use for more than two decades.

5.2 ENVIRONMENTAL SAFETY

Issue 3: Potential impact of gene flow on genetic diversity of brinjal in view of India being the centre of origin: Limited data on gene flow distance that cannot substitute for a risk assessment of potential harm from gene flow and is wholly inadequate to predict gene flow. Since India is the center for domestication and genetic diversity of brinjal, it is recommended that gene flow from Bt brinjal should be seriously considered and evaluated before commercialization.

Observations by EC-II:

- Genus *Solanum* is predominantly Central and South American. The question of Centre of origin of *S. melongena* is yet to be resolved (Khan R (1979). Evidence seems to indicate that it originated in Asia. S.W. Asia including Arabia, Indo-Burma region, Japan and China have been suggested as probable places of origin by different authors (Hooker, 1885; Vavilov, 1951; Bailey, 1947; Watt, 1908). It cannot be categorically concluded the brinjal originated in India.

- Karihaloo and Gottileb (1995) through their study on allozyme variation in *S. melongena* and similar wild and weedy forms suggested that *S. melongena* originated from an African species, *S. incanum.* Migration of S. *incanum,* or its derivative wild ancestor of *S. melongena,* into South and Southeast Asia would have taken place either by humans through land routes or by sea dispersal of fruits (D'Arcy and Pickett 1991; Lester and Hasan 1991).

- The impact of gene flow to wild relatives of cultivated brinjal (*S. melongena)* has been considered. It has been reported that there is no natural crossing among cultivated and wild species of brinjal including *S. incanum* and *S. insanum* (Rao, 1979). Under forced crossing situations, even if crossing was possible, the viability and subsequent development of stable crosses have not been successful.

- The crossability studies have been repeated by IIVR and it has been reported that crossing was not possible with representative wild relatives except *S. incanum* where limited crossing could be achieved through artificial pollination.

- The *cry1Ac* gene used in Bt brinjal event EE-1 confers no advantage to recipient plants in terms of aggressiveness or growth characteristics. Therefore, even if gene flow occurs in exceptional circumstances, it will not confer any fitness advantage to wild species because insect pests such as *Leucindes orbonalis* (FSB) are rarely found on them. FSB is a lepidopteran pest that prefers only brinjal and cry1Ac provides protection only against FSB and other lepidopteran pests. Since no lepidopteran pests are

prevalent on *Solanum* wild species, the matter of fitness advantage does not arise.

Issue 4: Effect on non target organisms : It has been stated that field trials are an inadequate basis to assess impacts on the agrosystem:

- Studies of long term effects are lacking,
- Studies on beneficial insects (e.g. natural enemies of target pests), as well as studies of abundance of secondary pests (which would have to be sprayed with insecticides) are lacking.
- Indirect effects (e.g. does the Bt toxin affect organisms that eat the target insect) are important in this regard.
- No laboratory studies have been performed to evaluate other lepidopteran insects

Observations by EC-II:

- M/s Mahyco has conducted extensive field trial across various agro-climatic zones to assess the effect of Bt brinjal event EE-1 on a variety of non target organisms as well as beneficial organisms. The protocols followed in these studies field evaluations are consistent with the international accepted procedures and therefore adequate to assess the impact on agro system.

Issue 5: Limited environmental studies on soil microflora in the rhizosphere: Limited environmental studies of Bt brinjal risks have been performed on an extremely little part of soil microflora, collembola, nematodes and earthworms. It is almost impossible through a few species measurements to get a whole view of a complicated ecosystem, moreover varying a lot from place to place in India.

Observations by EC-II:

- While it is correct that it is not possible to culture many soil fungi and bacteria, indicator species measured provide a framework for evaluation of soil effects. Hypothetical “evolutions and reactions” are not justifications for invalidating the studies conducted.

- Soil microflora studies and effects on collembola, nematodes and earthworms in Bt brinjal field plots have been extensively carried out in almost every growing season since 2003 in more than 50 locations in India and not a single instance of any impact on soil microflora has been noticed. The studies have been repeated in large scale trials conducted by IIVR. These studies reconfirmed that Bt brinjal does not have any significance effects on soil microflora both fungi and bacteria and soil invertebrates such as earthworm, collembola and nematodes. No Cry1Ac protein was detected in any of the soil samples of Bt brinjal field plots during the harvest period as well as in the post harvest period. This clearly demonstrates that Bt protein is rapidly degraded.

5.3 FOOD/FEED SAFETY

Issue 6: Cooking studies in Bt brinjal: Cooked forms of Bt brinjal are supposed not to contain Cry1Ac although the specificity and sensitivity of the assay does not form a part of the dossier. Thus this cannot be accepted as proof that the Bt toxin is not present in cooked Bt brinjal.

Observations by EC-II:

- All cooked forms of Bt brinjal have been tested using ELISA method for the presence of Bt protein, which is an established and accepted method for testing the presence of protein. The ELISA kit is based on a monoclonal antibody which specifically recognizes Cry1Ac/Cry1Ab antigen only. It will not detect any other Bt protein. The Cry1Ac ELISA plate is highly specific for Cry1Ac residues and Cry1Ab residues in plant extracts. It has a sensitivity of below 1%. The Limit of Quantification (LOQ) is 0.625 ng/ml and Limit of Detection (LOD) is 0.046 ng/ml.

- Further it has been demonstrated that Cry1Ac protein is heat labile and thus is not expected to be present in any cooked form of the brinjal. Thus ELISA results have confirmed the same

Issue 7: Nutritional, toxicity and allergenicity studies in cooked Bt brinjal: It is also expected that cooking degrades at least in part the Bt toxin. However there is no information on toxicity and allergenicity of the resulting products.

Observations by EC-II:

- Bt protein behaves like any other protein during the cooking process. Further it breaks down into common amino acids in the digestive system, which are part of the normal diet and are neither toxic nor allergic. The Cry1Ac protein has been extensively tested internationally in various digestive assays and found to be safe.

Issue 8: Studies for toxicity assessment and nutritional effects in mammals have been limited to a maximum of 90 days period or less, which is not adequate and there is a need for long term studies for assessment of chronic effects.

Observations by EC-II:

- The studies undertaken in Bt brinjal so far comply with the international guidelines as well as ICMR Guidelines accepted by GEAC.
- No long term studies are required because of the following reasons:
  - The Cry1Ac protein inserted into Bt brinjal event EE-1 has been extensively studied for its safety. It has been well established that the

Cry1Ac protein cannot cause any toxic effect in mammals because of lack of highly specific receptors and alkaline environment in the gut of mammals.

- Cry1Ac protein has a history of safe use for human and animal consumption as GM crops containing cry proteins including Cry1Ac protein have been consumed by millions of people without any adverse effects.
- It has been reported that 90-110 days of age (mating age) of rats is considered equivalent to 21-25 years age of humans (Laboratory Animals in the Study of Nutrition' in LAIS Centre News- No - 30, 1993).
- Cry1Ac protein has shown to be rapidly degraded (in 30 seconds) in simulated digestive fluids and thus is not detectable even in the short term studies.

Issue 9: Variation in the observations regarding the response of animals during the toxicity studies have been ignored and not been taken into consideration while drawing inference on the safety of Bt brinjal.

Observations by EC-II:

- In general toxicology and in safety testing the results are compared between test and control groups. If differences do occur which is but expected in dynamic biological systems such differences should be checked for:
  - Are they statistically significantly different
  - If yes, are the values or data in the normal physiological range
  - If there are more than one dose group then one should look for dose dependent changes in the parameters
  - Even if there are significant differences and they are in physiological range then one should correlate biochemical and haematological data with histopathological changes.
  - If there are significant differences and they are outside physiological ranges and they are associated with parallel histological changes. E.g. Elevation of aspartate aminotransferase (AST), alanine aminotransferase (ALT) with liver cell necrosis or changes in haematological parameters with corroborative changes in bone marrow. Then it is assumed that the test material could result in cellular changes. Significant changes given only at a single time point and not given at the end of the study are considered to be transient changes.

In the animal feeding studies conducted with Bt brinjal, no statistically significant changes have been observed in the parameters tested. All values are within the normal physiological ranges, and are not associated with any histopathological changes.

5.4 OTHER ISSUES

Issue 10: Impact on organic farming

Response of EC-II:

In organic farming, the pest management totally relies on the use of botanical insecticides like neem oil, pungam oil, iluppai oil or seed kernel extracts or leaf extracts, which act as repellant, antifeedant or in some cases as toxins. Like chemical pesticides, they are non specific in their mode of action. None of the botanical pesticides are expected to perform well against FSB since the pest hides itself from the sprays while staying inside the fruits/shoot. Further, several reports have confirmed the limited efficacy of botanical against FSB and other lepidopteran pests (Mishra and Dash 2007; Jena *et al.,* 2005; Mishra *et al.,* 2004; and Bharadiya and Patel 2005; Naik *et al.,* 2008; Sachan *et al.,* 2006; and Prasad *et al.,* 2005; Alagar and Sivasubramanian, 2006; Lalnuntluanga and Singh, 2008). In view of the heavy infestation by FSB and limited efficacy of botanicals, it is highly impossible to get economic yields in brinjal only with the application of organic inputs.

In spite of the above, the section of farmers who have a preference for organic farming can do so by following established agronomic practices such as maintaining isolation distance, differences in flowering time etc. for preventing cross contamination and ensuring identity preservation for organic produce. As described earlier, the rate of cross pollination from one field to other is quite low, and the frequency of such occurrence decreases with increasing distance from pollen source. Presently, the percentage of organic brinjal growers/exporters is negligible as compared to the total production and consumption of brinjal in the country.

Issue 11: Acceptance of data submitted by M/s Mahyco

Response of EC-II:

The data submitted by M/s Mahyco has been generated using GLP practices in accredited laboratories, public sector institutions and their own facilities. The certification of analysis has been provided for the test material for each study. The raw data as well as samples have been archived by the GLP laboratories. The above practices for data generation are in line with the national and international norms followed in case of other products such as pharmaceuticals.

Issue 12: Adequacy of information/data generated by M/s Mahyco

Observations of EC-II:

- The EC-II extensively deliberated on the list of studies recommended by Dr. P.M. Bhargava before release of a GMO into the environment. The

views of the EC-II on the specific points raised by Dr. Bhargava are placed at Annexure-1.

- The EC-II was of the view that several studies recommended by Dr. Bhargava are neither relevant nor applicable in the instant case. The EC-II had concluded that the guidelines and protocols prescribed by RCGM and GEAC are in line with the internationally accepted norms by FAO, WHO, OECD, and Codex Alimentarius. Further M/s Mahyco has completed with all the tests according to the prescribed protocols by the RCGM, GEAC and ICMR.

- Regulatory mechanism is a dynamic process which is continuously updated based on scientific developments and evidences. Therefore, the need for prescribing additional studies needs to be carried out on a case by case basis and consideration of data generated during the biosafety assessment. Raising the bar of the regulatory process as recommended by Dr. P.M. Bhargava based on hypothetical concerns and apprehensions would be highly detrimental for research and development in the area of agricultural biotechnology especially for public sector institutions.

*****

SECTION VI
CONCLUSIONS AND RECOMMENDATIONS

Bt brinjal event EE-1 developed by M/s Mahyco expressing the Cry1Ac protein, is resistant to fruit and shoot borer, one of the major pests that causes damage to the brinjal crop throughout its life cycle. Being a genetically engineered crop, Bt brinjal event EE-1 requires environmental clearance under the Rules, 1989 notified by MoEF.

Bt brinjal event EE-1 has been evaluated for its efficacy and safety as per the protocols and procedures prescribed under Rules, 1989 and relevant biosafety guidelines. The large scale field trials of Bt brinjal have been conducted under the supervision of Director, IIVR and the report has been submitted.

In accordance with the mandate given by the GEAC, the EC-II in its meetings held on July 30, 2009 and August 31, 2009, reviewed the findings of the data generated during the LST, biosafety data of Bt brinjal provided by the developer, studies conducted by various institutions outsourced by the developers , published literature, reports from international/national groups and representations from NGOs and other stakeholders.

The conclusions of the above review are as follows:

1. Damage by fruit and shoot borer is a major problem in brinjal production and there is an urgent need to have alternate strategies in place to control the same. The current practices of extensive use of chemical pesticides besides being expensive and unsustainable are also harmful to health and the environment.
2. The RCGM had considered and examined the biosafety data generated by M/s Mahyco and concluded that Bt brinjal is effective in controlling target pests, safe to the environment, non-toxic as determined by toxicity and animal feeding tests, non- allergenic and has potential to benefit the farmers.
3. The EC-I had recommended the conduct of large scale trials of Bt brinjal event EE-1 at 10-11 locations subject to certain conditions for reconfirmation of some biosafety data generated by M/s Mahyco. The status of compliance of conditions recommended by EC-I and stipulated by GEAC for large scale field trials indicate that there has been compliance with the prescribed conditions in most of the cases. Wherever there was a deviation, the same had the approval of GEAC. The results of the LST as well as biosafety studies conducted by IIVR and M/s Mahyco were in conformity with earlier information submitted by the applicant and available literature (Refer Table 2.2).
4. The data generated by the applicant with reference to food and feed safety assessment is complying with the “Guidelines for the safety assessment of foods derived from GE plant, 2008” .
5. The three genes introgressed by M/s Mahyco into Bt brinjal event EE-1 i.e. *cry1Ac* , *nptII* and *aad* gene have been extensively studied by researchers

and evaluated and approved by regulatory agencies worldwide in products such as Bt maize, Bt potato and Bt cotton. All the inserted genes and regulatory sequences (promoters and enhancers) have a history of safe use in view of their inherent characteristics/properties. Further, the expression of *cry1Ac* gene is consistent during the entire life of the crop, and the levels of Cry1Ac protein are sufficient for effective control of FSB in various agro-climatic conditions. This demonstrates that the insect resistance trait is stably integrated in the brinjal genome and there is no evidence or likelihood of genetic instability.

6. The protein encoded by the *cry1Ac* gene incorporated in Bt brinjal event EE-1 is 99.4% identical to native *cry1Ac* from *Bacillus thuringiensis* sub species *kurstaki*, and 100% identical to the one expressed in Bt cotton event MON-531 approved in India.

7. Environmental safety assessment studies on Bt brinjal event EE-1 demonstrated that introgression of *cry1Ac* gene has in no way affected outcrossing potential and weediness characteristics of *S. melongena*. There was no adverse impact on non target organisms including beneficial organisms and soil microflora. Further, no accumulation and persistence of Bt protein in the soil was observed.

8. S. *melongena* and its wild relatives including *S. incanum* have co-existed in nature for millennia. No instances of natural inter-specific hybridization with wild species have been reported for cultivated brinjal. The crossability studies have been repeated by IIVR and it has been reported that crossing was not possible with representative wild relatives except *S. incanum* where limited crossing could be achieved through assisted pollination.

9. The *cry1Ac* gene used in Bt brinjal event EE-1 confers no advantage to recipient plants in terms of aggressiveness or growth characteristics. Therefore, even if gene flow occurs in exceptional circumstances, it will not confer any fitness advantage to wild species because insect pests such as *Leucindes orbonalis* (FSB) are rarely found on them. FSB is a lepidopteran pest that prefers brinjal exclusively, and Cry1Ac provides protection only against FSB. Since no lepidopteran pests are prevalent on *Solanum* wild species, the matter of fitness advantage does not arise.

10. The food and feed safety assessment of Bt brinjal event EE-1 demonstrates that the expressed Bt protein is highly specific to lepidopteran pests and is neither toxic nor allergenic to human and animals.

11. Cry1Ac protein rapidly degrades (in 30 seconds) in simulated gastric and intestinal fluids and is not detectable even in short term digestibility studies.

12. The detailed compositional analysis consisting of proximate (protein, fat , ash, fibre, carbohydrate, moisture, calories), amino acid, fatty acid, minerals (Calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, selenium and zinc), vitamins (vitamin c, thiamin, riboflavin, niacin, vitamin B6, folic acid, Beta carotene, vitamin A, lycopene, vitamin E and vitamin K) and lipids demonstrates that Bt brinjal event EE-1 is substantially equivalent to its non transgenic counterpart.

13. Cry1Ac protein being heat labile is rapidly degraded upon cooking. Highly specific and sensitive ELISA tests conducted for the presence of Cry1Ac protein have confirmed that Cry1Ac protein is not detected in any cooked form of brinjal containing event EE-1.

14. Variations in the observations regarding the response of animals during the toxicity/feeding studies are commonly noticed in dynamic biological systems. Interpretation of data on safety studies are never done in isolation but as a meaningful holistic evaluation of the entire toxicological data. In the present case, it was observed that experimental observations are within the normal physiological ranges and statistically insignificant. Therefore, the studies conducted and inference drawn that no significant differences were observed between the animals fed with Bt brinjal *vis-à-vis* control non-Bt counterpart is valid.

15. Chronic toxicity studies are warranted only if any toxic effects are observed in acute or sub-chronic studies. Since no toxic effects were seen in acute and sub-chronic studies, there is no need and justification for any chronic or long term studies for evaluating the safety of Bt brinjal event EE-1.

16. Cry1Ac protein has a history of safe use for human and animal consumption as GM crops containing Cry proteins including Cry1Ac protein have been consumed by millions of people for over two decades without any adverse effects reported in the published scientific literature.

17. The cumulative results of more than 50 field trials demonstrate that Cry1Ac protein provides effective protection to brinjal crop from the FSB resulting in enhanced economic benefits accrued from higher marketable yield and lower usage of pesticide sprays.

18. The guidelines and protocols prescribed by RCGM and GEAC are in line with the internationally accepted norms prescribed by OECD, FAO, WHO,Codex Alimentarious etc.. M/s Mahyco has fully complied with the Indian regulatory requirements. Several studies recommended by Dr. P.M. Bhargava have no relevance and are not applicable in the present case, as discussed in Annexure-1.

RECOMMENDATIONS

In view of the above stated facts, EC-II concludes that the benefits of Bt brinjal event EE-I developed by M/s Mahyco far outweigh the perceived and projected risks. The EC-II submits the following recommendations for consideration by the GEAC:

1. Bt brinjal event EE-1 is safe for environmental release in India.

2. In accordance with the event based approval mechanism, the GEAC may consider approving all the Bt brinjal hybrids and varieties containing event EE-1 developed by M/s Mahyco, TNAU, Coimbatore and UAS, Dharwad and field tested so far.

3. Bt brinjal event EE-1 has been extensively tested for its biosafety and no additional studies/review are necessary.

4. Regulatory mechanism is a dynamic process which is continuously updated based on scientific developments and evidences. Therefore, the need for prescribing additional studies needs to be carried out on a case by case basis and consideration of data generated during the biosafety assessment. Raising the bar of the regulatory process as recommended by Dr. P.M. Bhargava based on hypothetical concerns and apprehensions would be highly detrimental for research and development in the area of agricultural biotechnology especially for public sector institutions and the benefits to the society at large.

*****

Annexure - 1

CONSIDERATION OF ISSUES RAISED BY NGOs, NATIONAL AND INTERNATIONAL GROUPS ON Bt BRINJAL BIOSAFETY STUDIES

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
A.	**Prof. G Serilini, University of Cannes, France**		
1.	Bt brinjal has been modified to produce an unknown chimeric insecticide toxin containing Cry1Ab and Cry1Ac modified sequences. In the toxicity tests on target and non-target insects, this chimeric toxin has not been used but instead, an improper Cry1Ac toxin was used because this control was easier. This could also make these tests not valid.	The *cry1Ac* gene inserted in Bt brinjal event EE-1 has been constructed by combining the first 1398 nucleotides of the *cry1Ab* gene (corresponding to amino acids 1 to 466) (Fischhoff *et. al.*, 1987) with nucleotides number 1399 to 3534 of the *cry1Ac* gene (corresponding to amino acids 467 to 1178). The resultant protein encoded by this gene is 99.4% identical to native Cry1Ac from *Bacillus thuringiensis* sub sp. *kurstaki*. This difference of 0.6% is attributed to the difference in presence of one amino acid at position 766 i.e. serine in place of leucine. The molecular characterization studies (Western blot) have confirmed that Cry1Ac protein expressed in EE-1 brinjal is equivalent to native *B.t.k* Cry1Ac as also Bollgard cotton event 531 already approved in India.	The EC-II concluded that the argument that this protein is unknown is incorrect as detailed characterization has been undertaken. The issue has been raised on the presumptions that the inserted construct is going to produce unknown chimeric protein. Based on the scientific facts, it is evident that large chunk of gene is cry*1Ac* and the expressed protein is also the same as per the experiments shown. Hence the control is appropriate.
2.	Two unnecessary antibiotic marker genes, called NPTII (neomycin phosphotransferase II) and aad (coding resistance to streptomycin or spectinomycin) have been used in Bt brinjal. Antibiotic resistance is recognized to be a major health problem and the commercialization of such a food is not advisable.	Antibiotic resistance markers have been extensively used in the production of GM plants. The health issues related to antibiotic marker genes have already been addressed by numerous peer reviewed publications and studies as well as reviews by regulatory authorities worldwide such as US FDA, Health Canada, EFSA, FSANZ etc. Though, the antibiotic resistance genes produce enzymes that can degrade antibiotics, it has been well researched and proven that the enzymes from these genes are produced at such low levels that is absolutely ineffective on the antibiotic. Numerous studies have also been carried out on the fate of antibiotic resistance marker from GE plants in digestives tracts. It has been well established that the probability of	The EC-II concluded that the two genes used in Bt brinjal Event EE1 i.e *npt II* and *aad* genes have already been accepted for use by regulatory authorities around the world, such as USA, EU, Australia, Philippines etc and that the crops containing the same have a history of safe use for more than a decade.

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
		transfer of transgenic from GM plant material to bacteria (including that normally inhabit stomach and intestine) is unlikely because of a series of well established barriers. All the above is supported by experimental evidence.	
3.	Cooked forms of Bt brinjal are supposed not to contain Cry1Ac although the specificity and sensitivity of the assay does not form a part of the dossier. Thus this cannot be accepted as proof that the Bt toxin is not present in cooked Bt brinjal.	All cooked forms of Bt brinjal have been tested using ELISA for the presence of the Bt protein, which is an established and accepted method for testing the presence of the protein. The ELISA kit is based on a monoclonal antibody which specifically recognizes Cry1Ac/Cry1Ab antigen only. It will not detect any other Bt protein. The Cry1Ac ELISA plate is highly specific for Cry1Ac residues and Cry1Ab residues in plant extracts. It has a sensitivity of below 1%, i.e. the plate can detect presence of Cry1Ac. The limit of quantification (LOQ) is 0.625 ng/ml and limit of detection (LOD) is 0.046 ng/ml. Further it has been demonstrated that Cry1Ac protein is heat labile and thus is not expected to be present in any cooked form of the brinjal. Thus ELISA results have confirmed the same.	The EC-II was of the view that no additional information regarding toxicity and allergenicity needs to be generated as the Bt proteins have a history of safe use since last more than one century, starting from whole bacteria to purified proteins.
3.	It is also expected that cooking degrades at least in part the Bt toxin. However there is no information on toxicity and allergenicity of the resulting products.	Bt protein behaves like any other protein during the cooking process. Further it breaks down into common amino acids in the digestive system, which are part of the normal diet and are neither toxic nor allergic. The Cry1Ac protein has been extensively tested internationally in various digestive assays and found to be safe.	The EC-II was of the view that no additional information regarding toxicity and allergenicity needs to be generated
4.	Studies for toxicity assessment and nutritional effects in mammals have been limited to a maximum of 90 days period or less, which is not adequate and there is a need for long term studies for assessment of chronic effects.	Safety assessment of a GM food crop requires an integrated stepwise and case by case approach. The evaluation of possible toxicity and allergenicity of gene products as well as consideration of the nutritional aspects is undertaken in a comprehensive manner through a multitude of tests. As per FAO/ WHO expert consultations and Codex principles, toxicity studies upto a 90 day repeat exposure study are	The EC-II concluded that no long term studies are required because of the following reasons: • The Cry1Ac protein inserted into Bt brinjal event EE-1 has been extensively studied for its safety. It has been well established that the

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
		sufficient to indicate the safety of the GM crops. Similarly, the guidelines have been provided for assessment of allergenicity and comparison of nutritional aspects. The studies undertaken in Bt brinjal comply with the international guidelines as well as ICMR Guidelines accepted by GEAC.	Cry1Ac protein cannot cause any toxic effect in mammals because of lack of highly specific receptors and alkaline environment in the gut of mammals. Cry1Ac protein has a history of safe use for human and animal consumption as GM crops such as Bt maize and Bt potato containing Cry proteins including Cry1Ac protein have been consumed by millions of people without any adverse effects. It has been reported that 90-110 days of age (mating age) of rats is considered equivalent to 21-25 years age of humans (Laboratory Animals in the Study of Nutrition' in LAIS Centre News- No - 30, 1993). Cry1Ac protein has shown to be rapidly degraded (in 30 seconds) in simulated digestive fluids and thus is not detectable even in the short term studies.
4.	**Sub-chronic feeding study in Goats**		
	a. There was significantly lower hay consumption in Bt group in week 11 in comparison to non Bt group. The authors do not conclude anything problematic from this.	a. Inter-animal variability and intra-animal variability at different time intervals in the feed/hay consumption is commonly observed in the feeding experiments. Such differences are expected in dynamic biological systems but such differences should be checked only if they are statistically significantly different In the present study, there is variability in the feeding pattern of animals at different intervals of time e.g. hay consumption of Bt brinjal treated group males during week 11 was reflected due to decreased consumption in only one male goat. This particular animal has shown	a. The EC-II opined that it is inappropriate to quote insignificant observations out of context as many such observations seen at one time point do not persist at the next time point or not observed in the other sex.

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
		similar variability in the hay consumption as seen in the goats with normal diet without brinjal as well as with non Bt brinjal. As the differences were not of any statistical significance, the decreased hay consumption of Bt brinjal treated males during week 11, was considered as incidental and not related to Bt brinjal treatment.	
	b. The feeding trial consisting of six male and six female but on page 323 it has been stated that the trial consisted of 6 male and 3 females	b. The phrase reads as 12 animals(6 males and 3 females)	b. The EC-II noted that this is clearly a typographical error. The entire dossier provides data on all six males and six females.
	c. The prothrombin time as well as total bilirubin was significantly higher in the GM-fed males at termination, and alkaline phosphatase was significantly lower.	c. As regards the prothrombin formation time, the values in the pretreated male goats as well as Bt brinjal treated animals were within the physiological ranges. These marginal changes in the Bt are a normal variation and of no statistical significance. Although some differences were noted in the total bilirubin, AST and ALT values in control Non-Bt brinjal and Bt brinjal treated groups in comparison with the Normal diet without brinjal but even the higher total bilirubin values were within the physiological control range. Additionally, there were no alterations in the hepatic parameters or histopathology of the liver Therefore these change were considered as normal variation and not related to Bt brinjal treatment.	c. The EC-II noted that no statistically significant changes have been observed in the parameters mentioned and the values are within the normal physiological ranges. Further significant changes given only at a single time point and not given at the end of the study are considered to be transient changes. These changes are also not associated with any histopatholgical changes and therefore do not warrant any attention.
	d. Growth curve in Bt fed-males are below the others from week 7. They gain a lot less weight. The feed consumption is lot less, even 25% less (week 5) only for this transgenic fed group. This is important although not clearly reported in the summary and obviously significant after curves observation. This appears to be a sexdependent effect like for endocrine	d. Barring the intra-animal variability, there is no statistical significance difference obtained in the feed consumption, body weight and body weight gains and the hay consumption (except for the incidence of decrease hay consumption in males during week 11) of Bt brinjal treated group and the Non-Bt brinjal treated group and Normal diet group. The scale used in the growth curves very small, even a subtle change is seen with magnification.	d. The EC-II noted that the test product findings need to be compared with the concurrent control and/or normal control findings, baseline control data/historical control data and published references wherever applicable and in this study, the generally accepted procedures

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
	diseases. Bt brinjal as an animal feed, or human food that it will be mostly, cannot be considered as safe with such results.		have been duly complied with.
5.	**Sub-chronic feeding study in rabbits**		
	a. There was a reduction of consumption at week 6 in the male Bt group in comparison to non Bt, the GM fed males consumed less in general, in the female group at week 11 (due to one animal, but the groups are too limited in numbers of animals unfortunately to calculate a real statistical significance) as if the Bt brinjals were less palatable. The females consumed less Bt brinjal. b. There was at interim blood sampling an increase in albumin and total bilirubin in GM fed males versus adequate controls, and of total bilirubin and lactose dehydrogenase in GM fed females; at terminal blood sampling again a significant increase of total bilirubin in males and females GM fed, increases in hepatic markers alanine and aspartate aminotransferases and sodium levels in GM fed males, a decrease of glucose levels in GM fed females. The authors of the study claim all the above differences as incidental and not treatment related, with no scientifically acceptable reasons. c. The platelet count was significantly reduced during the experiment as well as mean corpuscular haemoglobin concentration in the blood of Bt fed males in comparison to their controls, an increase in haematocrit value; prothrombin time was increased in females.	a. The data related to food consumption demonstrates that the average food consumption in the Bt brinjal and Non-Bt brinjal treatment group- males were 95 and 104 grams/rabbit/day, respectively. The decrease is very marginal (9 grams) and such variations are expected in dynamic biological systems. b. The changes in the total bilirubin were normal variations in biological systems. All the values were within the normal ranges and do not signify any organ pathology and hence were considered as incidental and not related to Bt brinjal treatment. c. The minor variations in various blood parameters are physiological changes which are observed even in the Normal diet treatment and the Non-Bt brinjal treatment and hence considered as not related to the Bt brinjal treatment. Further the testing lab as well as experts have reexamined the data and informed that all the figures are	In respect of variations reported in the sub-chronic feed study in rabbits (5 a to c), the EC-II opined that in general toxicology and in safety testing, the results are compared between test and control groups. If differences do occur which is but expected in dynamic biological systems such differences should be checked for: i. Are they statistically significantly different ii. If yes, are the values or data in the normal physiological range. iii. If there are more than one dose group then one should look for dose dependent changes in the parameters iv. Even if there are significant differences and they are in physiological range then one should correlate biochemical and haematological data with histopathological changes. v. If there are significant differences and they are outside physiological ranges and they are associated with parallel histological changes e.g. elevation of AST, ALT with liver cell necrosis or changes in haematological parameters with corroborative changes in bone marrow. Then it is assumed that

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
		within the normal ranges (e.g. the normal range of platelet count is between 1,00,000 to 5,00,000).	the test material could result in cellular changes. Significant changes given only at a single time point and not given at the end of the study are considered to be transient changes. In view of the above, the EC-II concluded that the findings of the study are in order and the variations observed are not attributable to Bt treatment.
6.	**Feeding study in (lactating, crossbred) cows:**		
	a. It has been claimed that Bt cotton is not detected in blood but there was only a short description of the method of detection and its limits and efficiency as well as repeatability were not indicated.	a. The method of detection that has been used is ELISA based on a monoclonal antibody which specifically recognizes Cry1Ac/Cry1Ab antigen only. It will not detect any other Bt protein.It has a sensitivity of below 1%. The limit of Quantification (LOQ) is 0.0625 ng/ml and Limit of Detection (LOD) is 0.046ng/ml.	a. The EC-II noted that the test has been standardized by M/s Mahyco and the required information has been provided.
	b. More milk production (14.3%) by GM fed cows, almost as if they were treated by a light hormone, in 42 days.	b. The data clearly indicates that there was no significant difference in weekly yield and feed intake between the cows fed transgenic and non-transgenic brinjal fruits.	b. The EC-II reiterated that insignificant changes have been unnecessarily highlighted.
	c. The ash content of the milk varied significantly for Bt brinjal-fed cows between the second and fourth week, by the end of the experiment they had significantly more roughage dry matter intake (10.5%).	c. The variations in ash content in milk in different weeks were also there in cows fed with non-transgenic brinjal fruits.	c. The EC-II noted that all the values of ash content were within the normal range in both the groups.
	d. It cannot be concluded from this experiment that there are no metabolic changes after Bt brinjal consumption in lactating cows and thus this feed cannot be considered as safe.	d. There was no significant difference between the cows fed transgenic and non-transgenic brinjal fruits and differences in weekly yield and feed intake.	d. The results of the study clearly demonstrated that consumption of Bt brinjal by cows did not result in any metabolic changes and showed no adverse effects.

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
7.	**Sub-chronic oral toxicity study in (Sprague Dawley) Rats:**		
	a. The first experiment of 14 days with rats allowed to the company to test two doses of Bt brinjal is badly designed experiment from a scientific point of view, increasing control animals by 2 in regard to treated rats. This was unexplained.	a. All protocols were designed as per the guidelines issued by DBT and approved by RCGM. Two controls (non-Bt counterpart and commercial Brinjal) were used in the study to demonstrate that there were no differences between non-Bt hybrid developed by the applicant and commercially available Brinjal.	The EC-II noted that increasing control animals in no away affects the scientific credibility of the experiments. The reviewer has unnecessarily tried to correlate common and incidental observations to the effect of Bt brinjal which is very unfortunate and indicates lack of familiarity with the subject. For example circling observed due to internal ear infection is a common observation in all rodents in cages and has taken place in both Bt and non Bt group. However, the same has been highlighted only in case of Bt group to mislead the readers about safety of Bt brinjal.
	b. Circling disorder and diarrhea were noticed only in the Bt brinjal group, males and females.	b. Circling was observed in one rat from non-Bt brinjal control group and one rat from Bt brinjal group. This is due to internal ear infection which is commonly noticed in rodents housed in cages. This does not affect normal living of rats. Hence it is not related to Bt treatment. Diarrhoea seen in only one female and two males out of ten animals in each group of rats, for only four days out of 90 days of study is an incidental observation and not related to treatment.	
	c. Moreover liver weight and relative liver to body weight ratio decreased in the dose range study in females, by 13% apparently significantly. Bt brinjal cannot be considered safe for rats considering these results.For the rats fed with Bt brinjal water consumption was 8-21% more than the non Bt brinjal group for some periods. The significance of this claimed to be null. However, all the scientific committees consulted agree with companies that statistical significant differences have been reported during 90 day studies between control and treated rats with different GMOs on numerous parameters, including blood composition and detoxification organs such as kidneys.	c. Minor differences in clinical parameters of Bt brinjal fed animals have been quoted out of context as such variations are fairly common in biological systems. Many observations seen at one time point do not persist at the next time point or not observed in the other sex or not significant against the non-bt brinjal.	Regarding the minor difference in clinical parameters, the EC-II reiterated its observations cited earlier (refer Point 5).

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
8.	**Primary skin irritation tests on (New Zealand white) rabbits** Three rabbits only were treated with Bt brinjal on a total of 12; this is not serious at all.	In the study, the Bt treatment was compared with three sets of controls, which does not in any way affect the results obtained with Bt treatment. The study was conducted in 2004 as per the "Guidelines for toxicity and allergenicity evaluation of Transgenic Plants", 1998.	The EC-II concluded that since the non allergenicity of Bt protein has been demonstrated through a series of studies using pure protein, this study is not much relevant and not required as per the new Guidelines for safety assessment of foods derived from GE plants, 2008 adopted by GEAC.
9.	**Mucous membrane irritation test and measurement of allergenicity in young adult Brown Norway rats are very limited tests to assess allergenicity**	Allergenicity assessment is undertaken using a weight of evidence approach based on FAO/WHO Consultation on Biotechnology and Food Safety. Internationally accepted methods such as the source of gene, heat stability, pepsin digestion, amino acid homology testing using bioinformatics have been used in allergenicity assessment of Cry1Ac protein in Bt brinjal. In addition, allergenicity tests in young adult Brown Norway rats were undertaken as per the regulatory requirements stipulated as per the "Guidelines for toxicity and allergenicity evaluation of Transgenic Plants", 1998. .	The EC-II concluded that the allergenicity assessment of Bt brinjal event EE-1 has been done as per the internationally accepted Codex guidelines. Further, the requirement for testing in young adult brown Norway rats is not mandatory in India as per the newly adopted Guidelines for safety assessment of foods derived from genetically engineered plants, 2008. This requirement has been dispensed with as there are no validated animal models for allergenicity assessment of GM foods.
10.	**GM brinjal consumption by birds (Feeding study in broiler chickens):**		
	a. 40 unsexed chickens received 5% Bt brinjal in their diet, 40 others, 10%, and 200 received different non GM diets. This was not a good design to detect any unintended GM effect in these conditions. In particular 10% is too low a percentage to clearly see unintended effects.	a. The experimental design consisted seven dietary treatments with two levels (5 and 10%) each of Bt, non Bt parental and a commercial brinjal along with a corn soya control treatment. Each diet was fed *ad libitum* to five replicated groups of eight unsexed chicks in both the grown phases i.e. starting 0 to 3 weeks and finishing 4 to 6 weeks phases following completely randomized design.	a-b. The EC-II opined that broiler chicken is the representative model and the experimental design is in conformity with the accepted guidelines. The EC-II was of the view that the reviewer has unnecessarily' chosen to highlight insignificant issues. All the six groups have lower and

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
		The experimental design is a widely accepted design and many research papers have been published in international journals with the similar design. The test diets contained 5 or 10% brinjal meal in dried powder form as part of total daily diet that for 42 days (starting from 1 day-old to 42nd day) *ad libitum* without any interruption in between. The 10 g dried brinjal meal in 100g of feed is equivalent to 67 grams of fresh brinjal for a broiler of about 1000g body weight. Hence, these levels of inclusions are much higher than generally consumed by human being.	insignificant differences compared to the control group. .
	b. Moreover there is only one species of bird studied for a limited period of time.	b. Poultry birds especially broiler chickens are used as model animals for bio-safety studies because of faster growth rate. Also since the broiler chickens are more vulnerable to any toxic / anti-nutritional factor (s) present in feed/feedstuffs they serve as good model animals. Six weeks (0-42 days) experimental period, when rate of growth is maximum, is sufficient to exhibit the toxicity of any substance, if any. Being rapid growth, 37 times of initial body weight, in the present context, in 42 day old broiler chickens were the most suited birds for conducting this experiment.	
	c. The feed intake for GM-fed broilers (10% Bt-brinjal) was 10% lower than in the corresponding control (10% non Bt brinjal in the diet) at different weeks (21-35 days of age) and then higher, the implication of this is a differential metabolism between both groups but the experimental report did not calculate the statistical significance of this difference. The blood glucose was also significantly different in the Bt groups.	c. The feed intake for GM-fed broilers was compared statistically week-wise and phase-wise. The mean values did not differ statistically/significantly either when analysed on weekly basis or on phase basis. The blood glucose was also significantly different in the Bt group in comparison to control diet, which was also evident in all the treatments fed brinjal from any source. Therefore, it is the characteristic of brinjal as a whole, not for Bt brinjal alone.	c-d. As regards the intra-animal variability in the results, the EC-II reiterated that such differences are expected in dynamic biological system. The EC-II further reiterated its observations in Point 5.

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
	d. The authors of the experiment write that there is no significant difference due to Bt brinjal consumption by chickens, but these differences lead instead to the conclusion that the Bt brinjal cannot be considered as safe according to this experiment.	d. The reviewers have interpreted the results based on the observations and statistical analyses. All the response criteria, related to growth and welfare evaluated in this study were not affected by feeding the brinjal of any source. Neither the heterophile to lymphocyte ratio nor the yield of visceral and immune organs, humoral and cell mediated immune response were affected with Bt brinjal. Hence, the conclusions drawn were based on scientific observations, rather than mere assumptions.	
11.	**Feeding studies on common carps**		
	a. There were numerous unnecessary non-transgenic control groups masking the significant effects between the two closest groups, Bt and non Bt. There were only 6 pools of 60 fishes (360) receiving Bt brinjal in the feed on a total of 24 pools, i.e. 1440 fishes, instead of having two main groups. This disproportion can mask a lot of significant effects if only a small group is compared with all the others.	a. The four groups of brinjal used in feeding trial for common carp were taken for better results to actually compare with each other. Secondly, the numbers of fishes per pool were enough (60/pool) for statistical analysis with two replicates (60 + 60 =120) for each test concentration (15%, 30% and 45%) of four groups of brinjal used totaling 360 fishes for each brinjal feeding trial. Thus, there was no harm to undertake the comparative study of Bt and Non-Bt with other groups of same type of brinjal available in the market. This was taken up for the comparative studies without adversely affecting the objectives.	a. The EC-II opined that the objection of disproportionate sample sizes between treatment group and control group is very unfortunate and unscientific. Comparison was made with Bt-brinjal group with non-Bt brinjal group, local variety and with a group that does not contain any brinjal. This means the Bt group is compared with three times its size. This is very robust and logical, since data in each group is provided separately with its own statistics reported. No pooling of the control group was done to mask the observations as claimed. Further growth was similar in all the groups, which nullify the possibility of any apprehension regarding the comparison between Bt and non-Bt brinjal.
	a. Average feed conversion and efficiency ratios were significantly higher in the Bt group versus closest control, at 45% brinjal in the diet. No safety can be concluded.	b. The Feed Conversion Ratio (FCR) and Feed Efficiency Ratio (FER) ranged between 2.8 + 0.02 to 3.3 + 0.10 and 0.35 $\pm$ 0.02 to 0.30 $\pm$ 0.02 respectively in all the feeding trials of four groups of brinjal. Furthermore, FCR and FER in the Bt group versus the closest control, at	b. The EC-II opined that these minor differences are in no way linked with the safety issues.

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
		45% brinjal in the diet were recorded as 3.1±0.2 & 3.3±0.10 and 0.32±0.03, 0.30±0.02 respectively which clearly demonstrate that there is no significant difference in feed conversion and efficiency among them. The only significant difference with respect FCR found at 45% level of feeding is unusually higher for fish. This difference might have been caused due to erroneous recording of feed intake data for the Bt brinjal fed group at 45% level. This can be explained as: $FCR = \frac{\text{Feed intake}}{\text{Weight gain}}$ The weight gain was similar in all the groups as explained earlier (table 9). Hence FCR value will only increase if feed intake value increases. Feed intake value is calculated after collecting the refusal. If something is leached out into water that is not estimated. There is maximum possibility of overlooking this factor, which otherwise gives a wrong impression of higher feed intake. Inclusion of 45% brinjal in a pelleted feed may definitely change the texture of the feed leading to leaching of the dry matter. The water stability of that diet was beyond the scope of the study.	
12.	**LIMITED TESTS OF Bt BRINJAL ON SOME SOIL MICROFLORA**		
	a. Limited environmental studies of Bt brinjal risks have been performed on an extremely little part of soil microflora, collembola, nematodes and earthworms. It is almost impossible through a few species measurements to get a whole view of a complicated ecosystem, moreover varying a lot from place to place in India. In addition, statistical tests that have been chosen appear to be limited, grossly inadequate as we have demonstrated in	a. The soil microflora studies and effects on Collembola, nematodes and earthworms in Bt brinjal field plots have been carried out in almost every growing season since 2003 over at least 50 locations in India and not a single instance of reduced soil fertility has been reported. The studies have been repeated in large scale trials conducted by IIVR as per the directive of GEAC. These studies demonstrated that Bt brinjal does not have any significance effects on soil microflora both fungi and bacteria and soil invertebrates such as earthworm, collembola and nematodes. No Cry1Ac protein was	The EC-II opined that it is not possible to culture many soil fungi and bacteria and indicator species measured provide a framework for evaluation of soil effects. It was concluded that sufficient soil impact analysis has been undertaken by M/s Mahyco and IIVR. Therefore, hypothetical "evolutions and reactions" are not a justification for invalidating the studies conducted.

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
	other studies (Séralini *et al*., Arch. Env. Contam.Tox. 52, 596-602, 2007). There are some severe limitations to the studies performed or that can be performed: • Culture media used do not allow for sure all bacteria and fungi to be measured. • Not all groups of invertebrates or insects have been taken into account. • Bt brinjal was cultivated only during 5 months before testing soil fertility, but most effects can appear after long11 term cultivations with pesticides treatments. • New Bt insecticide present in the soil due to GM brinjal and produced by it may be partially linked to particles and be released after rain or environmental changes, this has not been assessed either. • Significant differences have been observed in colembolla and earthworms populations between Bt and non Bt real control fields. Two additional controls mask the effects, by the end of the experiment (120 or 150 days), these don't have to be persistent to be of biological relevance since evolutions and reactions may exist in these complicated ecosystems that could alter in a long-term soil life and fertility. • Mortality of beings is often an insufficient parameter measured, reproduction capacity or physiological parameters are more pertinent for non-acute but chronic effects.	detected in any of the soil samples of Bt brinjal field plots.	Further the Bt cotton expressing the same gene is being grown extensively in the country since 2002. Bt cotton and Bt corn containing the same gene are being grown in more than 20 countries worldwide and no adverse reports have been reported.

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
13.	**Bt TOXICITY TESTS FOR NON TARGET INSECTS**		
	a. Effects on honey bees (7 days) or larvae survival were considered non significant at 20 ppm of Bt (NOEL: chosen as the No Observable Effect Level). Ladybird beetles, or green lacewing larvae, also beneficial insects, gave similar results for the company after 30 days. Unfortunately these tests are not relevant since they have been conducted with Cry1Ac which is not the insecticide produced by the Bt brinjal at all. As anyone can see, they are also very limited in time and doses.	a. The results reported by the applicant are comparable and relevant as they are tested with the protein that has been demonstrated to be biochemically and functionally similar to the one produced in Bt brinjal event EE-1 through a series of tests. The effects on honeybees, ladybird beetles or green lacewing are tested by adding the Cry1Ac protein at doses lethal to target insect pests and represent the direct effects, if any. The dose of 20 ppm is around 338 fold higher than the dose of 0.059 ppm required to kill the target insect or stop its growth.	a. The EC-II was of the view that the ecotoxicology tests for non-target insects have been always carried out using bacterially produced proteins as it is extremely difficult to extract the required protein from the plant material. The study reports submitted by the applicant have been reviewed and accepted by regulatory authorities in many countries *viz.* United States Environment Protection Agency, Canadian Food Inspection Agency, Office of Gene Technology Regulator, Australia, European Food Safety Agency, etc.
	b. Field trials are an inadequate basis to assess impacts on the agrosystem: • Studies of long term effects are lacking, • Studies on beneficial insects (e.g. natural enemies of target pests), as well as studies of abundance of secondary pests (which would have to be sprayed with insecticides) are lacking. • Indirect effects (e.g. does the Bt toxin affect organisms that eat the target insect) are important in this regard. • No laboratory studies have been performed to evaluate other lepidtoptera insects.	b. The field trials are well accepted methods for evaluating the impact on agro system. Extensive field trials have been conducted on Bt brinjal event EE1 over a period of five years at multiple locations representing different agro-climatic conditions. During these field trials the non-target insects (includes moths and butterflies) and beneficial insects have been recorded throughout the crop growth period. A total of 17 non-target and beneficial insect species are being recorded in these field trials comprising of insects from orders Lepidoptera, Coleoptera, Thysanoptera, Homoptera and Diptera, besides spiders (Arachnida). As regards long term effects, the reduction in insecticide use in a Bt crop will enhance the survival and reproduction of predators and parasitoids in an agroecosystem, which was already reported in several studies from Australia, China and the USA (Head *et al*.	b. The EC-II concluded that data generated during field trials coupled with laboratory and greenhouse evaluation and information on the biology of brinjal is adequate to assess the impact of Bt brinjal event EE1 on agro systems.

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
		2005, Chen *et al*., 2007). Laboratory studies have been performed to evaluate the impact on other lepidopteran insect as well and reported in the literature (Mendelsohn *et al*., 2003).	
	c. The harmful increase in secondary pests takes place after many years. Brinjal has also many insect pests (for example, sucking pests like whiteflies) that will not be controlled by this Bt toxin, and may increase over time. Thus will in turn increase chemical insecticide use compared to initial years of Bt brinjal use. This situation is difficult to predict, and would require monitoring after commercialization.	c. In the Bt resistance management program, the sustainability of efficacy of currently available insecticides will be considered, as Bt crops are part of holistic IPM program in a crop	c. The EC-II opined that appropriate post release monitoring mechanisms and IRM strategies would address this issue.
14.	**Bt toxicity tests for target insects:**		
	a. The toxicity of Bt toxin Cry1Ac to the larvae of a target fruit and shoot borer lepidopteran insect, Leucinodes orbonalis Gwen has been evaluated by the company. The Cry1Ac was from a commercial formulation and not purified from the Bt brinjal (surrogate protein), thus modifications of the protein in amino acids, structure and post-transcriptional modifications such as potential glycosylations have not been taken into account, limiting the significance of the results. b. Some lyophilized transgenic fruit powder was also used in bioassays but these lasted only 7 days. There were 12-14 fold variations in the results. The Bt protein was significantly toxic in this regard; this was the	a. The objective of the test is to study bioefficacy of the Cry1Ac protein on target fruit and shoot borer. Both commercial Cry1Ac protein and lypholized Bt brinjal powder have been found to be effective against fruit and shoot borer. The use of commercial Cry1Ac protein does not in any way limit the significance of the results as the protein produced is the same as present in Bt brinjal event EE-1. b. Bioassays have been done for seven days to demonstrate a dose response with Bt brinjal powder which is comparable to artificial diet bioassays. In an artificial diet bioassay, one can hold the diet with no bacterial and viral infections to a maximum of 8-10 days.	The EC-II concluded that the insect bioassays for bioefficacy against fruit and shoot borer have been undertaken as per the protocols approved by regulatory agencies. Further, the results of insect bioassays have been confirmed in field trials conducted in more than 50 locations across various agroclimatic zones in the country.

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
	goal of the GM brinjal on this insect.	The 12-14 fold variation presented in the results is a natural response observed in insect populations.	
15.	**Pollen flow studies:**		
	a. Cross pollination or pollen flow is a very small part of contamination possible by GM plants. Thus, pollen flow study alone has little impact on environmental risk assessment of dissemination per se because: 1) First, the seeds can be contaminated during the production when bought or taken by agricultural workers 2) the transportation and spreading of seeds for cultures is not full closed and cannot be restricted temporally to a particular designed field, 3) the cultivation can imply the sharing of workers or tools or even machines that bring contamination of pollen or seeds from one field to another, 4) the insects, birds, other animals such as rodents or mammals will bring fruits or parts of flowers or fruits from one place to another, 5) the harvest is made by tools that are shared and may mix the productions at low levels, 6) the storage is made in places that cannot be always fully dedicated to GM or non GM plants, 7) the markets or transformation factories or cookers may mix the fruits or seeds.	a. Pollen flow is a natural phenomenon in plants, which cannot be controlled and thus its impact needs to be evaluated. Issues related to dissemination mentioned by the reviewer are external factors, several of which can be controlled and the extent to which this aspect needs to be monitored is a trade related issue and not a part of environmental risk assessment.	The EC–II concluded that the pollen flow studies for four years as well as other environmental safety studies provide enough evidence of the safety of Bt brinjal to the environment. Other issues raised by the reviewer are hypothetical and out of the scope of the environmental risk assessment

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
	b. The sampling procedures are crude and limited and do not take into account the form and size of the field and the environment.	b. The sampling procedures are as per the standard practices and approved by RCGM and IIVR .	
	c. A maximum of 50 meters from the source has been studied for dissemination, this is not significant in comparison to the well known wave's effects of pollen disseminations depending on the wind blowing and insects and this has been demonstrated for several pollens (maize, oilseed rape...). Thus the assessment was incomplete and not extensive. pollen flow rates depend on a number of factors not addressed by the applicants. For example, in addition to proximity of fields, the relative size of brinjal fields can influence the rate and level of pollen contamination. Small conventional (non-GMO) brinjal fields planted near large Bt brinjal fields will have higher rates of contamination than large conventional brinjal fields in otherwise similar situations. Therefore, smaller conventional brinjal farmers may be at greater risk of higher levels of contamination than larger farmers. Further, the applicant did not consider that levels of contamination may be additive over time if a farmer saves non-GMO brinjal seed, and if neighboring Bt brinjal farmers continue to plant Bt brinjal. If more than one brinjal crop is planted in a year, this would accelerate this trend.	c. In the pollen flow studies conducted initially, the distance of 50 metres was used, but the studies were repeated as per the recommendations EC-I and conditions stipulated by GEAC upto 300 metre. The pollen flow was observed only upto 30 metres. Dissemination of pollen is dependent on wind blowing and insects, but most importantly it is crop specific and depends on the reproductive biology of that particular crop. Keeping in mind the size, pollen production, morphology and viability as well as environmental factors, pollen flow studies are designed. In no way, the results obtained in maize, oilseed etc. can be compared with brinjal. Size of field or repeated cultivation has no effect on the pollen flow.	
	d. The analysis of pollen flow also neglects other very important routes of contamination	d. The concerns regarding seed mixing are trade related issues and not a part of environmental risk assessment.	

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
	(e.g. by mixing seeds). Based on data from other countries on other genetically engineered crops, it seems likely that routes of contamination such as seed mixing are important. For example, in the U.S., levels (concentration) and rates (percent of the total crop) of contamination of soybean, a crop with low out-crossing rates similar to brinjal, were as high as for crops like corn that outcross at much higher rates. Since out-crossing occurs by pollen flow, these data suggest that other means of contamination are likely to be important ("A Growing Concern", Union of Concerned Scientists, 2004, http://www.ucsusa.org/assets/documents/food_and_agriculture/seedreport_fullreport.pdf).		
	e. Gene flow to wild weedy relatives may result in environmental harm. This important route of possible environmental harm is widely recognized, but apparently not considered by the applicant. Gene flow from Bt brinjal in India may occur with the sexually compatible wild weedy relative Solanum insanum. Another sexually compatible relative, and the progenitor species of brinjal, S. incanum, probably also occurs in India. Gene flow from GMO crops has occurred from a large scale field trial of creeping bentgrass (Agrostis stolonifera) in the U.S., and from commercialized canola in Canada	e. The crossability of different species of brinjal in India has been studied and reviewed by Rao, 1979. It has been reported that there is no natural crossing among cultivated and wild species of brinjal including *S. incanum* and *S. insanum*. Under forced crossing situations, even if crossing was possible, the viability and subsequent development of stable crosses have not been successful. Particularly in case of *S. incanum,* the crossability studies have been repeated by Indian Institute of Vegetable Research. It has been indicated that there was very limited crossing when *S. incanum* was used as female parent, whereas in the earlier study (2007-08), no crosses could be obtained. It can be concluded that gene flow from *S. melongena* to wild relatives of brinjal is not possible under natural conditions.	

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
	– in both cases involving a gene for glyphosate herbicide tolerance. Transfer of the Cry1 gene to these wild relatives may lead to harm to Lepidoptera or other non-target organisms that feed on these wild plants, or the wild plants may become more weedy due to suppression of herbivorous insects that may help keep their growth in check. Whether these possibilities occur depends on a number of factors that have not been tested by the applicant. For example, it must be determined whether these wild species grow in areas where brinjal is cultivated, which would allow gene flow to occur. Harm from such gene flow can only be determined through appropriate tests such as determining which organisms feed on these wild species, and whether they are sensitive to the Bt toxin. It should be noted that GM crops containing a Bt gene have not been commercialized in proximity to wild relatives anywhere in the world. Finally, gene flow to wild relatives may in some cases lead to reduce genetic diversity of the wild species. This is especially true for wild relative that grow near the crop, and occurs through the phenomenon of gene swamping when the crop is more numerous than the wild relative.		
	f. It is recognized that brinjal wild relatives may provide important pest resistance genes for brinjal diseases and insects, as well as other desirable traits. The possible reduction of	f. Genetic improvement by conventional plant breeding has not been successful due to the lack of resistance to FSB in brinjal germplasm. Some wild *Solanum* species showed high levels of resistance, but it has proved to be	

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
	such diversity could have negative implications for further improvement of the brinjal crop, and should therefore be carefully considered.	impossible to incorporate the genes for resistance from wild species into commercial cultivars due to breeding incompatibilities (Dhankhar *et al*., 1982).	
B.	**Dr. A. Velimirov (Austrian's study)**		
	Study focuses on biological effects of transgenic maize NK603 x MON810 fed in long term reproduction studies in mice. Three designs used including a multi generation study (MGS), reproductive assessment by continuous breeding (RACB) and a life term feeding study. The number of females without litters decreased with time in the GM and reference group, especially fourth generation. However, the production parameters i.e. average litter size and weight as well as number of weaned pups were in favour of the reference group. The results of MGS showed no statistically significant differences concerning parental biomass. However, the continuative investigations revealed effects on kidneys pointing to an effect on the GM crop on metabolic parameters. RACB trial showed time related negative reproductive effects of the GM maize under the given experimental conditions.	The study focuses on MON810 and NK603 corn technologies, which have a history of safe use since their introduction in 1997 and 2001, respectively. These products have been thoroughly tested and consumed for nearly a decade. Regulatory authorities in more than 20 countries have concluded that these products are as safe as conventional corn. One of the author of the study, Dr. Jurgen Zentek, has remarked that his team's three studies show inconsistent results and should be considered preliminary. The report has not been peer reviewed. The European Food Safety Authority's (EFSA) Scientific Panel on GM Food also assess the findings of the study and identified various deficiencies in data reporting, methodologies and statistical calculations, which do not allow any interpretation. EFSA has indicated that these data do not invalidate the safety of MON810.	Based on the scientific evidence and opinion available worldwide on the safety of transgenic maize, the EC-II opined that the findings of this study regarding reproductive effects of transgenic crops do not merit any consideration. The EC-II also opined that no such reproductive assessment is necessary.
C.	**Doug Gurian-Sherman**		
	a. Limited data on gene flow distance that cannot substitute for a risk assessment of potential harm from gene flow and is wholly inadequate to predict gene flow. Since India is the center for domestication and genetic	Issues raised herein have already been considered in Section III(2) of the report of EC-II. Further, similar issues have also been raised by Prof. Seralini which have been considered by EC-II (refer Point 15 of this table).	Refer Point 15 of this table

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
	diversity of brinjal, it is recommended that gene flow from Bt brinjal should be seriously considered and evaluated before commercialization.		
	b. Presence of sexually compatible wild relatives *S. insanum* (distributed throughout India) and *S.incanum* (restricted to Southern Indian scrub forests) demands an extensive tests on gene flow to avert the risks related to weediness, non target organisms and reduction in genetic diversity of wild relatives. .	Same as above	Same as above
	c. Better data could be gathered or generated as to the frequency of gene flow to *S.insanum* and *S.incanum,* and the fertility and fitness of hybrids between them and brinjal. Fitness of the gene in the wild relatives should also be determined.	Same as above	Same as above
	d. It should be determined whether wild relatives are important food sources for insects that feed on it.	Same as above	Same as above
D.	**List of studies recommended by Dr. P. M. Bhargava before release of a GMO into the environment.**		
1.	*DNA fingerprinting and proteomic analysis*	Transcriptomics (transcript profiling), proteomics (protein profiling) and metabolomics (metabolite profiling) are technologies, which facilitate a non-targeted approach and permit the measurement of thousands of variables simultaneously. These ‘‘omics” technologies applied to toxicology, also referred to as toxico-genomics, are currently in their infancy, but provide an opportunity to better understand the mechanism of action of chemicals and contribute to the development of alternatives to animal testing. However, further validation of these technologies and	The EC –II noted that the technologies such as transcriptomics (transcript profiling), proteomics (protein profiling) and metabolomics (metabolite profiling) involve long drawn expensive procedures with little value, and therefore not recommended for safety assessment of GM crops. These technologies are research tools and have not been validated for use in

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
		better knowledge of how to interpret the complex results is needed before they can be applied in routine safety assessment of food and feed derived from GM crops. The above views have been endorsed by EFSA while reviewing reviewed recent developments in molecular biology and analytical chemistry to evaluate the effect of chemicals in food and diet on mammalian cells at various integration levels (e.g. RNA, protein, metabolite) (EFSA, 2008).	evaluation of GM crops.
2.	*The total sequence of the transgene-flanking regions and the transgene, and identification of the site(s) of integration of the transgene in the GMO.*	The molecular characterization study for a GM crop provides the DNA sequence of the insert and flanking region.	Information/data is part of biosafety assessment.
3.	*Changes in the glycosylation pattern*	The protein characterization study includes an assessment of the glycosylation status of the expressed protein.	Information/data is part of biosafety assessment.
4.	*Determination of any selective increase in transcription and translation, thus including a study of the transcriptome*	Please refer response to Point 1.	Same as Point 1
5.	*Changes in relative concentration of major and important cellular metabolites*	Please refer response to Point 1.	Same as Point 1
6.	*Changes in surface properties that may affect normal interaction between species and with the environment, studied through electon microscope and atomic force microscope*	The interaction between species and with the environment is dependent on many factors particularly environmental factors rather than only surface properties.	The EC-II noted that this study would not be of any value, particularly when the interactions between species and the environment have been evaluated through a series of lab, greenhouse and field testing.
7.	*Reproduction interference*	The criteria and purpose of this study are not clear.	Irrelevant
8.	*Gene flow*	Impact of gene flow has been analyzed as part of environmental safety.	The EC-II concluded that the issues related to impact of gene flow have been adequately addressed.
9.	*Dispersal into areas where positive harm could be done (as happened with water hyacinth and*	The environmental assessment of GM crops includes a comprehensive evaluation of the potential for outcrossing to	The EC-II concluded that the environmental safety assessment has

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
	parthenium)	similar species and wild relatives, which could lead to the dispersal of the gene in the environment.	been satisfactorily completed in case of Bt brinjal.
10.	*Development of a technique to determine with accuracy 0.01 percent contamination with GMO or its product*	This requirement is not necessary as part of safety assessment. However, data is currently being provided to meet the Supreme Court-mandated requirement.	The EC-II noted that issue is already addressed.
11.	*In the case of GM food material, possible interaction with commonly used drugs, especially probiotics*	GM crops express low levels of digestible proteins that are readily degraded in the gut like other dietary proteins. There is no evidence to suggest that such foods have any greater potential to interact with commonly used drugs or probiotics than any other food.	The EC-II concluded that it is a hypothetical issue and does not merit any consideration.
12.	*Acute toxicity studies with native (not surrogate) protein, GM seeds and other GM plant material that is normally ingested by animals, including cattle. These studies should be done both on experimental lab animals and on farm animals such as goat, sheep and cows.*	Safety assessment of proteins expressed in GM crops is conducted according to internationally accepted criteria (Delaney *et al.*, 2008). These criteria include a bioinformatic assessment to ensure that the protein is not related to known allergens or toxins, in vitro digestibility studies to assess potential allergenicity and an acute toxicity study in a relevant mammalian species (typically mouse) to evaluate the potential for a protein to be acutely toxic. As discussed in Part 1c, p.3., a surrogate protein is used because of the limited quantities of the plant-produced (i.e., native) protein. The vast majority of proteins that are toxic to mammalian systems are known to act through acute mechanisms (Hammond and Cockburn, 2008; Delaney *et al*., 2008; Pariza and Johnson, 2001) and therefore, acute toxicity studies are sufficient to manifest their potential toxicity. In the case that (1) the mode of action of the expressed protein raises no safety concerns, (2) the protein is readily digestible, (3) there is a history of safe consumption of the protein, or proteins of related structure and function, the protein would be considered safe to consume. There is no rationale or precedent for evaluating acute protein toxicity in a non-rodent model,	The EC-II noted that the present guidelines for safety assessment of GE protein are in line with FAO/WHO and recommendations of Codex Alimantarius and therefore concluded that no additional studies are required.

[1] See http://www.fass.org/page.asp?pageID=52).

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
		such as a large mammal. Small mammals are suitable test systems to evaluate acute mammalian toxicity, including the potential for toxicity in humans or farm animals. Feeding studies with GM crops (expressing the native protein found in the crop) in large animals fed at maximum practical inclusion levels have been conducted and published.[1] The suggestion to conduct acute toxicity studies of the native protein in cattle or other ruminant species is not scientifically justified as these types of studies would add little to no value to the safety assessment of a protein. Furthermore, these studies are not required by international regulatory guidelines to ensure safety of proteins (Delaney *et al*., 2008; EFSA, 2006, 2008). Livestock will be exposed only to those levels in the crop or by-product at a maximum inclusion level for the feedstuff. Secondly, there is a highly proteolytic environment in the reticulo-rumen in which the probability of these native proteins surviving is very low.	
13.	*Chronic toxicity studies (including carcinogenicity) as above*	Same as above	Same as above
14.	*Effect on cattle GI microflora*	The effect of consuming GM material on cattle microflora has been indirectly measured in published studies. Studies of GM crops fed to cattle have measured feed intake, body weight, health parameters, and in the case of lactating dairy cows milk yield and composition (Flachowsky *et al*., 2005; CAST 2006). It is a well known established fact that the composition of the diet influences the microflora in reticulo-rumen as well as in the lower GI tract. Significant changes in the microflora that result in alteration of volatile fatty acids and microbial yields can be measured in terms of animal growth or changes in milk yield and composition. Significant negative effects on GI microflora may be manifested as reduced intake and performance and/or diarrhea. In all of the published GMO cattle feeding studies, no differences in	The EC-II concluded that no additional studies are required.

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
15.	*Effect on soil micronutrients in every region concerned (rain-fed, irrigated, semi-arid,) where GMO is likely to be released.*	performance or health were observed (CAST, 2006)[7]. Studies on soil microflora are undertaken in various agro-climatic zones and coupled with studies on accumulation and persistence of expressed protein provide sufficient evidence on the impact on soil.	The EC-II concluded that existing protocols are sufficient to assess impact of GE crops on soil and no additional studies are required.
16.	*Development of resistance to the trait that is introduced*	For the commercialization of Bt crops, regulatory agencies require that the applicant develop an insect resistance management plan to prevent development of resistance to the target pest. The plan includes the requirement to plant a non Bt refuge crop of that represents a specific percentage of the total growing area for that crop. The applicant is also required to develop a plan to monitor for the development of resistance. Such a plan has been implemented for Bt cotton in India.	The EC-II opined that appropriate post release monitoring mechanisms and IRM strategies would address this issue.
17.	*Increasing refuge requirements for refuge crops, if any.*	See response to Point 16 above.	Same as above
18.	*Increase in susceptibility to pests and infectious agents other than those expected to be killed by the transgene.*	Observations regarding susceptibility to important pests and infectious agents other than the target pest are routinely recorded as part of field trials.	The EC-II concluded that issue is already addressed
19.	*Comparison of the growth characteristics of the GMO and the parent organism.*	Agronomic growth characteristics and yield data, are routinely collected during field trials for a GM crop	The EC-II concluded that issue is already addressed.
20.	*Emergence of new dangers, for example super weeds ,following prolonged use of herbicide-resistant crops*	Issues related to impact on weediness are addressed through a series of tests and information a bout the biology of the crop, as part of environmental risk assessment. This is also followed by post release surveillance and monitoring.	The EC-II concluded that environmental safety assessment conducted as per the prescribed regulations is fairly comprehensive. Further, the regulatory process is dynamic and is continuously updated based on scientific developments to assess the safety of new products .

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
21.	*Effect of the population density on non-susceptible pests, following at least 5 successive plantations - for example in the case of GM Bt plants*	As per the regulatory requirements in India, the data on non target pests is to be recorded for a minimum of three years.	The EC-II concluded that the issues already addressed.
22.	*Automated karyotyping and gross chromosomal analysis*	The molecular characterization includes the DNA sequence of the insert which provides more precise information than would be obtained from automated karyotyping and gross chromosomal analysis	The EC-II opined that this does not merit consideration
23.	*If the GMO is a plant, its biomass productivity in comparison to the parent*	The purpose of this requirement is not clear as is not related either the safety or efficacy of the trait.	The EC-II opined that this does not merit consideration.
24.	*Comparison of inputs required for optimal growth of the GMO in comparison to the parent organism*	This is not a safety issue and is addressed while working out agronomic management practices for a particularly variety	The EC-II opined that this does not merit consideration.
25.	*Impact on ecology in controlled field trials (e.g., population of bees and other beneficial insects). This would require total mapping of insects in every region where the GMO is intended to be released over a substantial period of time.*	There is no reason to conduct such a study unless an adverse effect on non-target organisms is indicated during field trials.	The EC-II opined that this does not merit consideration.
E.	**Comments by Prof. Jack A. Heinemann, University of Canterbury, New Zealand**		
	The comments on the molecular characterisation of transformation methods and number of insertions, novel RNA and proteins and Cry1Ac toxin mode of action and non-target effects were considered. The EC-II concluded that most of the issues raised have been addressed in the preceding sections. Regarding presence of additional insertions of the transgene or fragments in the brinjal genome and presence of novel RNAs created by the insertions, the EC-II noted that M/s Mahyco has submitted relevant data pertaining to the molecular characterization of the event EE-I. In addition, blots have been generated using the entire pMON10518 plasmid as a probe, as well as the *nptII* gene and 7S terminator-right border regions as probes. No additional bands were detected using these probes, indicating that there are no additional fragments from the construct at other locations in the genome. Further, the insertion sites from Bt brinjal event EE-1 was isolated and sequenced. The 3' end of the *cry1Ac* gene was examined and found to have the expected stop codon, followed by the 7S terminator and right border. Genomic flanking sequence examined on either side of the insertion showed no significant matches any sequence in GenBank, and does not encode any open reading frames in all six frames. This suggests that no novel RNAs or proteins were generated as a result of the EE-1 insertion. Regarding the mode of action of cry1Ac and non target effects, the EC-II noted that the model of cry toxicity as presented by M/s Mahyco is an established model (Broderick *et al*., 2006; Broderick *et al*., 2009; Daly and Buntin 2005, Naranjo 2005; Wolfenbarger et al. 2008; Lawo et al. 2009; Rodrigo-Simo´n et al. 2006).		

S. No	Summary of issues raised	Observations of the EC-II based on responses received from the applicant, institutions involved in the study and international reports/regulatory dossiers	Concluding remarks
F.	**A review of Mahyco's GM Brinjal food safety studies by Dr Judy Carman, Australia**		
	The EC-II concluded that all the issues raised in the review have been addressed in the preceding sections.		
G.	**Letter from Ms Kavitha Kurunganti to members of the EC-II constituted on May 29, 2009, to review the biosafety studies and large scale trials of Bt brinjal**		
	The EC-II noted that the letter from Ms. Kurunganti is basically a recap of issues considered by EC-I, issues raised by Prof. Seralini and studies suggested by Dr. P.M. Bhargava. The EC-II concluded that all the issues raised in the letter have been addressed in the preceding sections.		
H.	**Appeals submitted to PMO.**		
	The EC-II noted that the appeals submitted to the PMO have expressed concern on the impact of Bt brinjal on human health. The concerns are based on issues raised in the Prof. Seralini's report and the Austrian study, which have been given wide publicity by the civil society. The EC-II has considered both the reports and the observations of the EC-II are reflected in the preceding sections.		
I.	**"I AM NO LAB RAT CAMPAIGN"**		
	The EC-II noted that the appeals submitted to the PMO have expressed concern on the impact of Bt brinjal on human health. The concerns are based on issues raised in the Prof. Seralini's report and the Austrian study, which have been given wide publicity by the civil society. The EC-II has considered both the reports and the observations of the EC-II are reflected in the preceding Sections.		

*****

REFERENCES

1. Agarwal, S.K. 2009. Pesticide Pollution. pp 332. A.P.H Publishing Corporation, New Delhi.
2. Agrawal, R.L. 1980. Seed Technology, Oxford and IBH Publishing Co., New Delhi, pp. 198-201.
3. Ahuja, A.K., Mohapatra, S., Sharma D. and Awasthi, M.D. 1998. Monitoring of vegetables for pesticide residue contamination at harvest. (Eds) Reddy, P.P., Kumar, N.K.K Varghese. Advances in IPM for horticultural crops, Proceedings of the First National Symposium on Pest Management in Horticultural Crops: environmental implications and thrusts, Bangalore, India. 15-17 October-1997. pp 243-246.
4. Alagar, M. and Sivasubiramanian, P. 2006. Efficacy of botanicals, insecticides and their combination on major pests of bhendi (*Abelmoschus esculentus*). *Indian Journal of Entomology***,** 68(4): 369-374.
5. ANZFA. 2001a. Final assessment report application A378: Food derived from glyphosate-tolerant sugarbeet line 77 (GTSB77). Report No. A378, Australia New Zealand Food Authority, Canberra, Australia.
6. ANZFA. 2001b. Food derived from glyphosate-tolerant cotton line 1445 - A safety assessment. Australia New Zealand Food Authority, Canberra, Australia.
7. Arora, S. 2008. Analysis of insecticides in okra and brinjal from IPM and non-IPM fields. *Environmental Monitoring and Assessment*. DOI 10.1007/s10661-008-0272-z.
8. Astwood, J.D., Leach, J.N. and Fuchs, R.L. 1996. Stability of food allergens to digestion *in vitro. Nature Biotechnology*. 14:1269-1273.
9. Atwal, A. S. 1976. *Agricultural pests of India and Southeast Asia*. pp 502. Kalyani Publishers, New Delhi.
10. Bailey, L.H. 1947: Original habitat is probably South West Asia. *The Standard Cyclopedia of Horticulture*. New York. III: 3182.
11. Bangladesh Agricultural Research Institute. 1995. Annual Research Report 1993-94. Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh.
12. Barbera, P.W. 1995. Toxicity/pathogenecity testing of *Bacillus thuringiensis* strain EG 7826 following acute oral challenge in rats. Report No. IITRI Project Number L08574, IIT Research Institute, Chicago IL.
13. Beck, E., Ludwig, G., Auerswald, E.A., Reiss, B. and Schaller, H. 1982. Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon Tn5. *Gene.* 19(19): 327-336.

14. Beena-Kumari, Madan, V.K., Singh, J., Singh, S. and Kathpal, T. 2004. Monitoring of pesticidal contamination of farmgate vegetables from Hisar. *Environmental Monitoring and Assessment.* 90(1/3):65-71.

15. Berberich, S.A., Leimgruber, R.M. and Regan, G.J. 1993. Preparation and verification of dose for a mouse acute oral toxicity study with neomycin phosphotransferase II protein (NPTII), study ML-91-409. Unpublished. Monsanto Report No. MSL: 13277. Monsanto Technical Report.

16. Betz, F.S., Hammond, B.G. and Fuchs, R.L. 2000. Safety and advantages of *Bacillus thuringiensis*- protected plants to control insect pests. *Regulatory Toxicology and Pharmacology.* 32: 156-173.

17. Bharadiya, A.B. and Patel, B.R. 2005. Study on the efficacy of ceratin insecticides against brinjal shoot and fruit borer *Leucinodes orbonalis* Guenee. *J. Plant Prot. Environ.,* 2(1): 113-115.

18. Broderick, N.A., Raffa, K.F., Handelsman, J., 2006. Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. *Proceedings of the National Academy of Sciences of the United States of America*, 103: 15196 -15199.

19. Broderick, N.A., Robinson, C.J., McMahon, M.D., Holt, J., Handelsman, J., Raffa, K.F., 2009. Contributions of gut bacteria to *Bacillus thuringiensis*-induced mortality vary across a range of Lepidoptera. *BMC Biology*, 7: 1-9.

20. Butani, D.K. and Jotwani, M.G. 1984. Insects in vegetables. pp 356. Periodical Expert Book Agency, Delhi, India.

21. Calgene, I. 1990. Request for advisory opinion- kan, gene: safety and use in the production of genetically engineered plants. Report No. FDA Docket Number 90A-0416.

22. Carter, J.N. and Ligget, M.P. 1994. Acute oral toxicity and infectivity/pathogenicity to rats of EG 7841. Report No. HRC Study Report number ECO 6/942538, Huntingdon Research Centre Ltd., Huntington Cambridgeshire England.

23. CAST. 2006. Safety of Meat, Milk, and Eggs from animals fed crops derived from Modern biotechnology. Animal agriculture's future through Biotechnology, Issue Paper 34.*Council for Agricultural Science and Technology*, USA.

24. Central Insecticides Board and Registration Committee (CIBRC). 2008. Ministry of Agriculture, Government of India. http://cibrc.nic.in/.

25. Chandrasekaran, S., Kuttalam, S. and Regupathy, A. 1997. Investigation on the level of contamination of insecticide residues in ready to market vegetables in Tamil Nadu. *Vegetable Science.* 24(2): 136-139.

26. Chen, M., Liu, Z.C., Ye, G.Y., Shen, Z.C., Hu, C. and Peng, Y.F. 2007. Impacts of transgenic cry1Ab rice on non-target plant hoppers and their

main predator *Cyrtorhinus lividipennis* (Hemiptera: Miridae) – a case study of the compatibility of Bt rice with biological control. *Biological Control.* 42: 242–50.

27. Chitra,G. A., Muraleedharan, V.R., Swaminathan, T. and Veeraraghavan, D. 2006. Use of pesticides and its impact on health of farmers in South India. *International Journal of Occupational and Environmental Health.* 12 (3): 228.

28. Choudhury, B. and Anothai-Choomsai, M.L. 1970. *Indian Journal of Agricultural Science*. 40:805-812

29. Choudhary, B. 1976. Evolution of Crop Plants. Ed. Simmonds, N.W. pp. 278-9. Longman Inc., London and New York.

30. Choudhury, B. 1971. Research on eggplants in India. Food Foundation/IITA/IRAT Seminar on vegetable crops research, Ibadan, Nigeria.

31. Coghlan, A. 2000. For the moment, the gene genie is staying in its bottle. *New Scientist.* 25.

32. Daly, T., Buntin, G.D. 2005. Effect of Bacillus thuringiensis transgenic corn for lepidopteran control on nontarget arthropods.*Environmental Entomology,* 34:1292 –1301.

33. D'Arcy,W.G. and Pickett, K. 1991. Salt water floatation of *Solanum* fruits and possible dispersal of eggplant. *Solanaceae Newsl.* 3:3 11

34. Delaney, B., Astwood, J.D., Cunny, H., Conn, R.E., Herouet-Guicheney, C., MacIntosh, S., Meyer, Privalle, L., Gao, Y.,Mattson, J. and Levine, M. 2008. ILSI International Food biotechnology Committee Task Force on Protein Safety Evaluation of protein safety in the context of agricultural biotechnology. *Food Chem. Toxicol.* 46:S71-S97.

35. Dhankhar, B. S., Kishore, N., Sharma, D. R. and Pandita, M. L. 1982. Susceptibility of *Solanum khasianum* to brinjal shoot and fruit borer (*Leucinodes orbonalis* Guen.). *Journal of Research Haryana Agricultural University*, **12**(3): 523-524.

36. Donegan, K.K., C.J. Palm, V.J. Fieland, L.A. Porteous, L.M. Ganio, D.L. Schaller, L.Q. Bucao, and R.J. Seidler. 1995. Changes in levels, species and DNA fingerprints of soil microorganisms associated with cotton expressing the *Bacillus thuringiensis* var. *kurstaki* endotoxin. *Appl. Soil Ecol*. 2:111–124.

37. Duara, B., Baruah, A.A.L.H., Deka, S.C. and Barman, N. 2003. Residues of cypermethrin and fenvalerate on brinjal. *Pesticide Research Journal.* 15(1): 43-46.

38. EFSA. Safety and nutritional assessment of GM plants and derived food and feed: The role of animal feeding trials. 2008. *Food and Chemical Toxicology*, 46: S2–S70.

39. English, L. and Slatin, S.L. 1992. Mode of action of delta-endotoxins from *Bacillus thuringiensis*: A comparison with other bacterial toxins. *Insect Biochemistry and Molecular Biology.* 22: 1-7.

40. FAOSTAT. 2007. http://www.fao.org

41. Fári, M., Nagy, I., Csányi, M., Mitykó, J. and Andrásfalvy, A. 1995. *Agrobacterium* mediated genetic transformation and plant regeneration via organogenesis and somatic embryogenesis from cotyledon leaves in eggplant (*Solanum melongena* L. cv. 'Kecskeméti lila'). *Plant Cell Reports.* 82-86.

42. FDA. 1994. Secondary direct food additives permitted in food for human consumption; food additives permitted in feed and drinking water of animals; aminoglycoside 3' phosphotransferase II. Federal Register 59:26700-26711.

43. Fischhoff, D., Bowdish, K., Perlak, F., Marrone P., McCormick, S., Niedermayer, J., Dean D., Kusano-Kretzmer, K., Mayer, E., Rochester, D., Rogers S., and Fraley, R. 1987. Insect tolerant transgenic tomato plants. *Bio-Technology*. 5: 807-813.

44. Fischhoff, D.A., Bowdish, K.S., Perlak, F.J., Marrone, P.G., McCormick, S.M., Niedermyer, J.G., Dean, D.A., Kusana-Kretzmer, K., Mayer, E.J., Rochester, D.E., Rodgers, S.G., and Fraley, R.T. 1987. Insect tolerant transgenic tomato plants. *Biotechnology* 5: 807-813.

45. Flachowsky, G., Chesson, A. and Aulrich, K. 2005. Animal nutrition with feeds from genetically modified plants. *Archives of animal nutrition.* 59(1): 1-40.

46. Flavell, R.B., Dart, E., Fuchs, R.L., and Fraley, R.T. 1992. Selectable Marker Genes: Safe for Plants? *Biotechnology* 10:141-144.

47. Fuchs, R. L., Heeren, R.A., Gustafson, M.E., Rogan, G.J., Bartnicki, D. E., Leimgruber, R.M., Finn, R.F., Hershman, A, and Berberich, S.A. 1993b. Purification and characterization of microbially expressed neomycin phosphotransferase II (NPTII) protein and its equivalence to the plant expressed protein. *Biotechnology (NY).* 11:1537-1542.

48. Fuchs, R. L., Ream, J.E., Hammond, B.G., Naylor, M.W., Leimgruger, R.M., and Berberich, S.A. 1993c. Safety assessment of the neomycin phosphotransferase II (NPTII) protein. *Biotechnology (NY).* 11:1543-1546.

49. Fuchs, R.L. and Astwood, J.D. 1996. Allergenicity assessment of foods derived from genetically modified plants. *Food Technology*. 50:83-88.

50. Fuchs, R.L., Berberich, S.A., Serdy, F.S. 1993a. Safety evaluation of genetically engineered plants and plant products: insect resistant cotton. pp 199-212. In: JA Thomas, LA Myers, eds. *Biotechnology and Safety Assessment*. Raven Press Ltd, New York.

51. Goswami, M.M., Deka, K..C. and Bhattacharya,D. 2002. Persistence of Triazophos in/on brinjal and lady's finger. Journal of the Agricultural Science Society of North East India. 15(1): 89-92.]

52. Goswami-Giri, A.S. 2007. Monitoring of Pesticidal Contamination of Vegetables from Maharashtra. Ed: V. S. Burkule, Seminar on Contaminants in Food and Beverages. October 06, 2007. Venue: Thorale Bajirao Peshwe Sabhagruha, 'Jnanadweepa', Thane College Campus, Chendani, Bunder Road, Thane (W) 400 601.

53. Graham Head *et al.,* 2002**,** No detection of Cry1Ac protein in soil after multiple years of transgenic Bt cotton (bollgard) use. *Environmental entomology*. 31(1): 30-36

54. Hammond, B. and Cockburn, A. 2008. The safety assessment of proteins introduced into crops developed through agricultural biotechnology; a consolidated approach to meet current and future needs in Agricultural Biotechnology. Hammond, B. (Edit.). pp. 259-288. CRC Press, Taylor and Francis Group, Boca Raton.

55. Handique, A.K. 1986. Breeding behavior of *Solanum khasianum* Clarke, *Euphytica* 35, 631.

56. Head, G., Moar, M., Eubanks, M., Freeman, B., Ruberson, J. and Hagerty, A. 2005. A multiyear, large-scale comparison of arthropod populations on commercially managed Bt and non-Bt cotton fields. *Environmental Entomology*. 34:1257–66.

57. Hoffmann, C., Vanderbruggen, H. V., Hofte, H., Van Rie, J., Jansens, S., and Van Mellaert, H. 1988. Specificity of *B. thuringiensis* delta-endotoxins is correlated with the presence of high affinity binding sites in the brush border membrane of target. insect midguts. *Proc. Natl. Acad. Sci. USA.* 85: 7844-7848.

58. Hooker. 1885. Flora of British India: “De Candolle says it is a native of Asia, not America, and Sendtner fixes its origin in Arabia; all this appears uncertain”.

59. http://www.ikisan.com;

60. http://www.tribuneindia.com/2001/20010611/agro

61. Jena, B.C., Srihari, B. and Mohapatro, R. 2005. Pesticidal mamnagement practices to control brinjal shoot and fruit borer *Leucinodes orbonalis* Guenee. *J. Plant Prot. Environ.* 2(2): 141-146.

62. Kabir, Roul, F.M.A., Islam, M.N., and Malaker, P.K. 1996. Efficiency of different insecticides in controlling brinjal fruit and shoot borer. *Leucinodes orbonalis* Guen. *University Journal of Zoology, Rajasthan University*, *Udaipur.* 13: 1-8.

63. Kale, V.D., Rane, S.D. and Dethe, M.D. 1997. Dissipation of quinalphos on brinjal fruits. Plant Protection Bulletin, Faridabad. 49(1/4): 4-5.

64. Karim, S., Riazuddin, S., Gould, F. and Dean, D.H. 2000. Determination of receptor binding properties of *Bacillus thuringiensis* delta-endotoxins to cotton bollworm (*Helicoverpa zea*) and pink bollworm (*Pectinophora gossypiella*) midgut brush border membrane vesicles. *Pesticide Biochemistry and Physiology.* 67:198-216

65. Kaur, R. 2008. Assessment of Genetic Damage in Workers Occupationally Exposed to Pesticides in Various Districts of Punjab, Department of Human Biology, Punjabi University, Patiala.

66. Kay, R., Chan, A., Daly, M. and McPherson, J. 1987. Duplication of CaMV 35S promoter sequences creates a strong enhancer for plant genes. *Science*. 236: 1299-1302.

67. Keck, P.J. and Mitsky, T.A. 1994. Comparative alignment of insecticidally-active *Bt.k.* HD-73 protein (*Batik.* protein) to known allergenic and toxic proteins using the FASTA algorithm. An unpublished study conducted by Monsanto Company. *Monsanto Technical Report.* MSL-13643 , St. Louis, MO, USA.

68. Khan, R. 1979. In: The Biology and Taxonomy of the Solanaceae Eds. J.G. Hawkes, R.N. Lester and A.D. Skelding. pp. 629-636. Academic Press. London.

69. Kimber, I., Kerkvliet, N.L., Taylor, S.L., Astwood, L.D., Salro, K. and Dearman, R.J. 1999. Toxicology of protein allergenicity: prediction and characterization. *Toxicological Sciences*. 48:157-162.

70. Knowles, B.H. and Dow, J.A.T. 1993. The crystal delta-endotoxins of *Bacillus thuringiensis*: models for their mechanism of action on the insect gut. *Bioassays.* 15: 469-476.

71. Lalnuntluanga, J. and Singh, H.K. 2008. Performance of certain chemicals and neem formulations against ginger shoot borer (*Dichocrocis punctiferalis* Guen.). *Indian J. Ent.,* 70(2): 183-186.

72. Lawo, N.C., Wackers, F.L., Romeis, J. 2009. Indian Bt Cotton Varieties Do Not Affect the Performance of Cotton Aphids. PLoS ONE 4(3):e4804. doi:10.1371/journal. pone.0004804

73. Lester, R.N. and Hasan, SIvIZ. 1991. Origin and domestication of the brinjal egg-plant, *Solanum melongena*, from *S. incanum*, in Africa and Asia. In: Hawkes, J.G., Lester, R.N., Nees, M. and Estrada, N. (eds) Solanaceae III. taxonomy, chemistry, evolution. pp 369-387. *Royal Botanic Gardens*, Kew.

74. Lorenz, E.S. 2009. UO198, The Pennsylvania State University.

75. Mancini, F., Van Bruggen, A.H., Jiggins, J.L., Ambatipudi, A.C. and Murphy, H. 2005. Acute pesticide poisoning among female and male cotton growers in India. *International Journal of Occupational and Environmental Health.* 11 (3): 221-33.

76. Mendelsohn, M., Kough, J., Vaituzis, Z. and Matthews, K. 2003. Are *Bt* crops safe. *Nature Biotechnology*. 21(9): 1003-1009.

77. Metcalfe, D.D., Astwood, J.D., Townsend, R., Sampson, H.A., Taylor, S.L. and Fuchs, R.L. 1996. Assessment of the allergenic potentials of foods derived from genetically engineered crop plants. *Critical Reviews in Food science and Nutrition.* 36(S): S165-S186.

78. Mishra, N.C. and Dash, D. 2007. Evaluation of synthetic and neem based pesticide schedules against shoot and fruit borer (*Leucinodes orbonalis* Guenee) on brinjal. *J. Plant Prot. Environ.* 4(2): 93-96.

79. Mishra, N.C., Ram, S., Swain, S. and Rath, S. 2004. Effect of insecticides and bioproducts on shoot and fruit borer incidence in brinjal. *Journal of Research (BAU)*, 16(2):257-260.

80. Morandin, L.A. and Winston, M.L. 2003. Effects of novel pesticides on bumble bee (Hymenoptera: Apidae) colony health and foraging ability. *Environmental Entomology*. 32: 555–563.

81. Naik, V.C.B., Arjunarao, P., Krishnayya, P.V. and Srinivasarao, V. 2008. Seasonal incidence and management of *Leucinodes orbonalis* Guenee on brinjal. *Ann. Pl. Protec. Sci.,* 16:329-332.

82. Naranjo, S.E. 2005. Long-term assessment of the effects of transgenic Bt cotton on the abundance of nontarget arthropod natural enemies. *Environmental Entomology*. 34:1193–1210.

83. National Centre for Integrated Pest Management (NCIPM). 2006. Extension Folder, Integrated Pest Management Studies for Okra and Brinjal, New Delhi. http://www.ncipm.org

84. Naylor, M.W. 1992. Acute oral toxicity study of beta-glucuronidase (GUS) protein in albino mice. Unpublished. Report No. Monsanto Report No. MSL: 12485, Monsanto Company, St. Louis.

85. Naylor, M.W. 1993a. One month feeding study with insect-resistant cottonseed meal in Sprague-Dawley rats. Monsanto Company The Agricultural Group, St Louise Missouri USA.

86. Naylor, M.W. 1993b. Acute oral toxicity study of *Bacillus thuringiensis* var. *kurstaki* [Cry1Ac] HD-73 protein in albino mice. The Agricultural Group Monsanto Company, St Louise Missouri USA.

87. Nielsen, K.M., Bones, A.M., Smalla, K. and Van Elsas, J.D. 1998. Horizontal gene transfer from transgenic plants to terrestrial bacteria - a rare event? *FEMS Microbiology Reviews.* 22: 79-103.

88. Nielsen, K.M., Gebhard, F., Smalla, K., Bones, A.M. and Van Elsas, J.D. 1997. Evaluation of possible horizontal gene transfer from transgenic plants to the soil bacterium *Acinobactor calcoaceticus* BD 413. *Theoretical and Applied Genetics.* 95: 815-821.

89. Nisha Kumari, Shahi, D.K., Sharma, A. and Sarkar, A.K. 2005. Occurrence of pesticide residue in farmgate samples of vegetables. *Journal of Research, Birsa Agricultural University*. 17(1): 11-14.

90. Odell, J.J., Nagy, F. and Chua, N.H. 1985. Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. *Nature.* 313:810-812.

91. Palm, C.J., Shcaller, D., Donegan, K.K. and Siedler, R.J. 1996. Persistance in sol of transgenic plant produced *Bacillus thuringiensis var kurstaki* Delta- endotoxin. *Canadian Journal of Microbiology*. 42:1258-1262.

92. Pariza, M.W., and Johnson, E.A. 2001. Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century. *Regul. Toxicol. Pharmacol.* 33:173-186.

93. Patel, B.A., Shah, P.G., Raj, M.F., Patel, B.K., Patel, J.A. and Talati, J.G. 1999. Chlorpyriphos residues in/on cabbage and brinjal. *Pesticide Research Journal*. 11(2): 194-196.

94. Pesticides Manufacturers and Formulators Association of India (PMFAI). 2008. MRL Values of Pesticides on Food Commodities. http://www.pmfai.org/stat.htm.

95. Picard-Nizou, A.L., Grison, R., Olsen, L., Pioche, C., Arnold, G. and Pham-Delegue, M.H. 1997. Impact of proteins used in plant genetic engineering: toxicity and behavioral studies in the honeybee. *J. Econ. Entomol.* 90: 1710-1716.

96. Prasad, S.S., Gupta, P. K. and Kanaujia, B.L. 2005. Efficacy and economics of promising granular insecticides against yellow stem borer on semi deep water rice. *Ann. Pl. Protec. Sci.,* 13:365-368.

97. Raj, M.F., Shah, P.G., Patel, B.K., Patel, B.A. and Patel, J.A. 1999. Dissipation of triazophos from brinjal and okra fruits. *Pesticide Research Journal.*11(1): 102-105.

98. Rajeswaran, J., Merlinkamala, I., Chandrasekaran, S., Jayakumar, R. and Kuttalam, S. 2004. Harvest time residues of carbosulfan in brinjal fruits. *Journal of Food, Agriculture and Environment.* 2(2): 276-277.

99. Rao N. 1979. The barriers to hybridization between *Solanum melongena* and some other species of Solanum, in The Biology and Taxonomy of the Solanaceae, Hawkes, J. G., Lester, R.N. and Skelding, A.D., Eds. *Academic press, NY* pp 605.

100. Ream, J. E. 1993. Assessment of degradation of neomycin phosphotransferase II in *in vitro* mammalian digestion models. An unpublished study conducted by Monsanto Company, Monsanto Study IRC-91-ANA-06, St. Louis, MO, USA.

101. Ream, J.E. 1994. Assessment of the *in vitro* digestive fate of *Bacillus thuringiensis* var. *kurstaki* HD-73 protein. A study conducted by Monsanto Company, Monsanto Study MSL-13299, St. Louis, MO.

102. Ream, J.E., Sims, S.R. and Leach, J.N. 1994. Aerobic soil degradation of *Bacillus thuringiensis var kurstaki* HD-73 protein bioactivity, Monsanto Company Lab Project, MSL No.13267, St. Louis.

103. Reddy, D.D.R. and Rao, A.P. March, 2000. Acharya N.G. Ranga Agricultural University Information Bulletin Number 11. pp 58. Hyderabad.

104. Reddy, D.J., Rao, B.N., Sultan, M.A. and Reddy, K.N. 1998. Pesticide residues in farmgate vegetables. *Journal of Research ANGRAU*. 26(3/4): 6-10. College of Agriculture, Rajendranagar, Hyderabad.

105. Rodrigo-Simon. A., de Maagd R.A., Avilla, C., Bakker, P., Molthoff, J., Gonzales-Zamora, J.E., Ferre, J. 2006. Lack of detrimental effects of Bacillus thuringiensis Cry toxins on the insect predator Chrysoperla carnea: a toxicological, histopathological, and biochemical analysis. *Applied and Environmental Microbiology*. 72:1595–603.

106. Rogers, S.G., O'Connell, K., Horsch, R.B. and Fraley, R.T. 1985. Zaitlin, M., Day, P., Hollaender, A. and Wilson, C.A. (eds.). *Biotechnology in plant science.*Academic Press Inc, New York. 219-226.

107. Rupa, D.S., Reddy, P.P. and Reddi, O.S. 1991. Reproductive performance in population exposed to pesticides in cotton fields in India. *Environ Res.* 55 (2):123-8.

108. Sachan, S.K., Singh, D.V. and Chaudhary, A.S. 2006. Field evaluation of insecticides against rice stem borer and leaf folder. *Ann. Pl. Protec. Sci.,* 14:469-470.

109. Sambandam, C.N. (1962). Natural cross pollination in eggplant, *Econ. Bot.*, 18:128-131.

110. Sandanayake, W. R. M. and Edirisinghe, J. P. 1992. *Trathala flavoorbitalis*: parasitization and development in relation to host-stage attacked. *Insect Sciences and its Applications,* **13**(3): 287–292.

111. Schlüter, K., Fütterer, J. and Potrykus, I. 1995. Horizontal gene transfer from a transgenic potato line to a bacterial pathogen (*Erwinia chrysanthemi*) occurs- if at all- at an extremely low frequency. *Bio/Technology*. 13: 1094-1098.

112. Schuler, M.A., Schmitt, E.S. and Beachy, R.N. 1982. Closely related families of genes code for the alpha and alpha' subunits of the soybean 7S storage protein complex. *Nucleic Acids Res.* 10: 8225-8261.

113. Sharma, K.K. 2007. Pesticide Residue Analysis Manual, Indian Council of Agricultural Research (ICAR), New Delhi.

114. Shitole, D.M. March, 2005. Plant Protection Agrochemicals in Indian Market, pp 177-178. Akash Krishi Prakashan. Pune.

115. Sims, S. R. 1995. *Bacillus thuringiensis var. kurstaki* (Cry1A(c) protein expressed in transgenic cotton: Effects on beneficial and other non-target insects. *Southwestern Entomologist.* 20: 493-500.

116. Singh, I.P. and Mukherjee, U. 1992. Persistence and degradation of fenvalerate in/on brinjal [aubergine] fruits. *Journal of Research, Birsa Agricultural University,* 4(2): 129-132.

117. Singh, S. and Sharma, N. 2000. Neurological syndromes following organophosphate poisoning. *Neurol India.* 48 (4):308-13.

118. Singh, S., Ranjit, A., Parthasarathy, S., Sharma, N. and Bambery P. 2004. Organo-phosphate induced delayed neuropathy: report of two cases. *Neurol India.* 52 (4):525-6.

119. Srinivas, P., Ramesh Babu, T., Sultan, M.A., Reddy, D.D.R and Gour, T.B. 1996. Dissipation and decontamination of monocrotophos, fenvalerate and carbaryl in/on okra. *Indian Journal of Plant Protection.* 24 (1&2): 86-89.

120. Srivastava, K. P. and Butani, D. K. 1998. *Pest management in vegetables*, Part 1. pp 294. Research Periodical and Book Publishing House. Houston, USA.

121. Stotzky, G. 2000. Persistence and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis* and of bacterial DNA bound on clays and humic acids. *Journal of Environmental Quality* 29: 691-705.

122. Syvanen, M. 1999. In search of horizontal gene transfer. *Nature Biotechnology* 17: 833.

123. Taylor, S.L. and Lehrer, S.B. 1996. Principles and characteristics of food allergans. Critical Reviews in Food Science and Nutrition. 36:S91-S118.

124. Tewari, G.C. and Sardana, H.R. 1987. An unusual heavy parasitisation of the brinjal shoot and fruit borer, *Leucinodes orbonalis* Guen, by a new braconid parasite. *Indian Journal of Entomology.* 52: 338–341.

125. US FDA. 1998. Guidance for industry: use of antibiotic resistance marker genes in transgenic plants.1-26. http://vm.cfsan.fda.gov/~dms/opa-armg.html.

126. Van Rie, J., Jansens, S., Höfte, H., Degheele, D., and Mellaert, H.V. 1990. Receptors on the brush border membrane of the insect midgut as determinants of the specificity of *Bacillus thuringiensis* delta-endotoxins. *Appl. Environ. Microbiol.* 56: 1378-1385.

127. Van Rie, J., Jansens, S., Hofte, H., Degheele, D., and Van Mellaert, H. 1989. Specificity of *Bacillus thuringiensis* endotoxins: importance of specific receptors on the brush border membrane of the mid-gut of target insects. *Eur. J. Biochem.* 186: 239-247.

128. Vishwanathan, T.V. 1975. On the occurance of natural hybridization between *Solanum incanum* L. and *Solanum melongena* L. *Current Science.* 44: 134.

129. Watt. 1908. Watt does not consider the eggplant to be native of India. The Commercial Products on India, 1966, New Delhi (Cited by Khan, 1979).

130. Wolfenbarger, L.L., Naranjo, S.E., Lundgren, J.G., Bitzer, R.J., Watrud, L.S. 2008. Bt crop effects on functional guilds of non-target arthropods: a meta-analysis. PLoS ONE 3(5): e2118 18461164.e2118. doi:10.1371/journal.pone.0002118.

*****