

Bt Brinjal Event EE1

Bt Brinjal: The scope and adequacy of the GEAC environmental risk assessment

DAVID A. ANDOW
DEPARTMENT OF ENTOMOLOGY
UNIVERSITY OF MINNESOTA



Bt Brinjal:

The scope and adequacy of the GEAC environmental risk assessment

Contents	Page
Preface	ii
Summary and Conclusion	1
Context and Need	8
Characterisation of Transgene	15
Inserted transgene	16
Transgene expression	18
Product quality of EE-1 Bt brinjal	20
Scientific opinion on regulatory compliance	21
Environmental Risk Assessment	22
Gene flow and its consequences	23
Risks to biological diversity	31
Resistance risk in target species	38
Socioeconomic Analysis	47
Profitability – utilitarian analysis	47
Economic security – utilitarian analysis	57
Health, religion and culture	58
Large-Scale Indirect Socioeconomic Effects	58
Sequencing Needed Risk Assessment Activities	60
About the Author	64
References Cited	69

List of Boxes

Box	Page
Box 1. Backcrossing and Transgene Characterisation	16
Box 2. Surrogate Proteins	18
Box 3. Why the experimental gene flow estimates from Bt brinjal may be underestimated	23
Box 4. Taxonomy of Solanum melongena L.	26
Box 5. Some important synonyms	27
Box 6. Taxa known to form fertile hybrids with Solanum melongena L.	28
Box 7. The Root Rhizosphere	34
Box 8. Scientific Validity	35
Box 9. Adverse Consequences of Resistance and Benefits of Managing Resistance	39
Box 10. Definition of Resistance	40
Box 11. High Dose and Low Dose	42
Box 12. Brinjal Yield Gap: Overestimation of Yield Benefit of Bt Brinjal	49

Preface

On 14 October 2009 the Bt Brinjal Expert Committee II Report (EC-II), dated 8 October 2009 was tabled at the 97th Meeting of the apex Regulator, the GEAC (Genetic Engineering Appraisal Committee). The GEAC accepted the recommendations of the EC-II and approved the commercialisation of Bt brinjal, Event EE1 developed by: The Maharashtra Hybrid Seeds Company Ltd. (Mahyco), Mumbai, a subsidiary of Monsanto Company, and the University of Agricultural Sciences (UAS), Dharwad and Tamil Nadu Agricultural University (TNAU), Coimbatore.

On 15 October 2009, the MOS, (Minister of State), Independent Charge, (MoEF (Ministry of Environment and Forests), the Honourable Shri Jairam Ramesh, intervened to stop the approval process of Bt brinjal because of the nationwide criticism of the EC-II and the apparent haste with which the GEAC gave its assent. The Minister instituted a process by which he invited documented responses to the EC-II. The Minister also contacted the United States National Academy of Sciences (US-NAS) for a scientific evaluation of the EC-II. The research arm of the US-NAS organised and solicited comment from a small group of about 10 noted US scientists, including the author of this report, who are internationally-acknowledged experts on the environmental risks of genetically engineered crop plants. The US-NAS is held in great esteem by its ability to generate balanced, objective scientific comment to complex social controversies, injecting logic and structure to help policy makers better understand the issues and take more reasoned decisions. The author of this report along with other solicited scientists submitted such comments to the Minister, which were duly included among the responses he received to his invitation.

Minister Jairam Ramesh's initiative is unique, and the global response he received is also unique. The global scientific community responded to his invitation with an outpouring of comment, significant both in number and quality. Together, these provided a searching critique of Mahyco's Bt brinjal bio-safety dossier and the EC II Report. Such a response is astonishing because it is based purely on altruistic concerns – none of the commenting scientists received compensation for their efforts – but it reflects the importance of the implications of releasing the world's first major GM food crop, the brinjal and in a centre of diversity and origin of that crop.

On 9 February 2010, Jairam Ramesh declared a moratorium on the commercial approval of Bt brinjal, citing the need for further safety testing.

This Report is the outcome of a series of discussions with several scientists, at the request of Aruna Rodrigues, Lead

Petitioner to the Supreme Court, following the moratorium on Bt brinjal called by Minister Jairam Ramesh. The aim of those discussions was to provide a comprehensive appraisal of the bio-safety impacts of Bt brinjal and the regulatory protocols that were used by the GEAC. In a meeting in Delhi during 19-21 February 2010, it was decided that the author with the assistance of Dr. G. K. Veeresh would present an analyses of the EC-II assessment of the environmental effects of Bt brinjal. This is expected to be the first of two appraisals of EC-II to be produced during 2010. The other analysis is expected to address the human and animal health effects of Bt brinjal, and will be undertaken by other scientists. An initial draft of this report was completed in March 2010.

This report, "Bt Brinjal: The scope and adequacy of the GEAC environmental risk assessment," addresses the scientific issues related to the risk assessment and moratorium on Bt brinjal. The experiences of the author during the 19-21 February 2010 meeting in Delhi and subsequent study of the scientific literature relevant to the issue has deepened, broadened, and increased verisimilitude of the original comment submitted by the author to Minister Jairam Ramesh via the US-NAS. The value of the many scientific submissions to the Minister and subsequent deliberations will only be realised if they help guide the emergence of a comprehensive and accurate bio-safety regulatory protocol in India for Bt brinjal and other GM crops. This report is designed to examine and assess EC-II and associated materials to highlight areas where the regulatory protocols can be improved with respect to environmental impacts.

I would like to thank Dr. G. K. Veeresh, Association for the Promotion of Organic Foods, Bangalore, and formerly Vice Chancellor of the University of Agricultural Sciences, Bangalore for his many insightful observations about vegetable production in India, extensive comment on early drafts of this report and assistance in acquiring some of the publications cited herein. I also acknowledge with gratitude critical reviews of parts of this report from Professor Norman Ellstrand, University of California, Riverside, Professor Allison Snow, The Ohio State University, Dr. Douglas Gurian-Sherman, Union of Concerned Scientists, and Dr. Jack Heinemann, University of Canterbury. I thank Dr. Gabor Lövei, Danish Institute for Agricultural Science, University of Aarhus for stimulating discussions that helped my thinking about values and Professor Paul B. Thompson, Michigan State University for some comments that lead to the framing of this report. Finally, I thank Aruna Rodrigues whose efforts brought forth this report and whose comments helped focus and clarify it. All this attention considerably improved this report, but responsibility for its content and faults rests with the author.

David A. Andow
St. Paul, MN, USA
August, 2010

Summary and Conclusion

This report evaluates the scope and adequacy of the environmental risk assessment (ERA) for hybrid EE-1 Bt brinjal requested by the Genetic Engineering Approval Committee (GEAC) in response to the Maharashtra Hybrid Seeds Company Ltd., Mumbai (Mahyco) application for permission to commercialise hybrid EE-1 Bt brinjal. *The assessment is reported in the Report of the Expert Committee (EC-II) on Bt Brinjal Event EE-1.* Event EE-1 expresses a genetically engineered crystalline (Cry) protein toxin from the soil bacterium *Bacillus thuringiensis* Berliner (Bt). The Cry toxin is similar but not identical to Cry1Ac. It is a chimeric protein of Cry1Ac and Cry1Ab, and designated Ccry1A in this report. EE-1 was inserted into improved brinjal hybrids, which are modern cultivars of *Solanum melongena* L. [Solanaceae: *Leptostemonum*]. It was developed to control the brinjal fruit and stem borer, *Leucinodes orbonalis* Guenee [Lepidoptera: Crambidae], which is abbreviated BFSB in the rest of this report. This report does not address the potential risks of hybrid Bt brinjal as human food or animal feed, and does not fully analyse the need for, requirements of, and methodologies for post-commercialisation monitoring of hybrid Bt brinjal.

The report is based on an analysis of EC-II, the eight volumes of supporting information to the EC-II, which are referred to as the Dossier in the rest of this report, Supplemental Materials submitted to the GEAC by Mahyco, which comprise 13 technical reports, and publicly available scientific literature on Bt brinjal, BFSB and brinjal.

The main thesis of the report is that the GEAC set too narrow a scope for environmental risk assessment (ERA) of hybrid Bt brinjal, and it is because of this overly narrow scope that the EC-II is not an adequate ERA.

Context and Need

This report holds the EC-II environmental risk assessment (ERA) to the standard of assessing outcomes about concrete environmental risks of Bt brinjal, and does not dwell much on the more narrow concern of whether the EC-II ERA meets the regulatory standards of the GEAC. By doing so, this report points out where the scope of the EC-II ERA is too narrow and challenges the adequacy of the ERA framework established by GEAC and under which Bt brinjal has been assessed. At many points in this report, the EC-II is criticised not for whether it has accomplished what it set out to do, but whether it set out to do the right thing in the first place. Such errors of omission are sometimes invisible, especially when everyone focuses on potential errors of commission. However, these omissions are probably more critical for India, in that serious concerns are left unexamined.

Brinjal plays a unique role in Indian society, and it will be important to evaluate if and how Bt brinjal may affect these values. Brinjal is one of the most important vegetable crops in India, especially for the rural and urban poor. About 61% is grown in the three eastern states of West Bengal, Orissa and Bihar by small-scale resource-poor farmers. These States have banned the use of Bt brinjal. India is the centre of the world's biological diversity in brinjal with over 2500 varieties grown in the country. Some local varieties have significant religious and cultural value.

EE-1 Bt brinjal is proposed as a hybrid, and is unlikely to fit well in the small-scale production systems relying on open-pollinated varieties (OPVs) of brinjal. For small-scale resource-poor farmers it is critical for creating economic security, primarily by facilitating cash flow, insuring against income variation from other crops, and providing families with needed food and nutrition. It may fit in large-scale commercial brinjal production systems that already use brinjal hybrids. Brinjal is produced for net income by large-scale commercial producers.

Brinjal fruit and shoot borer (BFSB) causes significant economic damage to brinjal throughout India for all farmers, probably averaging about 30% yield loss (a comparison of yield with pest management versus no pest management). Its perceived significance by farmers often lead them to overestimate the loss to BFSB, precipitating an over-use of insecticides significantly beyond that justified by the actual economic significance of BFSB. Real losses from BFSB are higher for large-scale commercial producers than for small-scale resource-poor producers because of the way damaged fruit can be used by the small-scale resource-poor farmers. EC-II vastly over-estimated losses to BFSB.

Some management for BFSB is practiced by all brinjal farmers. Most farmers overuse synthetic insecticides by a considerable amount. Insecticide use can be reduced substantially using integrated pest management (IPM). Useful alternative production systems for control of BFSB are being tested, actively used, and promoted in India. These include IPM, traditional pest management, organic production and other locally-derived methods that reduce costs associated with external inputs.

Main Conclusion:

The potential advantages of hybrid EE-1 Bt brinjal seem marginal and uncertain for most Indian farmers, and the environmental risks (including socioeconomic risks) to Indian farmers and consumers remain very uncertain, despite efforts by the EC-II to assess these risks. The potential advantages can be better estimated by careful consideration of the context of different kinds of brinjal farmers in India. Several significant environmental risks have not been considered and nearly all of the others have been inadequately considered. These can be better characterised, eliminating the excessive uncertainty, and most of this can be done without releasing Bt brinjal into the field. Once these risks are better characterised, risk management measures may be developed to address the remaining uncertainties. Until the risks can be better understood or managed, there seems little reason to approve commercial use of hybrid Bt brinjal at this time.

Transgene Characterisation

Conclusion 1. The EE-1 Bt transgene needs to be characterised more fully 1) to demonstrate that there is only one transgene insert in Bt brinjal, 2) that the transgene expresses the intended Cry protein, 3) that it does not interrupt a functioning plant gene, 4) to provide expression levels in additional plant tissues, and 5) to demonstrate empirically that the marker genes are not expressed in Bt brinjal.

The description of the transgene in Bt brinjal EE-1 is inadequate to support environmental risk assessment. There is at least one transgene incorporated into EE-1 Bt brinjal. It is not known that there is only one transgene incorporated into the brinjal genome, that the one known inserted transgene expresses the intended gene product, or that the transgene does not interrupt a functional plant gene. Additional transgenes, expression of an unintended product and interruption of a plant gene could create additional environmental risks that have not been considered or assessed.

Adequate characterisation of expression of the transgene is essential for effective environmental risk assessment. The description of the chimeric Cry1A protein (Ccry1A) is inadequate for this purpose. The amino acid sequence of Ccry1A as expressed in brinjal is needed. The expression level of Ccry1A in pollen of Bt brinjal EE-1 is needed. There is no need to use Cry1Ac, a protein that is not identical to Ccry1A, in any the experiments used to support risk assessment, as was done in EC-II and the Dossier. The expression level of the marker genes is assumed to be low. This should be demonstrated empirically.

Conclusion 2. The EE-1 transgene may be a second-rate Bt brinjal product. EE-1 was probably produced in the late 1980s or early 1990s. More recently produced commercial transgenes have significant improvements over EE-1. EE-1 has relatively low control of BFSB, and other Cry toxins might perform better.

The EE-1 transgene does not kill nearly all young BFSB larvae. This is unlike other transgenes used to control other lepidopteran pests in the Crambidae, the same family as BFSB. Indeed all other target pests in the Crambidae are controlled by a Bt transgene with >99% mortality. Control of lepidopteran pests in other insect families is not as good, but it should be possible to get much better control of BFSB if the proper transgene were used. EE-1 is a very old transgene, and while it may not be exactly transgene dumping, India would do better to wait for a more efficacious transgene before seriously considering approval of Bt brinjal.

Conclusion 3. The EC-II assessment does not comply with scientific aspects of transgene characterisation described in the *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants* (Codex Alimentarius, 2003, CAC/GL 45-2003).

The GEAC would like to conclude that the EC-II report is in compliance with India's international obligations under all relevant treaties, but especially the Codex Alimentarius. The Codex has a key role in the World Trade Organization (WTO), and compliance to Codex would mean that India is compliant with related obligations in the WTO. The lack of compliance of EC-II to Codex highlights a serious deficiency in the EC-II assessment.

Environmental Risk Assessment

Conclusion 4. Most of the possible environmental risks of Bt brinjal have not been adequately evaluated; this includes risks to local varieties of brinjal and wild relatives, risks to biological diversity, and risk of resistance evolution in BFSB.

This conclusion is supported by the following three conclusions. Briefly, EC-II relied on dubious scientific assumptions, did not focus on realistic environmental concerns, inadequately evaluated some important environmental concerns, and ignored other real environmental concerns.

Conclusion 5. Brinjal has considerable valuable genetic diversity in India that could be threatened by gene flow. EC-II, the Dossier, and the Supplemental Materials are inadequate for concluding that Bt brinjal has no significant effects on the biological diversity or weediness of brinjal or its wild relatives.

EC-II and the Dossier argued that Bt brinjal does not outcross enough to create environmental risks, but this is dubious. It is highly likely that cultivated brinjal (*Solanum melongena*), including local varieties and landraces have crossed with feral populations of *S. melongena*, and it is possible that cultivated varieties can revert to wild phenotypes and establish feral populations. Therefore, the possible effects of intraspecific gene flow from Bt brinjal to other varieties and populations of brinjal should be examined. In addition, there is likely to be natural crossing between Bt brinjal and wild species related to brinjal. Hybridisation with perhaps as many as 29 wild relative species needs to be evaluated carefully and the consequences of any hybridisation that occurs needs to be evaluated.

There is insufficient evidence that 1) wild or weedy relatives of brinjal would not obtain a fitness benefit from a Bt transgene should gene flow occur; 2) wild relatives of brinjal will not suffer reduced genetic diversity from the introgression of the Bt transgene; and 3) non-GM brinjal will remain uncontaminated by Bt brinjal. All of these risks need to be evaluated.

Conclusion 6. Most of the main kinds of possible adverse effects of Bt brinjal on biological species diversity and ecosystems have not been evaluated sufficiently. The scientific evidence does not support the EC-II conclusion (page 42), Bt brinjal event EE-1 “has no adverse impact on non-target organisms including beneficial organisms and soil microflora.”

There are six kinds of potential adverse environmental effects that Bt brinjal could have on biological diversity. These are: increased secondary pests, either through direct enhancement or indirectly through the reduction of natural enemy controls or other means; reduction in soil quality or health, adversely affecting crop production in either the short or long term; reduced value of non-crop economic activities (such as honey production or wild food harvesting); reduced cultural value by affecting a cultural icon or a species of cultural significance (e.g., Monarch butterfly in the United States); increased conservation concern, such as an adverse effect on an endangered species; and reduced environmental quality through an effect on an ecosystem service such as pollination.

EC-II argues that most species are not at risk from Bt brinjal because the Ccry1A protein has a narrow toxicity spectrum. By doing so, EC-II ignores the considerable scientific uncertainty about the toxicity spectrum of Ccry1A, and the over-reliance on this assumption (EC-II, page 37) is not scientifically justified. In addition, Bt brinjal can have effects not directly related to toxicity, and these potential indirect environmental effects may be more significant.

EC-II also draws conclusions about the absence of environmental risk in the absence of supporting data. For example, EC-II (page 41) concludes “there is no accumulation of the [Bt] protein in the soil associated with production of Bt brinjal,” but this is not supported by any scientific data.

Many of the experiments submitted by Mahyco in the Dossier do not address real concerns for India. Only 1 of the 7 species tested in laboratory bioassays to assess environmental risk even occur in brinjal fields in India. These laboratory tests provide little relevant information about the potential impact of Bt brinjal on species in India, and therefore do not assess any real concern in India. The soil studies were not designed to evaluating effects on soil health, brinjal productivity, or the productivity of other crops grown by brinjal farmers, which are the real concerns for Indian farmers. Instead, they measure microbial populations, which have little relevance for assessing these real concerns.

EC-II does examine, in part, one of the six potential adverse effects of Bt brinjal on biological diversity. The multi-site field trials (MST) report data on the risk of potential non target pests. EC-II, however, overstates conclusions based on the limited and highly variable data. The MST data suggest that phytophagous mites will not become secondary pests of Bt brinjal, but the data are insufficient to draw conclusions about whether the other important non-target pests will or will not become secondary pests. The EC-II assessment of effects on species of conservation concern, species of cultural concern, other economically significant species (such as silkworm), and other ecosystem services is incomplete or insufficient.

Conclusion 7. The evolution of resistance in BFSB to overcome Bt brinjal is a real risk that must be managed. EC-II does not acknowledge this risk, and the Dossier does not propose effective means to manage it.

Any major pest control practice will select for resistant individuals in the target pest population. If enough individuals become resistant, the control fails, the pest becomes abundant and crop yields decline. The evolution of resistance to Bt crops is a real risk and is treated as such throughout the world.

Event EE-1 Bt brinjal poses several unique challenges because the likelihood of resistance evolving quickly is high. Without any management of resistance evolution, Bt brinjal is projected to fail in 4-12 years. Effective use of 20% non-Bt refuges can extend this time by 25% or more. The reason for this high risk is that EE-1 is a “low dose” event, by virtue of the relatively low control efficacy already mentioned under conclusion 2. It will require extra effort to delay resistance substantially. Resistance risk would be substantially reduced if EE-1 Bt brinjal were never released commercially and India were to wait for a “high-dose” Bt brinjal. Resistance to EE-1 would be a stepping stone for more rapid resistance in any subsequent, improved event.

The Dossier presents a plan to delay resistance in BFSB. EC-II makes no reference to this plan. The plan in the Dossier is purely voluntary and contains no incentives for farmers to comply voluntarily. In the plan, only a voluntary non-Bt refuge is likely to delay resistance evolution, but farmers are unlikely to comply. The planned education program to encourage adoption of non-Bt refuges is aimed both to ensure sales of Bt brinjal seed and affect the risk of resistance. Farmers will likely hear this mixed message and cooperate in undermining the voluntary plan. New approaches for resistance management must be developed, and some strategies are suggested.

Resistance monitoring in BFSB should begin as soon as possible, initiated first in the large-scale commercial production regions where hybrid Bt brinjal may first be adopted, and then expand to include rural areas at risk.

Socioeconomic Analysis

Conclusion 8. Hybrid Bt brinjal may increase profitability for large-scale, commercial brinjal producers by at most Rs.23,439/ha. For these producers, a reduction of 6.5 insecticide applications may also occur, but this potential benefit may be unrealised if secondary pests arise. Small-scale resource-poor farmers may improve profit by only Rs.3,250/ha, and many may see no benefit from hybrid Bt brinjal. In contrast, adoption of brinjal IPM will almost certainly increase profitability, perhaps by Rs.66,794/ha for small-scale resource-poor farmers. Farmers are expected to retain only 10% of the increase in profitability from Bt brinjal, but are expected to retain 63% of the increase from brinjal IPM.

Bt brinjal has been suggested to improve brinjal profitability in India through increased marketable yield and reduced insecticide applications. A narrow utilitarian analysis can be used to evaluate effects on profitability using data on yield and insecticide use in field production systems. The multi-site field trials (MST) and large-scale field trials (LST) were conducted under controlled conditions on experimental plots. They were designed to estimate yield and insecticide use in hybrid Bt brinjal compared to genetically similar non-Bt hybrids under conditions appropriate for large-scale commercial brinjal production. The data are not representative of the production systems used by small-scale resource-poor farmers. Consequently, the data are probably appropriate for only about 4% of the brinjal producers in India. The estimated yields probably greatly overestimate the yields that will be obtained on farm. Yield gaps are prevalent and large between experimentally estimated yield and average farmer yield for any agricultural technology. To take the yield gap into account, yields of Bt and non-Bt brinjal reported in the MST and LST experiments should be multiplied by 0.54 to estimate the yield benefit for the average large-scale commercial farmer. Taking into account the differences between production systems of large-scale commercial farmers and small-scale resource-poor farmers, the expected maximum potential yield benefit from Bt brinjal is probably ≤ 43.7 q/ha for large-scale commercial farmers and ≤ 7.2 q/ha for small-scale resource-poor farmers; about 16 % of the time Bt brinjal is not expected to out-yield non-Bt brinjal. Insecticide use might decline in large-scale commercial Bt brinjal production systems by an average of 6.5 applications. However, other factors may modulate this substantially, and new secondary pests would result in more insecticide use. Because this risk is not adequately evaluated, it is not possible to develop an unbiased estimate of insecticide use for Bt brinjal. It is not

possible to estimate how insecticide use might change if Bt brinjal were used by small-scale resource-poor farmers, but it is likely to be a reduction of 6.5 applications or less. For the purposes of estimating profitability, an average of 6.5 applications was used for both large- and small-scale farmers. By doing so, it is likely that profitability will be overestimated.

Hybrid Bt brinjal may improve profitability of large-scale commercial farmers by at most Rs.23,439/ha and of small-scale resource-poor farmers by at most Rs.3,250/ha. In comparison, brinjal IPM has improved net returns of small-scale resource-poor farmers by Rs.66,794/ha.

The increase in profitability will not accrue in full to the farmer. Some will be passed on to the consumer and some will be retained by the seed company. Farmers are likely to receive only a small part of the economic benefits from hybrid Bt brinjal. Summed across all farmers expected to use either brinjal IPM or hybrid Bt brinjal, the total profit (= economic surplus) for brinjal IPM (Rs.326 million for the Birbhum District of West Bengal only) is significantly proportionately larger than for hybrid Bt brinjal (Rs.1008 million for all of India). More significantly, farmers are expected to keep 63% of the economic surplus from brinjal IPM but only 10% of the economic surplus from hybrid Bt brinjal if Mahyco charges a seed price that optimises their profits. About 48% of the economic surplus from hybrid Bt brinjal is expected to be retained by the seed company. The remaining economic surplus is expected to go to the consumer.

The social economic benefits from brinjal IPM will be much greater than those from hybrid Bt brinjal. Increased public investment, greater promotion, and strengthened public policy for brinjal IPM relative to investment in hybrid Bt brinjal will result in greater social benefits in India and a major increase in actual net returns for small-scale resource-poor farmers.

Conclusion 9. No analysis of the effects of Bt brinjal on the economic security of Indian farmers has been conducted or cited. Small-scale resource-poor farmers using hybrid Bt brinjal may have increased risk of economic failure. An analysis of the effect of hybrid Bt brinjal on the economic security of brinjal farmers should be conducted.

Economic security is important for Indian farmers to enable them to continue with their livelihood from year to year and also to meet financial obligations throughout the year. The high suicide rate among Indian farmers is an indication of the lack of economic security provided by society. Brinjal farmers in India have a quite low suicide rate, because brinjal helps cash flow and buffers income variation in other crops. An analysis of the effect of hybrid Bt brinjal on the economic security of large-scale commercial farmers and small-scale resource-poor farmers is essential.

Conclusion 10. There are many important human values outside of utilitarian values that are important for economic development. Hybrid Bt brinjal may adversely effect human health, religion and culture through the economic system. These potential concerns should be assessed.

Nobel prize winner, Amartya Sen, has eloquently argued that utilitarian values are too narrow a guide for economic development. He suggests that other factors associated with human freedom are essential for humans to flourish under economic development. Three such elements have emerged in the environmental assessment of Bt brinjal, effects on human health, religion and culture. If utilitarian values are allowed primacy, the resulting economic development could have indirect adverse effects on human health, religion, and Indian culture. None of these have been assessed ex ante as a potential effect of Bt brinjal.

Conclusion 11. Bt brinjal will require extension support to maintain any social benefits, and it may lead to loss of local brinjal varieties, and inhibit government plans to create a strong vegetable export market. These risks should be considered prior to commercialisation of Bt brinjal.

Bt brinjal is not a panacea and will require new scientific information to maintain benefits for society even over relatively short time periods of 5-10 years. This implies that India will need a geographically extensive extension service that is prepared to provide timely information about new demands for pest control as Bt brinjal is commercialised. Without this support, many of the potential social benefits of Bt brinjal will likely be lost soon after commercialisation.

Bt brinjal may also have several indirect socioeconomic effects that merit assessment. These include an indirect loss of brinjal varietal diversity, interference with vegetable export market expansion, and increased economic risks to brinjal farmers.

Timing of Needed Environmental Risk Assessment Activities

Although the GEAC and Mahyco have already invested considerable time and effort into environmental risk assessment (ERA) for EE-1 hybrid Bt brinjal, much of the effort was misdirected and did not assess actual adverse environmental consequences in India. The needs for ERA that are outlined here are developed with an eye towards improving oversight of genetically engineered crops in India, and redirecting efforts to issues that actually matter to India.

Nearly all of these needs can be assessed without releasing Bt brinjal into the environment. Even those needs that require the release of Bt brinjal in the environment are conditioned on certain results being found in prior tests conducted in the laboratory or greenhouse. Thus, there is little need for additional exposure of Bt brinjal to the environment to complete an ERA that evaluates serious concerns for India.

This report does not cover post-commercialisation monitoring because this is a very complex issue. There are two main classes of justifications for monitoring: 1) a need to monitor specific, anticipated adverse environmental effects with significant risk, and 2) a need to compensate for uncertainty about specific or undefined adverse environmental effects. If one is not sure that something bad will happen, monitoring can substitute for this uncertainty because by monitoring, one can detect the bad thing in time to mitigate, avoid or remedy the situation. However, there will be continual pressure to reduce the cost and effectiveness of this second kind of monitoring rendering it unstable even on medium-range temporal scales. An in-depth analysis of these issues would be a welcome addition to the literature on ERA for genetically engineered organisms.

Context and Need

Environmental risk assessment (ERA) has changed substantially since it was initiated about 50 years ago to address point-source pollution, environmental siting decisions, and pesticide regulation. Since that time, its scope has expanded to address such diverse activities as global climate change, invasive species, non-point source pollution, comparative and cumulative environmental risk, and the subject of this report, transgenic organisms.

As the scope of ERA expanded, the original methodologies were pulled, stretched, and cut to fit the topic, much like a Procrustean bed. In the process the whole purpose of ERA became obscured and was sometimes lost as people sought to adapt old means to new ends. Indeed, the scope and adequacy of the US regulatory structure for transgenic organisms came under considerable criticism in a series of reports from the US National Academy of Sciences (NRC 2000, 2002, 2004).

These considerations lead to the following problematic, which has not been adequately addressed by the GEAC in India. An ERA may fulfil the regulatory requirements of a country and be deemed acceptable relative to those requirements, but at the same time, it may not assess any substantive environmental risks because the regulatory structure is too slow to change and relies more on procedural standards than on outcome-based standards. For example, a common way for an applicant to meet regulatory requirements is to complete whatever study is requested by the regulator. Whether that study actually tells anyone anything about an outcome about environmental risks can be forgotten in the frenzy to set and meet regulatory standards.

In other words, an ERA that is “good” according to certain regulatory standards may not be the ERA that ought to have been done to assess actual potential environmental risks. No matter how “good” it may be relative to this narrow regulatory standard, it may be no good for the environment.

For transgenic organisms, including Bt brinjal, an ERA ought to identify the possible concerns that the transgenic organism could affect in the environment in concrete terms.

It should then proceed to determine if any of these concrete concerns are so small as to not matter, are so large that we must be concerned, or somewhere in the middle that we have to figure out what to do about them. A concrete concern must identify an environmental value at risk (e.g., the endangerment of a species, the loss of the economic security of a farmer, a reduction in productive capacity of the soil, etc.), identify an ecological entity through which the value is reduced (e.g., a particular endangered species, the security of small-scale resource-poor brinjal farmers in India, the nutrient or micronutrient content of the soil, etc.), and specify all of the means by which the transgenic organism could affect the identified ecological entity. At this point, but not before, it is possible to assess the significance of the potential environmental risk.

This report holds the EC-II ERA to the standard of assessing outcomes about concrete environmental risks, and does not dwell much on the more narrow concern of whether the EC-II ERA meets the regulatory standards of the GEAC. By doing so, this report points out where the scope of the EC-II ERA is too narrow and challenges the adequacy of the ERA framework established by GEAC and under which Bt brinjal has been assessed by EC-II. At many points in this report, the EC-II is criticised not for whether it has accomplished what it set out to do, but whether it set out to do the right thing in the first place. The main thesis of the report is that the GEAC set too narrow a scope for ERA, and it is because of this overly narrow scope that the EC-II is not an adequate ERA.

The report first describes the context into which Bt brinjal is meant to be introduced in India. The described context is more nuanced than that provided by EC-II and contradicts EC-II in many significant ways. The report next addresses the scope and adequacy of the characterisation of the transgene, EE-1, that is incorporated into Bt brinjal. The focus is on what must be characterised to enable an effective ERA. It does not address how to characterise a transgene so that risks to human and animal health can be assessed. Within this topic, the genetic and phenotypic characterisation of the transgene is addressed, followed by an evaluation of the quality of the EE-1 transgene, and a scientific opinion on compliance of the EC-II ERA to international

standards for ERA. The third major topic is on the assessment of environmental risks. This is divided into three major themes: gene flow and its consequences, effects on biological diversity, and the risk of resistance evolving in brinjal fruit and stem borer (BFBS) to Bt brinjal. Although there is no perfectly good division of environmental risks, the first addresses risks that are associated with genetics and evolution of plants, including brinjal and its wild relatives. The second addresses risks that are ecologically mediated and do not require distinction and analysis of the genotypes within species. The third focuses on risks associated with the genetics and evolution of non-plant species, in particular, the target pest, BFBS. The report concludes with a section on socioeconomic analysis of Bt brinjal in the context of pest control alternatives now available in India. This section also summarises the kinds of studies that are needed to provide an adequate assessment of environmental concerns that cover a realistic and significant scope for ERA.

Finding 1. Brinjal is one of the most important vegetable crops in India, especially for the rural and urban poor. Most is grown in the three eastern states of West Bengal, Orissa and Bihar by small-scale resource-poor farmers.

After potato, and perhaps onion, brinjal is the most important vegetable crop produced and consumed in India. About 9.5 million tonnes of brinjal is produced in a year, nearly all of which is internally consumed (APEDA 2009). Only 0.04% of the brinjal produced is exported, mainly to the UK, France and Saudi Arabia. During the hot and rainy season, brinjal is one of the few vegetables that can be afforded by the rural and urban poor (Dr. Prabhakar, IIHR, personal communication).

India and China are the largest producers of brinjal by far. Together these two countries produce about 2/3rds of the world's brinjal, with India slightly behind China. Projected population growth in India suggests that the demand for brinjal will increase in the short term.

Nearly all brinjal farmers in India are small-scale resource-poor farmers, who farm <1ha for all of their crops (DES 2008). They grow brinjal in small (~65 m²) plots, and sell to local village and town markets. Large-scale commercial brinjal producers grow >0.4ha of brinjal and purchase many of their farm inputs (APEDA 2009). They comprise less than 10% of the total brinjal growers and often sell to the large urban markets. This simple structural breakdown of brinjal farmers is critical for understanding the potential value of Bt brinjal, as will be discussed in considerable depth in this report.

Within India, about 61% of the brinjal is grown in the three eastern states of West Bengal, Orissa, and Bihar (APEDA 2009). Additional production occurs in Assam, Meghalaya, Jharkhand and the other small eastern states. This area receives heavy monsoons and brinjal can be cultivated around the year because there is sufficient water even during the “drier” seasons. A larger proportion of the farmers in this region are small-scale resource-poor farmers than elsewhere in the country.

The remaining ~36% of the brinjal is produced throughout the rest of India, with the largest concentration in Gujarat. Smaller, but still significant quantities are produced in Andhra Pradesh, Maharashtra, Karnataka, Chhattisgarh, and Madhya Pradesh. Overall, there are proportionately more large-scale producers in these parts of India, but these tend to be located in peri-urban areas (the areas surrounding the large cities).

Finding 2. India is the centre of the world's biological diversity in brinjal with over 2500 varieties grown in the country. Some local varieties have significant religious and cultural value.

Brinjal is open-pollinated with 12 pairs of chromosomes (Doganlar et al. 2002) with scientific name *Solanum melongena* L. in subgenus *Leptostemonum* in the large, mostly tropical Solanaceae. The National Brinjal Germplasm Bank holds nearly 3555 and 784 wild species, and more than 2500 different varieties of brinjal have been identified in India (National Bureau of Plant Genetic Resources, New Delhi; Sharma SK and Pandey S, 2009). There is probably additional varietal variation in India that has not yet been identified. India has released ~200 modern brinjal varieties, including 54 hybrids, some of which are commonly grown in India (IIHR 2007, 2008). These facts provide a substantive context for the assessment of gene flow risks, addressed below.

Each local region within a state, typically has a characteristic variety that is most commonly grown in the region, but not commonly grown elsewhere (V. Ramprasad, personal communication). Some of the locally recognised varieties are being registered as “Geographical Indicators” under the TRIPS Agreement. For example, in Karnataka four varieties including *Hirangere*, a >10cm, oblong, yellow-greenish brinjal grown around Mysore, and *Kudochi* and *Malapur* in Northern Karnataka

are being considered for GI status. The fourth variety, *Matti gulla* has been granted Geographical Indicator status (Raghuram 2010). It is a special brinjal variety grown only in Mattu, a village between the Udyavar and Pangala rivers in the Udupi district of Karnataka. It is a small, green, round brinjal (<100g) with a thorned hook. Many of these local varieties are used in the preparation of special brinjal dishes that are specialties of the region, for example a special preparation of *Malapur* goes well with Jola Roti (Jowar Bread). In addition, some varieties have significant cultural value. *Malapur* is an essential accompaniment at festivals, marriages, and other social functions in the Darwad region of Karnataka. *Matti gulla* gives two crops a year and a part of the first crop is offered to the Udupi Krishna temple (Raghuram 2010). These religious and cultural values are discussed in greater depth in the socioeconomic analysis below.

Finding 3. EE-1 Bt brinjal is proposed as a hybrid, and is unlikely to fit well in small-scale production. It may fit in large-scale commercial brinjal production.

Brinjal production practices vary geographically and are strongly influenced by the environment. In the east where water is plentiful, brinjal can be cultivated around the year, and 2 crops per year are common. The time of planting can vary considerably. In other parts of India, the dry season tends to push brinjal to one crop per year, and planting times are reasonably synchronised to take advantage of the rainy season (*kharif*). Winter season production (*rabi*) is less than *kharif* production, and occurs under irrigation, including manual, localised and surface irrigation.

More significantly, brinjal production varies according to farm size and access to resources. Small-scale resource-poor farmers typically cultivate 1-4 local brinjal varieties, in dooryard production of a dozen plants up to a few small plots. These small plots are visited daily and sometime multiple times in a day. Because the fields are so small, they are often tucked away in special or otherwise unused space. Large-scale producers typically cultivate one commercial variety of brinjal in a few larger fields. These fields are also visited frequently, but their larger size precludes daily walking all parts of every field.

EE-1 Bt brinjal is a commercial hybrid. Because small-scale resource-poor farmers do not grow commercial varieties, acceptance of EE-1 Bt brinjal by these farmers may be low. Hybrids must be purchased anew every crop from a seed producer, and these farmers often cannot afford to use their limited capital in this way. Moreover, as indicated above, many of the OPVs that these farmers use have important characteristics and hybrids are a poor substitute.

EE-1 Bt brinjal may be more suited for the large-scale brinjal producers who already use commercial seed. Many of these farmers already use hybrid seed, so depending on the cost of hybrid Bt brinjal seed, it may be readily incorporated into their production systems. However, a substantial fraction of these farmers use modern improved OPVs, and these farmers may be less willing to shift to a Bt brinjal hybrid because of the increased seed cost for each crop.

It has been proposed that Mahyco will introduce the Bt gene into open-pollinated varieties (OPVs) of brinjal and make them available to small-scale resource-poor farmers at minimal cost. These varieties are being developed at several agricultural universities in conjunction with Mahyco. Presently Mahyco is indicating that the licence for OPV-Bt brinjal will be free, but Mahyco's pricing policy for the seed itself has not been determined. It would be presumptuous to speculate about these OPVs in this report because there has been no application to commercialise these varieties yet. Hence any speculation would be a non-essential distraction to the present discussion about EE-1 in hybrid brinjal varieties.

Finding 4. Brinjal is produced for net income by large-scale producers. For small-scale resource-poor farmers it is critical for creating economic security, primarily by facilitating cash flow, insuring against income variation from other crops, and providing families with needed nutrition.

Brinjal plays a different role in the economy of large-scale farmers than that of small-scale resource-poor farmers. Large-scale commercial brinjal producers produce to generate higher net income from their brinjal crop from which they purchase other needed goods and services. They do this by maintaining sufficient credit to purchase many of their farm inputs (APEDA 2009)

and seek to increase their profits by increasing brinjal yield. They typically purchase hybrid brinjal seed from certified seed suppliers to obtain high-yielding varieties and they then protect the plants from pests with pesticides. Large-scale commercial producers are common around the large cities, where they have a ready market to supply the urban demand for brinjal.

In contrast, small-scale resource-poor farmers produce brinjal to help cash flow, insure themselves against income variation in other crops, and provide their families with needed nutrition from a vegetable crop. For these farmers, brinjal production is essential for their overall economic security and well-being (APEDA 2009). This is not to say that they don't try to generate higher net income as well, but that the other goals are more important. These farmers obtain nearly all of their farm inputs for brinjal locally, except for some insecticides. They recycle OPV brinjal seed and exchange seed with other local farmers via a reciprocity system. Seed of local varieties may be held temporarily in informal local seed banks. Brinjal is harvested daily, and the surplus over daily household needs is sold in local village and town markets. In this way, brinjal provides a small daily cash income over a long period of time, helping stabilise cash flow, which can be used to purchase clothing and school materials. In addition, it provides a critical buffer against income variation from other crops and important nutrition to the family. As one measure of economic security, the suicide rate among small-scale resource-poor brinjal farmers is among the lowest in India (Karnataka 2002)

A switch to hybrid Bt brinjal seed by a small-scale resource-poor farmer may jeopardise their economic security. The extra expense of purchasing hybrid brinjal seeds also entails purchase of additional inputs suggested by the seed company to ensure capture of the yield benefits of the hybrid. This reduces cash flow potential and increases the risk in producing brinjal so that it no longer functions as insurance against variability in other crops. In addition, Bt brinjal hybrids, which enter a mass market, will not have the same local market as the local non-hybrid brinjal varieties, which enter a local niche market based on their local characteristics. Consequently farmers may have more difficulty selling Bt brinjal through their normal channels and may need to find and access additional markets to sell them.

Consequently, EE-1 Bt brinjal may have little trouble slotting into the production system of some large-scale brinjal farmers. However, if adopted by small-scale resource-poor farmers, it may disrupt their economic security.

Finding 5. Brinjal fruit and shoot borer (BFSB) causes significant economic damage to brinjal throughout India for all farmers, probably about 30% yield loss compared to no control. Many factors reduce losses, including soil status and cultivation conditions. Its perceived significance by farmers may precipitate an over-response relative to its true economic significance. Real losses from BFSB are higher for large-scale commercial producers than for small-scale resource-poor producers because of the way damaged fruit can be used. EC-II vastly over-estimated losses to BFSB.

Brinjal fruit and stem borer (BFSB), *Leucinodes orbonalis* Guenee is a medium-sized moth [Lepidoptera: Crambidae] in the subfamily Pyraustinae (Fig. 1). The larvae (caterpillars) cause damage to brinjal by boring into stems and fruits. The adults cause no damage.

BFSB populations and economic damage vary by time of season, cultivation practice, brinjal variety, geographic location and soil conditions. Damage is typically higher during the *kharif* season than the *rabi* season, although it can be high during the *rabi* season. Its damage is greatest during the six weeks from late July to early September. Populations and damage are lowest under crop rotation, high soil organic matter content (SOM), good soil drainage, low plant residue, low plant density, and intercropping. They are highest under continuous cropping, low SOM, easily waterlogged soils, high plant residue, high plant density, and monoculture. Geographic locations with a longer monsoon season give BFSB only a limited time to build up populations during the summer, and damage is low. For example the *Udipigulla* variety, which is grown in the west coastal region of Karnataka where heavy rainfall confines BFSB to a short summer season, suffers little economic damage from BFSB.

Fig. 1. Brinjal fruit and stem borer adult (*Leucinodes orbonalis* Guenee). Copyright 1994-2010 Len Willan and CSIRO Entomology

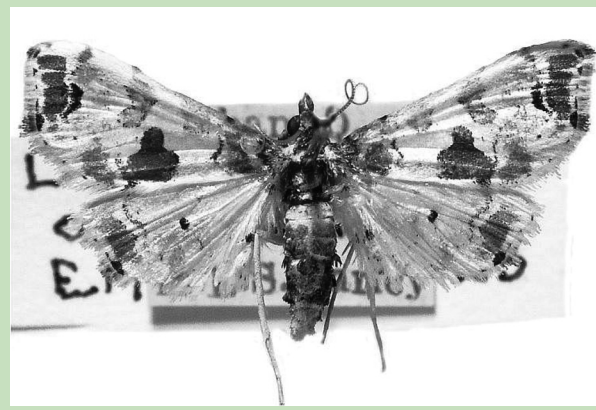


Table 1. Brinjal losses to BFSB in India. Yield loss is calculated for a damaged fruit either being totally lost or the damaged part trimmed away (not consumed) so that the undamaged parts of damaged fruits are still considered in yield. Fruit damage is a measure of the proportion of fruits that have BFSB damage at any particular time. Probably all of the listed studies were conducted on the land of the Agricultural College. NA = not available. The NA season is most likely kharif. The NA varieties are most likely high yielding varieties (HYVs).

State	Season	Variety	Yield loss (%)		Comment	Reference
				Not consumed		
West Bengal	NA	NA	5.3*		IPM neem v. control	Chakraborti 2001
West Bengal	NA	NA	25.2*		Chemical v. control	Chakraborti 2001
Orissa	Kharif	NA	10.8-54.8		Range, 2 years	Paitnaik et al. 1997
Uttar Pradesh	NA	Local	25.3	7.3		Haseeb et al 2009
Uttar Pradesh	NA	NA	13.1	3.6		Mall et al. 1992
Delhi	NA	NA	20.7	9.7		Peswani and Lall 1964
Gujarat	NA	NA	5.3			Thanki and Patel 1988
Rajasthan	kharif	NA	40.8-71.8*		Range, 4 years	Pareek and Bhargava 2003
Madhya Pradesh	kharif	HYV	23.3-47.8*		Range during growth	Dhamdhere et al. 1995
Madhya Pradesh	ziyad	HYV	17.2-30.9*		Range during growth	Dhamdhere et al. 1995
Maharashtra	kharif	NA	53.1	12.52**	High yeild v.control	Naitam and Markad 2003
Andhra Pradesh	rabi	Local	53.4		Best v.control	Naik et al 2008
Andhra Pradesh	rabi	Local	34		Neem v. control	Naik et al 2008
Karnataka	kharif	NA	26.0-45.0*		Range planting density	Shukla and Prabhakar 1985?
Tamil Nadu	NA	NA		1.67**	clusterbean polyculture/ brinjal monoculture	Elanchezhyam et al 2008

* Difference in fruit damage, not yield

While there have been a number of studies on yield loss to BFSB throughout India wherever brinjal is grown, many of the studies are in publications available in certain Indian States and are difficult to obtain. A partial review of this rather large literature is given in Table 1. These are the reported yield losses to BFSB comparing no control with some control measure, and are therefore estimates of the maximum yield loss from BFSB. As discussed below, few farmers do nothing to control BFSB, so actual yield losses suffered by farmers are much less than reported in Table 1. This means that the actual yield benefits of Bt brinjal need to be estimated as the marginal benefits (or costs) over presently available control practices. This will be elaborated in more detail in the socioeconomic analysis below.

Although losses from BFSB can reach the high levels (70%, Table 1), under most conditions the losses are significantly lower, and average losses are much less. Based on these experiments conducted on Agricultural College lands, 50% yield loss was not unusual, but typically, yield loss was observed to average around 30% (Table 1). EC-II reported 60-70% yield loss even when insecticides are used; this is obviously an over-estimate of yield loss to BFSB. Moreover, the data reported in Table 1 probably overestimate yield loss. As will be discussed in greater depth in the socioeconomic section, yields on experiment stations and agricultural colleges tend to exceed the yields farmers can obtain on their own fields. This “yield gap” implies that

the reported yield losses (Table 1) will exceed the losses experienced by farmers in their own crop. Thus, these figures provide an upper bound on typical yield losses from BFSB.

Commercial production of brinjal using synthetic fertiliser in peri-urban areas probably suffers higher yield losses than elsewhere. Door-yard brinjal production of brinjal is done in tiny plots, often involving a dozen or so plants of a special local variety. Door-yard production is common among small-scale, resource-poor farmers, who may grow these special varieties in specialised locations that minimise attack by BFSB. For example, *Hirangere* is grown in the silt outwash immediately below the cattle water tanks and typically suffers little or no damage from BFSB. These microsites are well-drained and have higher than average soil organic matter.

Brinjal produced in small plots by small-scale, resource-poor farmers also suffers significant losses from BFSB. Nearly all of the yield loss experiments that have been conducted (Table 1) use high-yielding varieties, which are rarely used by these farmers. The few trials on local varieties in Table 1, however, indicate that BFSB can significantly damage local varieties, although perhaps at a lower rate than high-yielding varieties. Again, these observed yield losses may be overestimated due to the yield gap. Small-scale resource-poor farmers typically fertilise with manures, which elevates soil organic matter, and plant at lower plant density. Both of these factors tend to reduce yield loss from BFSB.

Farmers typically overestimate losses from BFSB. In bunchy brinjal varieties, distortion of fruits is often erroneously attributed to BFSB. In addition, similar to many other plants, brinjal can compensate some yield loss. If damaged fruits are promptly removed from the plants, brinjal compensates by developing additional flowers and fruits. In addition, farmers all over the world tend to apply insecticides against borers when damage is seen, which is often too late for the insecticides to control the pest. These “revenge” applications cost farmers substantial amounts of money, but have little economic value beyond the grim satisfaction of trying to kill the pest.

More significantly, small-scale resource-poor farmers manage yield loss by trimming off the damaged parts of the brinjal fruits and consuming the undamaged parts. This reduces yield loss to 1/3rd its nominal value (Table 1). In West Bengal, 54% of the farmers sell damaged fruits on the market, 47% consume the damaged fruits themselves, and 86% feed them to cattle (Baral et al. 2006). Large-scale commercial farmers do not have the ability to absorb their larger quantity of damaged fruits in the same way.

Finding 6. Some management for BFSB is practiced by all brinjal farmers. Most overuse synthetic insecticides by a considerable amount. This can be reduced substantially using IPM.

Both large-scale commercial farmers and small-scale resource-poor farmers spend considerable amounts of capital to control BFSB. Small-scale farmers throughout India overuse insecticides. In West Bengal the *kharif* crop receives an average of 32 applications of synthetic insecticide and the *rabi* crop received an average of 54 applications of synthetic insecticide (Baral et al. 2006). Nearly all small-scale farmers in Gujarat applied synthetic insecticides >40 times on the *kharif* crop (Alam et al. 2006). In eastern Uttar Pradesh, 86% of small-scale farmers applied synthetic insecticide 2-3 times a week (Alam et al. 2006), which is 40-60 applications for the entire growing season. Insecticides are the main cash expense for these farmers, exceeding 50% of production costs (Alam et al. 2006). Considerable research in India shows that 4-8 applications of insecticides provide economic control even without integrating other pest control measures. This means that these small-scale producers can eliminate 30-50 applications of insecticide and suffer no change in brinjal yield. Integrated pest management (IPM) would greatly benefit these farmers. This will be discussed further in the Socioeconomic section of this report.

Small-scale resource-poor farmers also use other methods of control, but the frequency of use is poorly known. At the other extreme from over-intensive use of synthetic insecticides described previously, farmers may stop harvesting from a small plot, rouge out the bad fruits and wait for the damage to decline. Only 12% of the farmers surveyed in Gujarat rouged out damaged stems and fruits, while 85% of farmers surveyed in Uttar Pradesh did so (Alam et al. 2006). About 28% of West Bengal farmers used practices other than insecticides. Many other traditional methods have been used to control BFSB (CIKS 2010a), including use of Kochila-cow dung compost and tobacco-soaked water with soap (CIKS 2010b).

Management under large-scale commercial production typically relies on the application of synthetic insecticide on a calendar basis (see University of Agricultural Sciences, Bangalore: packages of practices for horticultural crops). In the peri-urban areas

with endemic BFSB populations, brinjal is sprayed every week or fortnightly on a calendar basis. A calendar basis means that insecticides are applied at set time intervals whether or not there is a threat of loss from BFSB. Consequently, some of these brinjal fields receive >12 insecticide applications. Large-scale commercial fields with no risk of loss from BFSB typically still receive 2-4 applications of insecticide for BFSB control on a calendar basis. Insecticide use on large-scale commercial brinjal can be reduced substantially by using IPM.

Finding 7. Useful alternative production systems for control of BFSB are being tested, actively used, and promoted in India. These include IPM, organic production and other methods that reduce costs associated with external inputs.

Indian farmers have several resources available to use to control BFSB instead of synthetic insecticides and Bt brinjal. These include local insecticides based on neem, including neem cake and neem seed kernel extract. Farmers can also rouge damaged stems and fruits to reduce BFSB populations. High levels of phosphorous in the fertiliser will promote more rapid maturation of the fruits and a hardening of the plant tissues, which enable plants to escape being injured by BFSB (Prabhu et al. 2005). In addition, BFSB sex pheromone and BFSB egg parasitoids have also been examined.

Small-scale resource poor farmers can use low plant density, residue removal, local insecticide technology based on neem, and hand-rouging damaged plant parts to manage BFSB (APOF 2004). The ubiquitous neem tree can be used to produce a local insecticide, and hand-rouging can be very effective and efficient at removing the local BFSB population before it can increase because there is a small number of brinjal plants involved. Application of 100kg per acre neem cake with 5 to 8% residue oil to the base of the plant, pheromone traps, egg parasitoids and clean cultivation comprise one of the recommended package of practices for brinjal pest management, including BFSB.

IPM for BFSB has been tested and is being promoted in India (Alam et al. 2006, Barel et al. 2006). This is based on using damage thresholds to determine when to apply an insecticide (which can be a neem product), use of pheromone traps (Cork et al. 2003) constructed out of readily available materials, ¹hand-rouging, and residue removal. IPM has enjoyed widespread acceptance and has provided substantial value to farmers. This project will be discussed more in the Socioeconomic section of this report.

India has launched a major initiative in Organic Agriculture and Bt brinjal is not compatible with organic production (NPOP 2005). Indeed, Bt brinjal could jeopardise this developing organic vegetable export market. India is already facing the problem of organic cotton contamination with Bt cotton in the Vidarbha region of Maharashtra. India accounts for about half the global supply of organic cotton. About 200 'A' category organic farmers affiliated to Vidarbha Organic Farmers Association (VOFA) in Yavatmal district of Maharashtra did not get their cotton certified because "their cotton samples tested positive for Bt contamination". (Aparna Pallavi, Down to Earth Vol. 19, No. 19, Feb 16-28 2010, page 20). Organic production practices control BFSB effectively. These include recipes for local insecticides, such as mixing 2 ton of compost with 200 kg of neem cake and 2 kg *Trichoderma*, holding at 60% moisture for a week, and applying at transplanting. In addition, pheromone traps coupled with one of the many proven indigenous local insecticidal technologies has been effective at controlling BFSB.

Large-scale farmers also have some control options that are not readily usable by small-scale resource-poor farmers. Net house production systems have been advocated for large production systems by the Indian Institute for Horticultural Research (IIHR). These exclude BFSB with netting and although the netting is a substantial capital expense, in experimental applications a farmer cleared Rs.2,000,000 on less than one hectare of brinjal in one season (Dr. Prabhakar, IIHR, personal communication).

¹(<http://www.takingroots.in/>)

Characterisation of Transgene

Genetic Modification of Bt Brinjal

Conclusion 1. The EE-1 transgene needs to be characterised more fully to 1) demonstrate that there is only one functional or non-functional insert in Bt brinjal, 2) that the transgene expresses the intended Cry protein, 3) that it does not interrupt a functioning plant gene, 4) provide expression levels in additional plant tissues, and 5) demonstrate empirically that the marker genes are not expressed in Bt brinjal.

Bt brinjal EE-1 contains three transgenes:

1. A chimeric Bt toxin gene which is formed from *cryIAc* and *cryIAb*, which should be called *ccryIA* (a chimeric *cryIA* gene).
2. A kanamycin resistance gene *nptII* and
3. A streptomycin resistance gene *aad*.

Characterisation of the transgene involves describing the inserted transgene(s) and the expression of the inserted transgene(s). The transgene description focuses on the transgene as it exists inserted in the plant. A description of the inserted plasmid, the cassette, or the planned insertion is insufficient for risk assessment. The transgene description includes the number of transgene inserts, the DNA sequence of the inserted transgene, and the DNA sequence of regions flanking the transgene.

Inserted Transgene

Finding 8. The description of the transgene in Bt brinjal EE-1 is inadequate to support risk assessment. It is not clearly known that there is only one transgene incorporated into the brinjal genome, that the inserted transgene expresses the intended gene product or that the transgene does not interrupt a functional plant gene.

(A) Number of inserts. The Dossier indicates that one transgene insert remains in Bt brinjal EE-1. Based on the evidence provided, the conclusion is not fully justified. There are several sources of uncertainty that weaken the conclusion, and three of these sources of uncertainty may affect risk. Two may increase risk and one may increase or decrease risk.

- Uncertainty arises because the size of the digoxigenin (DIG)-labelled Bt probe is not specified. If it is too large, it may not detect independently inserted fragments; if it is too small, it may not detect independently inserted fragments not associated with the probe.
- Uncertainty also arises because probes for other parts of the plasmid, such as the nptII gene and several others, are not used. If fragments are inserted in such a way that a new open reading frame is created that is associated with a plant promoter, novel gene products may be expressed at high rates.
- The third source of uncertainty is associated with the breeding program. Although the file makes it clear that the transformed plant is backcrossed to elite brinjal germplasm, it is not clear how many backcross generations were done. Each backcross generation reduces the probability that an additional integration event is retained into the final brinjal product. In other words, the more backcross generations, the less likely an undetected integration event is retained in the final brinjal product (Box 1). Because the number of backcross generations is not specified for each of the Bt brinjal products, it is uncertain how well the inserted transgene has been characterised.

(B) Sequence of the inserted transgene. Under the assumption that there is only one transgene inserted into Bt brinjal EE-1 as asserted by the file, the sequence of this one transgene was not reported. The sequence of the inserted transgene is typically required by regulatory authorities around the

Box 1

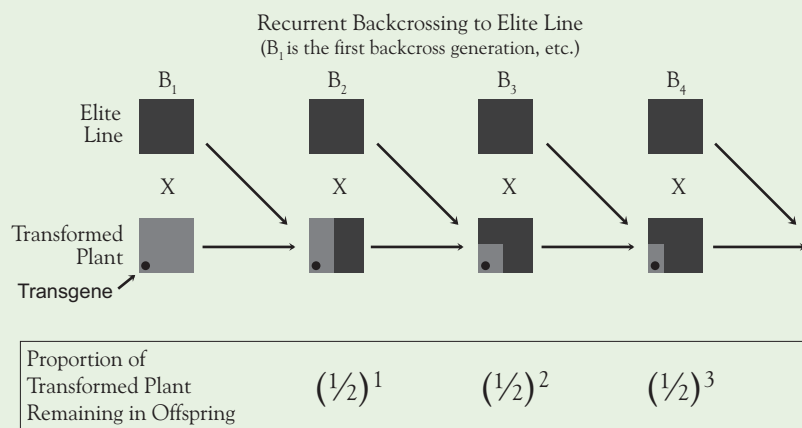
Backcrossing and Transgene Characterization

Backcrossing is one way to create crop varieties that are similar to previous elite varieties with a few new traits added. It is also a useful way to clean up “dirty” transformation events that generate transgenic plants.

At the time Bt brinjal event EE-1 was created, all know methods for creating transgenic events created “dirty” transgenic events, and EE-1 is no exception. A “dirty” event is one in which there are multiple transgene insertions, often with inversions of the transgene, interspersions of plant DNA, artificially created introns, and transgene fragments. All of the “dirty” elements of the event could create serious risks to the environment.

The multiple insertions typically end up on different plant chromosomes or some distance apart on the same chromosome. Recurrent backcrossing can help to clean up these dirty events.

Once a transgenic plant cell is created, it must be regrown into a whole plant. Backcrossing involves crossing the newly created transgenic plant with an elite variety or line that the breeder wants the transgenic plant to resemble. Recurrent backcrossing involves multiple generations of crossing offspring back to the same elite variety or line as diagrammed in the figure below.



For brinjal's 12 linkage groups (Doganlar et al. 2002), about 10 backcross generations are necessary to eliminate all but the chromosome with the transgene with 99% probability. Ten BC generations would also eliminate all but 0.1% (on average) of the genetic material associated with the transgene. If only 5 backcross generations were used, the probability that all other chromosomes have been eliminated is only 62%, and about 3% of the chromosome associated with the transgene remains. If only 5 backcross generations were used, a significant amount of germplasm that potentially contains unintended transgenes remains in the final variety. This creates unnecessary risks to the environment that could have been easily reduced with a bit more breeding effort. Hence the number of backcross generations is an essential element to characterizing a transgene.

world. Although the sequence of the Bt gene, and all of the marker genes was known (but not provided), this is the sequence of the plasmid, not the sequence of the inserted gene. There are typically differences between the two. The sequence of the inserted transgene is necessary to substantiate the logic behind the reasoning that the marker genes pose no risk, and that the Cry1Ac protein is virtually identical to other Cry1Ac proteins that have been studied previously. The sequence will also enable identification of unintended open reading frames (ORFs), which could result in unintended protein expression.

(C) Flanking regions. Information on flanking regions is not provided. Although there are limitations on the extent of genetic (DNA) analysis that can be done on brinjal because the entire brinjal genome has not yet been sequenced, there remain several significant and important elements that were not done. Typically an inserted transgene will be flanked by a mixture of plasmid and plant DNA, which is further flanked by only plant DNA associated with the original plant chromosome. Sequencing through the mixed flanking regions to the plant flanking regions is now routinely required by many regulatory authorities around the world. The flanking sequences are needed to determine if the transformation process has inadvertently created novel open reading frames and to determine if a functioning plant gene was interrupted by the insertion event. Lacking the full brinjal sequence, it is not possible to determine unambiguously if a plant gene was or was not interrupted, but because of the high correspondence of the brinjal and tomato genomes, the flanking region sequences can be blasted against the tomato genome until such time that a full brinjal sequence is available.

Needed Experiments and Observations

1. **Probe sensitivity.** Provide the size and sequence of the DIG-labelled Bt probe and identify the part of the transgene to which it is expected to bind.
2. **Transgene number.** Use additional probes that provide complete coverage of the original plasmid to explore for incorporated transgene fragments associated with other parts of the transgene. Report the size of these probes and the detection limits of the Southern blots.
3. **Transgene sequence.** Sequence the inserted transgene and any other inserted fragments identified in 1) and 2).
4. **Flanking regions.** Sequence the regions flanking the inserted transgene (and transgene fragments) until continuous brinjal DNA is encountered.
5. **Gene disruption.** Identify the brinjal DNA in the flanking regions and determine if it is associated with a potentially functioning brinjal gene. As long as the brinjal genome has not been sequenced, the tomato and potato genomes can be used for this purpose. If it is associated with a potentially functioning brinjal gene, determine the function and its potential effects on the plant.

Transgene Expression

Finding 9. The description of Ccry1A is inadequate for supporting risk assessment. Expression level of Ccry1A is needed in pollen of Bt brinjal EE-1. There is no need to use a protein not identical to Ccry1A in any the experiments used to support risk assessment. The expression level of the marker genes is assumed to be low. This should be demonstrated empirically.

Amino acid sequence of toxin

The Bt toxin gene is intended to be a chimeric protein of *cry1Ac* and *cry1Ab*. No data are provided that describe definitively the protein(s) that is(are) actually expressed in EE-1. Consequently the expressed protein is not fully known, which is unacceptable for developing a risk assessment. What is known is that it has a molecular weight that is approximately as expected and it reacts to certain antibodies the same as Cry1Ac. In the rest of this document, the protein will be referred to as Ccry1A (a chimeric Cry1A, probably closely related to Cry1Ac), the product of the *ccry1A* gene.

Use of surrogate proteins

Surrogate Cry1Ac protein (Box 2) is used for some of the environmental risk assessment tests. There is not an adequate biological justification for accepting this choice, and GEAC does not adequately consider the problems involved with it.

All of the toxicological experiments performed in the laboratory that are reported in EC-II, the Dossier and the Supplemental Materials relied on a surrogate Cry1Ac protein. The surrogate protein is surrogate in both the sense that it is produced heterologously in a genetically engineered *E. coli* bacterium and because it does not have the identical amino acid sequence as EE-1 is expected to have (Box 2).

In the present case, because the amino acid sequence of the Ccry1A toxin in EE-1 is not actually known, it is not really possible to know how similar the surrogate toxin is to it. This deficiency must be addressed before it is possible to interpret accurately the results of the toxicological experiments.

BOX 2

Surrogate Proteins

A surrogate protein is one that is substituted for the actual protein. “Surrogate” has at least two possible meanings in the present context.

Most commonly, a surrogate protein is one that is produced from a surrogate expression system, often *Escherichia coli*. This is also called heterologous production of the protein. In this meaning, the surrogate protein has the same amino acid sequence as the actual protein, but it is produced in a different way. Such proteins may be different from the actual protein if the actual protein is modified post-translationally in the plant. Heterologous systems are not able to replicate post-translational modification, and as a consequence it is possible for there to be structural and functional differences between the actual protein and the one that is produced heterologously.

A second and more problematic meaning of the term “surrogate” is a protein that does not have the identical primary amino acid sequence. This is more problematic because differences in amino acid sequence are known to affect the structural and functional properties of proteins. In other words differences should be expected unless proven otherwise. To say otherwise is a bit like saying that cow’s milk is the same as goat’s milk in the hope that people will believe it because they had never tasted both of them.

Typically, the justification for using surrogate proteins is that they are significantly less expensive to acquire than the actual protein. Heterologous production is often much less expensive than isolation and purification of a protein from living plants. However, production of a protein with identical amino acid structure as the actual protein is not that much more expensive than creating a protein that is merely similar, but not identical. Thus there are economic reasons to rely on heterologous proteins in risk assessment, except when assessing the most important risks. However, there is little justification for using similar but not identical proteins in any risk assessment test.

The surrogate Cry1Ac toxin used in several of the environmental risk assessment experiments is surrogate in both senses of the term. Biologically, each of these extrapolations due to surrogacy requires some scientific data and justification that the extrapolation is valid for the purpose of risk assessment.

In addition, GEAC asserts that the toxicological properties of EE-1 are the same as the properties of the heterologous Cry1Ac. No data are provided to justify this conclusion, so it is difficult to conclude that the two proteins have identical or even “similar enough” biological properties (however one defines “similar enough”).

It is not unreasonable to suggest that the toxicological experiments should have been conducted using a protein with the identical amino acid sequence as in EE-1 Bt brinjal. The cost to produce such a protein would not be very expensive. This would involve the following: 1) determination of the structure of Ccry1A, and 2) transformation of a bacterium to produce Ccry1A, instead of a “similar amino acid structure. Given the ease at which bacteria can be transformed, it is not clear why this alternative was not used.

Toxin expression in plants

Expression levels of Ccry1A are provided, but results for pollen are not reported. These data are necessary to interpret some of the non-target experiments. Expression levels are much lower in roots and somewhat lower in stems than in leaves, fruits, shoots and flowers. It is not clear if concentrations decline as fruits age.

Expression of marker genes

For environmental risk assessment, the most important issue related to marker genes is their expression in the plant, rather than horizontal transfer of the genes themselves. While there remains some debate about the environmental effects of the antibiotic resistance marker genes (*nptII* and *aad*) that probably remain in EE-1 brinjal, it is likely that their expression in EE-1 brinjal is low, because they are associated with bacterial promoters. The plant gene transcription apparatus does not easily recognise bacterial promoters, so expression is quite low. The environmental effect of these antibiotic resistance genes is not clear, but considerable evidence in other GM plants indicates that it is likely to be small because expression levels are low. Modern transgene constructs typically have eliminated these marker genes in the final product. Regulatory systems in Europe and other countries require that antibiotic marker genes are removed. This is because it does not take much effort to remove them (or to design the transgene vector so that they can be easily eliminated), so even if they cause a small risk, the risk can be reduced to nil at very small cost. EEII should have addressed why the marker genes were not removed. In any event, some empirical data should be presented to demonstrate that expression levels of these antibiotic resistance genes is in fact low.

Needed Experiments and Observations

1. Amino acid sequence. Provide the amino acid sequence of the expressed Ccry1A protein.
2. Ccry1A expression. Quantify expression levels of Ccry1A in pollen.
3. Marker expression. Quantify expression levels of antibiotic marker genes.
4. Heterologous system. Develop a heterologous expression system to produce sufficient quantities of Ccry1A that can be used for risk assessment research.

Product Quality of EE-1 Bt Brinjal

Conclusion 2. The EE-1 transgene may be a second-rate Bt brinjal product. EE-1 was probably produced in the late 1980s or early 1990s, its control of BFSB is low, and other Cry toxins might perform better.

During the late 1980s, Monsanto developed Event 531, the *cryIAc* transgene in Bt cotton Bollgard I. This transgene was formed from a plasmid with *nptII* and *aad*, which are marker genes for kanamycin and streptomycin resistance respectively (see, e.g., Zambryski 1992). During the early 1990s, Monsanto developed Event 15985, the *cry2Ab* transgene in Bt cotton Bollgard II (McCabe and Martinell 1993). This transgene was formed by co-transformation with two plasmids. One contained *nptII* and the other *uidA*, which was eliminated from the final product by segregation. During the early 1990s, Monsanto developed Mon810, the *cry1Ab* transgene in Bt maize, Yieldgard (AGBIOS 2003). This transgene was formed by co-transformation with two plasmids. Segregation eliminated some of the undesired elements from the final product, which contained only *nptII*. More recent methods used by Monsanto produce transgenes with no antibiotic markers. Because EE-1 contains both *nptII* and *aad*, this suggests that it was produced by Monsanto sometime during the late 1980s or early 1990s, before Event 15985 and Mon810.

As will be reviewed below, efficacy of EE-1 is low. It provides only 73% control of BFSB in the MST field trials (Dossier vol. 6) and sometimes requires an insecticide application to control BFSB. BFSB is in the family Crambidae in the order Lepidoptera. Several other stem boring Crambids have been targeted for control by other Bt crops. These include *Ostrinia nubilalis* (European corn borer), *Diatraea grandiosella* (southwestern corn borer), *Diatraea saccharalis* (sugar cane borer), and *Chilo partellus* (spotted stemborer). Bt maize typically shows 100% control for all these Crambid species.

There may be a better transgene than Ccry1A for control of BFSB. Mon810 is based on the Cry1Ab toxin, which affects the high level of control of Crambids. A comparison of susceptibility in *O. nubilalis* to Cry1Ab and Cry1Ac suggests that it is more susceptible to Cry1Ab than Cry1Ac (Denolf et al. 1993, Marçon et al. 1999). Thus, it is possible that a more modern transgene based on Cry1Ab would control BFSB better and reduce concerns associated with the marker genes.

Given these considerations, it seems clear that the applicant (either Monsanto or Mahyco) has invested little in the development of a useful Bt brinjal product for India. Indeed, an inflammatory characterisation of the process so far would be a case of “transgene dumping.” It would seem in India’s best interest to require a modern transgene that exhibits much better efficacy against BFSB. Such a request, if fulfilled, would eliminate several risk issues associated with inadequate transgene characterisation of EE-1 and would diminish other environmental risk issues, especially related to resistance evolution in BFSB.

Scientific Opinion on Regulatory Compliance

Conclusion 3. The assessment does not comply with the *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (Codex, 2003, CAC/GL 45-2003)*.

Compliance to Codex (2003), *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (Codex, 2003, CAC/GL 45-2003)* is a complicated issue, and this report is not intended to address this issue in a comprehensive manner. Instead, the report focuses narrowly on scientific dimensions related to compliance to Codex (2003). In addition, this report does not evaluate whether or not the applicant has complied with the Indian regulations or the requests of the Indian regulators.

The justification for evaluating compliance to Codex (2003) from a scientific perspective lies in the statement: “The goal of each safety assessment is to provide assurance, in the light of the best available scientific knowledge, that the food does not cause harm when prepared, used and/or eaten according to its intended use. (Codex 2003, paragraph 21, our emphasis).”

This section raises the issue of scientific standards for risk assessment. There are two reasonable standards that could be considered: (1) scientific knowledge that is commonly used by GMO regulatory authorities around the world and (2) new scientific knowledge that can be used for the evaluation of Bt brinjal. Standard (2) would imply that India be a leader in risk assessment and evaluate and adopt new approaches as they become available. Standard (1) only requires that India follow regulatory practice that is commonly used. The purpose here is not to conduct a comprehensive analysis of how the present assessment does not comply with Codex, but to provide a few illustrative examples, using the much lower and conservative standard in (1). Under this standard, it takes only one instance of non-compliance to demonstrate non-compliance to Codex (2003). Here is presented two instances.

Example 1. Information on the DNA insertions into the plant genome should include: “the organisation of the inserted genetic material at each insertion site including copy number and sequence data of the inserted material and of the surrounding region (Codex 2003, paragraph 31C).” Regulatory authorities in the USA, Canada, the European Union, and many other countries require the DNA sequence of the inserted transgene and flanking regions. This information is not reported in EC-II, the Dossier or the Supplemental Materials.

Example 2. “Information should be provided ... to demonstrate whether 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 (Codex 2003, paragraph 33B).” Regulatory authorities in the USA, Canada, the European Union, and many other countries require this information. EC-II and the Dossier report that there are several deliberate modifications made to the amino acid sequence of Ccry1A in the chimeric transgene, but neither provides any information to evaluate potential changes in post-translational modification. In addition, neither document provides information to evaluate their claims that the amino acid modifications do not affect sites critical for determining the structure or function of Ccry1A.

Environmental Risk Assessment

Conclusion 4. Most of the possible environmental risks of Bt brinjal have not been adequately evaluated; this includes risks to local varieties of brinjal and wild relatives, risks to biological diversity, and risk of resistance evolution in BFSB.

This conclusion is developed in considerable detail in the following sections. A brief overview of a few of the main lines or argument are abstracted here as a guide for the reader.

- **Gene Flow.** EC-II and the Dossier did not assess gene flow risks adequately.
- **Biological Diversity.** Some risks to biological diversity were evaluated, but many risks were not considered or not evaluated sufficiently.
- **Resistance Evolution.** Resistance risks were not acknowledged by EC-II. Although the Dossier presented a plan to manage these risks, the plan will probably be ineffective.

India is a world centre of diversity of brinjal and harbours many wild relatives of brinjal, including wild populations of *S. melongena* and allied species. Risks associated with gene flow from Bt brinjal were inadequately evaluated. EC-II and the Dossier did not identify all of the *Solanum* species and subspecies of *S. melongena* that are at risk from Bt brinjal and did not fully consider the risks to existing land races of brinjal. EC-II and the Dossier argued that Bt brinjal does not outcross enough to matter; this is dubious. Many *Solanum* species, *S. melongena* subspecies and brinjal landrace varieties are at risk from gene flow from Bt brinjal. These risks need to be assessed.

There are six kinds of adverse environmental effects that Bt brinjal could have on biological diversity. Only a part of one of these is considered effectively by EC-II. EC-II inappropriately over-relies on assumptions about the specificity of Ccry1A. By doing so, EC-II assumes away many of the potential environmental concerns. It also draws overly broad conclusions about the absence of environmental risk from a limited empirical base. Consequently, the more serious concerns about biological diversity are inadequately assessed, and unnecessary effort was expended on issues of uncertain significance. The more serious concerns need to be assessed.

A real concern is that BFSB will evolve resistance to Bt brinjal, rendering it useless. Resistance is expected to occur within a few years at the localities where hybrid EE-1 Bt brinjal is adopted at a high rate. This is because EE-1 is a low dose event and the GEAC set no requirements to reduce the risk of resistance. In addition, EE-1 may jeopardise better Bt brinjals that could be developed in the future by providing a stepping-stone for rapid resistance evolution.

Gene Flow and Its Consequences

Conclusion 5. Brinjal has considerable valuable genetic diversity in India that could be threatened by gene flow. EC-II, the Dossier, and the Supplemental Materials are inadequate for concluding that Bt brinjal has no significant effects on the biological diversity or weediness of brinjal or its wild relatives.

Brinjal has many local varieties and wild relatives in India that could be threatened by gene flow from Bt brinjal. India is the world's centre of biological diversity of brinjal (land races, cultivars, door-yard plants, etc.), with over 2500 local varieties known. Brinjal has at least one conspecific wild relative in India can introgress with brinjal, *S. melongena* var. *insanum*. Brinjal also has several heterospecific wild relatives in India that can hybridise with brinjal. Thus, the potential threat to the genetic diversity of brinjal and its wild relatives and the potential weediness of brinjal and its wild relatives must be assessed. EEII, the Dossier, and the Supplemental Materials suggested that cultivated brinjal does not form wild populations and gene flow among brinjal populations and from cultivated brinjal to its wild relatives is so low that the risks associated with gene flow are negligible. As detailed below, the data presented are inadequate for concluding that these risks are negligible.

Finding 10. It is highly likely that cultivated brinjal (*Solanum melongena*), including local varieties and landraces have crossed with feral populations of *S. melongena*, and it is possible that cultivated varieties can revert to wild phenotypes and establish feral populations. Therefore, the possible effects of intraspecific gene flow from Bt brinjal to other varieties and populations of brinjal should be examined.

EC-II and the Dossier assert that brinjal does not form volunteer or feral populations. This assertion is not adequately demonstrated, and it is quite possible that brinjal can form feral populations in India. Working in southern India, Deb (1989) noted that "feral forms [of brinjal] are sometimes found." Indeed, wild populations of *S. melongena* are known to occur in India (Choudhury 1995; Sekara et al. 2007). Therefore additional studies are needed to determine the extent of these wild populations and their weediness. In addition, the possibility that Bt brinjal is able to revert to a wild or weedy

phenotype must be examined, because the Bt transgene could spread rapidly by this means into other natural populations of *S. melongena* and transfer the Bt transgene to many local brinjal varieties.

Additional studies on the potential reversion of brinjal cultivars to wild and weedy phenotypes should be conducted. These could be conducted on the parental varieties to Bt brinjal. It is important to evaluate progeny past the F1 generation, as rapid

BOX 3

Why the experimental gene flow estimates from Bt brinjal may be underestimated

- A. The flowering times of the Bt brinjal and the surrounding non-Bt brinjal were not coincident. Relevant data to determine the degree of coincidence were not provided.
- B. The location of the honeybee colonies at the corners of the Bt brinjal plot may enable bees to take very short inter-plant movements to harvest sufficient pollen and/or nectar, thereby underestimating the degree of gene flow (most inter-plant movements would be among Bt brinjal plants or among non-Bt brinjal plants rather than between Bt and non-Bt brinjal plants). By positioning the colonies farther away, bees would have to move farther to find the plot, and then might be more likely to move more frequently between Bt and non-Bt plants, resulting in high rates of outcrossing.
- C. Slight differences in flower morphology between the Bt and non-Bt brinjal might lead bees to specialise foraging on one or the other (but not both), leading to underestimated levels of gene flow.
- D. Native pollinators may be more efficient pollinators of brinjal than honeybees. Most solanaceous species, such as brinjal, are more effectively pollinated by buzz pollinators than by honeybees. If these native pollinators were absent or rare, gene flow would be underestimated.

adaptation or introgression can occur after a weak F1 generation (Mikkelsen et al. 1996, Warwick et al. 2007).

Even if Bt brinjal cannot revert to feral forms, gene flow from Bt brinjal to wild populations of *S. melongena* could result in significant risks. EC-II reported published outcrossing estimates of 0.248% for brinjal in various environments. Thus gene flow from brinjal can be low or high, and the factors determining these rates are poorly known. The Dossier presents information from one (or possibly two; the details were not clear) outcrossing experiment with Bt brinjal conducted during 2002-3 (Volume 5). The Supplemental Materials present the results of an additional gene flow experiment conducted during 2009 conducted at two sites (IIVR 2009).

The level of gene flow from Bt brinjal that was measured was probably close to 1% or less in the 2002-3 experiment (the actual value is not reported). During 2009, it was 0.43% or 0.85% at the two sites. EC-II used these low measured rates in its assessment and ignored the higher rates from the published literature. Gene flow can vary substantially from experiment to experiment, depending on a number of factors (Box 3), so these data provide only weak support for low gene flow from Bt brinjal. Thus, it is possible that the rate of gene flow from Bt brinjal will be low, but it is also possible that the rate of gene flow will be substantially higher under normal conditions of cultivation.

Indeed, based on the sampling design and published literature, it is likely that the gene flow distance was underestimated in the experiments described in the Dossier and Supplemental Materials. Gene flow up to 30 meters was reported in the Dossier (Vol. 5), but the farthest distance examined was 50 meters during 2002-3. The 2009 experiments made observations up to 300 meters away, but sampling was intensive only in the first 30 meters (1200 plants per site = 400 plants per 10 meters of distance from Bt brinjal). After 30 meters, fewer plants were sampled per location with a much lower density of sampling. Previous studies on bee-pollinated plants demonstrate that gene flow distances can be hundreds of meters and even several kilometers (St. Amand et al. 2000, Rieger et al. 2002, Pasquet et al. 2008). Thus, it is possible that the distance of gene flow is farther than considered.

What are significant and insignificant levels of gene flow? Although this is a challenging problem, rates as low as 0.5% can be biologically significant. If the trait has an advantage in the recipient population and/or the trait is recurrently introduced (Haygood et al. 2003), such “low” rates of gene flow could result in rapid introgression in the recipient population. Thus, even the experimentally measured rates of gene flow could be biologically significant, and EC-II is incorrect to assert that “low” rates of gene flow are sufficient by themselves to conclude that there are low gene flow risks.

Needed Experiments and Observations

It could be useful to conduct additional outcrossing experiments to establish that gene flow does occur at evolutionary significant rates. These outcrossing experiments should be conducted under conditions facilitating gene flow, examining distances farther than 30 meters with larger sample sizes or alternate methodologies (see St. Amand et al. 2000, Rieger et al. 2002, Pasquet et al. 2008). The densities of wild bees that are capable of pollinating brinjal, the occurrence of bee visitation between source and recipient brinjal, and the overlap of flowering times between source and recipient brinjal should be monitored during these experiments.

However, conducting these additional gene flow experiments could be a waste of time, unless it is possible to estimate the true mean gene flow rate and its variance. Such work would take a considerable amount of time and effort, because there are many experimental factors that bias estimates downward. A simpler alternative might be to estimate a reasonable worst-case value for gene flow and evaluate the consequences stemming from such a worst-case estimate. It is a standard method of risk assessment to evaluate worst-case scenarios because they provide considerable support to a risk assessment when the risks and effects are found to be low even under the worst-case assumptions.

A more productive experimental approach for risk assessment would be to assume that intraspecific gene flow is sufficiently high that risks could possibly occur, and focus efforts on evaluating the consequences of gene flow. As noted above, rather small amounts of gene flow can be significant, and the present data are insufficient to conclude that the rates of gene flow are so low that there are no significant risks. In addition, the extant scientific literature on gene flow (Ellstrand 2003) also suggests that it might be reasonable to conclude that intraspecific gene flow occurs at evolutionarily significant rates.

1) Fitness of intraspecific hybrids and backcrosses. Risk assessment research should shift to investigate the consequences of

this gene flow by studying the relative fitness of hybrids and backcrosses between Bt brinjal and brinjal landraces and between Bt brinjal and intraspecific wild relatives, such as *S. melongena insanum*. Such investigations would enable estimation of potential weediness and effects on genetic diversity.

Finding 11. There is likely to be natural crossing between Bt brinjal and wild species related to brinjal.

Hybridisation with perhaps as many as 29 wild relative species needs to be evaluated carefully and the consequences of any hybridisation that occurs needs to be evaluated.

EC-II (page 56) states “It has been reported that there is no natural crossing among cultivated and wild species of brinjal including *S. incanum* ... (Rao, 1979).” However, EC-II and the Dossier contain contradictory evaluations of the possibility of crossing with other *Solanum* species. The Dossier (Vol 1, page 25) states that hybrids between *S. melongena* and *S. incanum* can be produced, yet later, it states that risks associated with such hybrids need not be evaluated because they do not occur. EC-II bases their conclusion of no natural crossing between cultivated brinjal and wild species on a single, older paper by Rao (1979). Rao (1979) actually found that *S. melongena* and *S. incanum* can cross and produce fertile offspring, but he thought that crossing was difficult and speculated that interspecific crosses were unlikely in nature.

The Supplemental Materials report the results of a literature review (Singh 2009?) and two experiments examining between-species hybrid formation (IIVR 2007a, b). Singh (2009?) concludes that the centre of origin of brinjal is not conclusively known, although India is perhaps as likely as any of the other two possibilities.

The location of the centre of origin of brinjal is not the most important consideration for risk assessment. It is more critical to know if there is significant brinjal biological diversity in India that could be threatened by Bt brinjal. There is no doubt that India is the world’s centre of biological diversity for brinjal, with over 2500 varieties known.

Singh (2009?) focuses his argument on the potential effects of hybridisation between *S. melongena* and *S. incanum*. He argues that there will be no adverse effects from any hybridisation between Bt brinjal and *S. incanum*. He states,

“It is known that *S. melongena* and its wild relatives such as *S. incanum* and *S. insanum* co-exist in nature since ages. Even though 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. This clearly establishes that the natural cross-pollination does not affect weediness characteristic and wild relatives and vice-versa.”

At its surface this seems to be a compelling argument. Essentially, Singh is suggesting that the natural barriers to gene flow will prevent *S. melongena* from influencing the evolution of *S. incanum*, despite the fact that hybrids between the species can be formed. This argument, however, does not hold up to scrutiny. First, there are many cases where hybridisation between two plant species was low, yet the hybrids still managed to influence the evolution of the parental species (Ellstrand 2003). In other words, the argument is not generally valid. Second, the concern is over two specific issues – can the Bt transgene change *S. incanum* to either make it more weedy or to reduce its genetic diversity. If the Bt transgene gives *S. incanum* a selective advantage or if gene flow is recurrent, the Bt transgene could spread quickly even if the rate of hybridisation is low. Third, one of the key premises of the argument is not known. We do not know if *S. melongena* and *S. incanum* have not affected each others’ diversity in natural populations. It seems equally likely that they have affected each others’ diversity but we have not detected it. No longitudinal data can be provided to adjudicate between these alternatives. Thus, Singh’s (2009?) straightforward argument is too weak to support the conclusion that Bt brinjal will not have any significant effects on *S. incanum*. This theoretical argument is insufficient, and empirical data are needed to evaluate potential effects of Bt brinjal on *S. incanum*.

IIVR (2007a) reports that interspecific crosses between *S. melongena* and *S. incanum* occur readily when *S. melongena* is the seed parent, but not very often when *S. incanum* is the seed parent. Such asymmetrical hybridisation has been used to suggest that the Bt transgene will not leave Bt brinjal for *S. incanum*, even though *S. incanum* genes could enter the Bt brinjal genome.

Unilateral incompatibility, where brinjal pollen does not cross out, but brinjal can receive pollen from other *Solanum* species remains a gene flow risk because farmers can plant and spread these interspecific hybrids, which may be fully (not unilaterally) compatible with the wild relatives. If the hybrids are fully compatible, then the transgene can readily cross into the wild relative. Thus, it is essential to evaluate the crossability of the F1 hybrid to the wild relative (F1 backcross) before it can be concluded that genes from *S. melongena* cannot invade wild relatives. EC-II, the Dossier and the Supplemental Materials have not evaluated any F1 backcrosses.

In conclusion, hybridisation between brinjal and *S. incanum* does occur, and gene flow risks from Bt brinjal to *S. incanum* have not been properly assessed.

Gene flow risks associated with other wild relatives of brinjal have been poorly evaluated. The taxonomy and phylogeny of *S. melongena* and its wild relatives is still being developed (Box 4). Brinjal is in the monophyletic subgenus *Leptostemonum*, which contains 495 taxa. The classical classification of the subgenus into Sections and Series is not valid, and even Whalen's (1984) excellent work dividing the subgenus into species groups probably contains a few errors. Modern phylogenetic work is not complete enough to place all of these species into monophyletic clades, and the relationship of many of the species to brinjal remains uncertain. Twenty-six species in *Leptostemonum* are known to occur in India (Box 4), and it is possible that additional species occur, but have not been reported. The taxonomic affinity of 12 of these species to brinjal is not known. Many of these species have been assigned names that are different from the names used in the literature cited in EC-II, the Dossier, and the Supplemental Materials. Most of the names used in EC-II, the Dossier and the Supplemental Materials are invalid synonyms. A list of several of the wild relatives of brinjal in India with their common synonyms is given in Box 5. A quick perusal of this list should convince the reader that the taxonomy of the species in subgenus *Leptostemonum* is complex and not for the faint of heart. These synonymies are essential to understand the literature on interspecific hybridisation because many papers are published using synonyms.

BOX 4

Taxonomy of *Solanum melongena* L.

The phylogenetic relationships among taxa related to brinjal is still poorly understood. Brinjal (*Solanum melongena* L.) is a member of the subgenus *Leptostemonum* (Levin et al. 2006), one of the 13 major clades within the genus *Solanum*. *Leptostemonum* contains 495 valid taxa (SS 2010) in 10 clades (Levin et al. 2006). Brinjal is in the Old World clade, which is the largest in the subgenus. The classically identified Sections and Series are only partially supported by modern data, so these classical groups should not be relied on for understanding species relationships and crossability. The phylogenetic relationships within the Old World clade remains uncertain (Levin et al. 2006, Isshiki et al. 2008).

Table B2.1. Species in subgenus *Leptostemonum* with a South Asia distribution (CNBD = Could Not Be Determined; H = fertile hybrids known). Data from SS (2010) and Choudhary and Gaur (2009)

Species	Clade within subgenus <i>Leptostemonum</i>	Status in South Asia
<i>Solanum aculeatissimum</i> Jacq.	Acanthophora	
<i>Solanum aethiopicum</i> L.	Old World	H
<i>Solanum anomalum</i> Thonn.	Acanthophora	H
<i>Solanum barbisetum</i> Nees	CNBD	
<i>Solanum capsicoides</i> All.	Acanthophora	introduced
<i>Solanum chrysotrichum</i> Schldl.	CNBD	possibly naturalised
<i>Solanum coagulans</i> Forssk.	CNBD	
<i>Solanum cordatum</i> Forssk.	CNBD	
<i>Solanum forskalii</i> Dunal	CNBD	possibly introduced
<i>Solanum giganteum</i> Jacq.	Old World	probably monophyletic
<i>Solanum hirtum</i> Vahl	Lasiocarpa	probably introduced
<i>Solanum hovei</i> Dunal	CNBD	
<i>Solanum incanum</i> L.	Old World	H
<i>Solanum lasiocarpum</i> Dunal	Lasiocarpa	
<i>Solanum macrocarpon</i> L.	Macrocarpon	H
<i>Solanum multiflorum</i> Roth ex Roem. & Schult.	CNBD	
<i>Solanum pubescens</i> Willd.	CNBD	
<i>Solanum robustum</i> H.L.Wendl.	Robustum	possibly introduced
<i>Solanum torvum</i> Sw.	Torva	
<i>Solanum trilobatum</i> L.	CNBD	
<i>Solanum undatum</i> Lam.	CNBD	
<i>Solanum viarium</i> Dunal	Acanthophora	long naturalised
<i>Solanum violaceum</i> Ortega	Old World	H
<i>Solanum virginianum</i> L.	Old World	H
<i>Solanum wightii</i> Nees	CNBD	
<i>Solanum wrightii</i> Benth.	CNBD	

Brinjal is not known to hybridise successfully with any species of *Solanum* outside of the subgenus *Leptostemonum*. Hybridisation within the subgenus probably occurs more readily between brinjal and its more closely related species, however, because the taxonomy of the subgenus is only now beginning to be understood using modern phylogenetic methods, it is not yet possible to know the relationships of all of the species to brinjal.

To determine if there are possible risks associated with gene flow to wild relatives, it is essential that a risk assessment identify the possible recipient species. EC-II, the Dossier and the Supplemental Materials do not identify the possible recipient species. This hampers their ability to conduct a valid risk assessment of the gene flow risks.

Weediness risks can be assessed by identifying wild relatives that have the potential to become weedy. The most logical relatives are those that may already be weedy. This has not been considered in EC-II, the Dossier, or the Supplemental Materials.

Species that are invasive in India, or that have recently invaded may be actual or potential weeds. Several species in *Leptostemonum* are possibly or probably naturalised or introduced into India (Box 4). These include *Solanum capsicoides* All., *Solanum chrysotrichum* Schltld., *Solanum forskalii* Dunal, *Solanum hirtum* Vahl, *Solanum robustum* H.L.Wendl., and *Solanum viarum* Dunal. In addition, *Solanum sisymbriifolium* has probably been introduced into India. Any of these species could be weedy or have their weediness enhanced by the Bt gene in Bt brinjal. This risk has not been adequately assessed and should be assessed.

Other gene flow risks can be partially assessed first by examining the potential for hybrid formation. Several species in the subgenus *Leptostemonum* are known to form fertile hybrids with *S. melongena* (Box 6). These include close relatives, *S. incanum* and *S. violaceum*, but also much more distant species, including *S. macrocarpon*, *S. aethiopicum* and *S. virginianum*. Four additional species, which are phylogenically even more distant from *S. melongena*, are also known to form fertile hybrids. Given the uncertainty associated with the phylogenetic relationships within the subgenus, it is not feasible to predict which species in the subgenus can and cannot form fertile hybrids with *S. melongena* without testing each species.

Cross-compatibility experiments are known to be notoriously difficult. Singh (2009?) notes that successful hybridisation is lowest when hand-pollination is used and highest when the natural pollinators are allowed to visit flowers. IIVR (2007a, b) rely on hand pollination to estimate hybridisation rates, so their results should be considered an underestimate of the true rate of hybridisation. IIVR (2007a) reports on interspecific hybridisation rates with five species of *Solanum*. The exact species used in these tests is not precisely known, because IIVR (2007a, b) did not report full taxonomic information about these species. Presumably, the species in IIVR (2007a) were *Solanum incanum* L., *Solanum violaceum* Ortega, *Solanum sisymbriifolium* Lam., *Solanum torvum* Sw., and *Solanum nigrum* L. IIVR (2007b) examined only *Solanum incanum* L. The results for *S.*

Box 5

Some important synonymies

Table B3-1. Some species synonymies. *S. indicum* Roxb. and *Solanum tomentosum* Herb. Hasselt. ex Miq. and all names under *S. torvum* are invalid names. Data from SS (2010)

Valid species name	Synonyms
<i>Solanum melongena</i> L.	<i>Solanum insanum</i> L. <i>Solanum oviferum</i> Salisb. <i>Solanum ovigerum</i> Dunal <i>Solanum esculentum</i> Dunal <i>Solanum edule</i> Schumach. & Thonn. <i>Solanum aethiopicum</i> var. <i>violaceum</i> Dunal
<i>Solanum aethiopicum</i> L.	<i>Solanum gilo</i> Raddi. <i>Solanum integrifolium</i> Poir.
<i>Solanum anomalum</i> Thonn.	<i>Solanum warneckeanum</i> Dammer <i>Solanum lescrauwaetii</i> De Wild. <i>Solanum marquesii</i> Dammer
<i>Solanum grandiflorum</i> Ruiz & Pav.	<i>Solanum macranthum</i> Dunal
<i>Solanum incanum</i> L.	<i>Solanum unguiculatum</i> A.Rich. <i>Solanum melongena</i> var. <i>incanum</i> (L.) Kuntze
<i>Solanum macrocarpon</i> L.	<i>Solanum dimorphum</i> Matsum. <i>Solanum atropo</i> Schumach. & Thonn. <i>Solanum mors-elephantum</i> Dammann
<i>Solanum torvum</i> Sw.	<i>Solanum campechiense</i> Hort. <i>Solanum maccai</i> Bertero <i>Solanum mammosum</i> Herb.
<i>Solanum undatum</i> Lam.	<i>Solanum cumingii</i> Dunal <i>Solanum tomentosum</i> Herb. Hasselt. ex Miq.
<i>Solanum violaceum</i> Ortega	<i>Solanum indicum</i> L. <i>Solanum kurzii</i> Brace ex Prain <i>Solanum chinense</i> Dunal <i>Solanum pubescens</i> var. <i>lobatum</i> C.B.Clarke <i>Solanum indicum</i> Roxb.
<i>Solanum virginianum</i> L.	<i>Solanum virginicum</i> L. <i>Solanum mairei</i> H.Lév. <i>Solanum xanthocarpum</i> Schrad. & J.C.Wendl. <i>Solanum surattense</i> Burm.

BOX 6

Taxa known to form fertile hybrids with *Solanum melongena* L.

The taxonomy of *Solanum melongena* L. and related species has changed over the years as modern taxonomic methods have been brought to bear. Consequently the status of published work documenting hybridization with *S. melongena* should be re-evaluated in light of these more modern taxonomic concepts.

1. The species concept of *Solanum melongena* L. now includes wild relatives that used to be considered separate species, the most notable of which was *S. insanum*, now *S. melongena* var. *insanum*. Cultivated varieties of *S. melongena* can cross with *S. melongena* var. *insanum* (Ali and Fujiera 1989, Deb 1989, Lester and Hasan 1991a, Karihaloo and Gottlieb 1995, Karihaloo et al. 1995, Kashyap et al. 2003).
2. *Solanum incanum* L. has been considered to be closely related to *S. melongena* L. Modern methods show it is a distinct taxon from *S. melongena*, and it may be closely related to *S. melongena* (Furini and Wunder 2004), or as distantly related as *S. macrocarpon* L. (Isshiki et al. 2008) or of intermediate status (Levin et al. 2006). Fertile hybrids of *S. melongena* and *S. incanum* occur (Deb 1989, Lester and Hasan 1991a, Karihaloo and Gottlieb 1995, Karihaloo et al. 1995, Behera and Singh 2002) and Viswanathan (1975) found a putative hybrid between *S. melongena* and *S. incanum* growing along a roadside in Kerala, India.
3. *Solanum violaceum* Ortega has been considered to be closely related to *S. melongena*, but not as close as *S. incanum*. *Solanum indicum* L. has been synonymized to *S. violaceum* Ortega. Modern methods place it closer to *S. melongena* than *S. macrocarpon* (Levin et al. 2006, Isshiki et al. 2008), but it may be a closer (Isshiki et al. 2008) or more distant (Levin et al. 2006) relative than *S. incanum*. Fertile hybrids are formed with *S. melongena* (Ali and Fujieda 1990, Patel et al. 2001, Behera and Singh 2002 [as pollen parent]), although some hybrids are only partially fertile (Kashyap et al. 2003).
4. *Solanum macrocarpon* L. has classically been included in the *Macrocarpon* Series or the *S. macrocarpon* species group (Whalen 1984), which is separate from the close relatives of *S. melongena* L. Modern taxonomic studies support this separation (Furini and Wunder 2004, Levin et al. 2006, Isshiki et al. 2008, Polignano et al. 2010). Fertile hybrids have been formed (Schaff et al. 1982), but not always (Gowda et al. 1990).
5. *Solanum aethiopicum* L. has classically been included in the *Anguivi* Series or the *S. anguivi* species group (Whalen 1984) and modern analysis suggests that it is more closely allied with *S. macrocarpon* than *S. melongena* (Furini and Wunder 2004, Levin et al. 2006). Fertile hybrids are produced (Ali and Fujieda 1990, Behera and Singh 2002 [as pollen parent]), but some hybrids are sterile and some are only partially fertile (Kashyap et al. 2003).
6. *Solanum virginianum* L. has been considered more distant than any of the previously mentioned species. Modern methods place it closer than *S. incanum* and *S. macrocarpon* (Isshiki et al. 2008) or more distant than all of the species discussed here (Levin et al. 2006). Partial fertility was established by Ali and Fujieda (1989), and Kashyap et al. (2003) report that hybrids are mostly fertile.
7. Four additional species have been reported to form viable hybrids with *S. melongena*. These are *Solanum anomalum* Thonn., which forms viable hybrids as the seed parent (Behara and Singh 2002), *Solanum grandiflorum* Ruiz & Pav. (Rameriz 1959), *Solanum sodomium* (Tudor and Tomescu 1995), and *Solanum undatum* Lam. (Capinpin et al. 1963, Fukusawa 1964). The affinities of these taxa with *S. melongena* has not been investigated with modern techniques. *S. sodomium* has been classically grouped with *S. virginianum* in the *Sodomela* Series.

incanum have already been addressed above, so the other four species are considered here. One of the species tested, *S. nigrum* is not in the subgenus *Leptostemonum* (SS 2010), and therefore cannot hybridise with brinjal. The remaining three species are in *Leptostemonum*. No viable hybrids were formed with any of these species, *S. violaceum*, *S. sisymbriifolium*, and *S. torvum*. *Solanum sisymbriifolium* is a South American species that must have recently invaded India. Fertile hybrids between *S. melongena* and either *S. sisymbriifolium* or *S. torvum* have never been reported, despite multiple attempts (Kashyap et al.

2003). IIVR (2007a) can be considered to confirm these previously published results. However, multiple papers have reported successful hybridisation between brinjal and *S. violaceum* (Box 6). Consequently the results in IIVR (2007a) for *S. violaceum* must be considered anomalous. Taking all of these issues into consideration, as a whole, the data in IIVR (2007a) are rather uninformative for assessing the gene flow risks associated with Bt brinjal.

Nine species of *Leptostemonum* are known to hybridise with brinjal (Box 6), and six of these species are known to occur in India. Twenty-six species of *Leptostemonum* occur in India, and the brinjal hybridisation potential for almost 20 of these species is unknown. Only three of the 26 species were even considered by EC-II, the Dossier, or the Supplemental Materials. Thus there was insufficient information for concluding that gene flow risks are low.

Needed Experiments and Observations

Additional analysis and experimentation should be conducted so that gene flow risks to other species of *Solanum* in subgenus *Leptostemonum* can be adequately assessed (numbering is continued from previous section):

2) Identification of wild relatives. Additional effort should go into determining if there are more species of *Leptostemonum* that occur in India. The nation's herbaria should be examined and taxonomic experts in the group should be consulted.

3) Hybridisation. The 26 species listed in Box 4, the three additional species listed in Box 6, and any new species identified in the previous efforts in 1) should be carefully analysed to determine which species are known not to hybridise with brinjal.

4) Overlap. For the species that remain from 2), if the data exist, the timing of flowering and the geographic distribution should be characterised to determine the extent of overlap with brinjal.

5) Cross-compatibility. For species with overlap (and species for which the information is insufficient to conclude that there is no overlap), cross-compatibility between the species and brinjal should be evaluated experimentally using methods that will provide more accurate estimates of successful hybridisation. To include relevant genetic diversity, the hybridisation studies should use recently collected wild and weedy genotypes (not older accessions) from a range of habitats and geographic areas in India (e.g., see Karihaloo and Gottlieb 1995), especially regions where brinjal production is concentrated. These experiments should be designed to confirm previously published results on successful and unsuccessful hybridisation so that the data on new species can be considered to be reliable. If the previous studies cannot be confirmed, it will be necessary to conduct additional experiments to explain why. By doing this, the data from the hybridisation experiments will be more reliable.

6) Fitness of interspecific hybrids and backcrosses. For the species in Box 6 and any other species forming fertile hybrids with brinjal, experiments should be conducted to determine the relative fitness of crosses between the hybrids and the wild parent (this cross is called an F1 backcross). If the fitness of the backcross progeny is greater than the F1 hybrid, then the species should be considered at risk of gene flow.

7) Gene flow rates. It will probably be a waste of time to conduct additional field experiments to estimate gene flow rates from Bt brinjal to the wild species. It is difficult to obtain unbiased estimates of gene flow, and for the purpose of risk assessment it can be assumed that gene flow is high enough to be evolutionarily and ecologically meaningful.

Finding 12. There is insufficient evidence that wild or weedy relatives of brinjal would not obtain a fitness benefit from a Bt transgene should gene flow occur. There is insufficient evidence that wild relatives of brinjal will not suffer reduced genetic diversity from the introgression of the Bt transgene. There is insufficient evidence that non-GM brinjal will remain uncontaminated by Bt brinjal.

EC-II (page 56, our emphasis) asserts "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." No evidence is provided to substantiate these claims. There are no published studies on the arthropod herbivores (including lepidoptera) inhabiting wild populations of *S. melongena*, *S. melongena insaanum*, or *S. incanum* or any of the other wild relatives potentially at risk (Box 4 and Box 6). Thus, it is possible that some wild relatives of brinjal would gain an advantage from the Bt transgene.

The host range of BFSB is uncertain. Even if EC-II is correct in that BFSB prefers to feed on brinjal, it is the suitable host range, not the preferred host range that is relevant for assessing this risk. All things being equal, the preferred host will

suffer greater damage than the less preferred host. However, in real ecological conditions, all things are never equal, and there are many circumstances where a less preferred plant is damaged more than the more preferred one. Thus, the suitable host range of BFSB provides the best basis for understanding the effects of gene flow on attack by BFSB. The suitable host range of BFSB is quite broad (Table 2), and includes some plants not even remotely related to brinjal. Thus, it must be concluded that the host range of BFSB is not well known.

Moreover, the known host range of BFSB includes two species with which brinjal can hybridise. These are *Solanum violaceum* Ortega and *Solanum virginianum* L. It is also likely that BFSB feeds on wild populations of *S. melongena*, such as *S. melongena* var. *insanum*. All of these taxa could obtain a fitness benefit from the Bt transgene, yet these possibilities are not considered by EEII, the Dossier, or the Supplemental Materials. Overall, the assessment of weediness is overly simplistic and ignores of the published literature related to the problem.

The aggressiveness study cited in EC-II is insufficient to assess weediness. The negative results from the aggressiveness test are inconclusive (Dossier, vol. 5), because there is no information about the level of seed deposition. It is not possible to know if the negative results are due to the lack of aggressiveness of brinjal or because of the lack of seed deposition.

Moreover, the results do not address the more serious potential aggressiveness associated with wild or feral populations of *S. melongena*, such as *S. melongena* var. *insanum*. Because brinjal can form wild populations, the results from the aggressiveness study focusing only on cultivars may be misleading.

There is no effort to determine if the genetic diversity of any of the wild relatives could be adversely affected. Hybridisation with *S. incanum* occurs at significant enough rates that the threat to its genetic diversity should be considered. Effects on genetic diversity of the recipient population are likely only when introgression is very high (Haygood et al. 2002, Ellstrand 2003). Thus, there is an *a priori* expectation that loss of genetic diversity is unlikely. However, empirical data coupled to a theoretical understanding of the problem are needed to provide assurance that the *a priori* expectations hold in this case.

There is also no effort to determine if Bt brinjal will contaminate non-GM brinjal, thereby affecting the quality of non-GM brinjal. Several kinds of impacts need to be considered:

- Organic brinjal production. Will contamination result in loss of organic status, classification or price?
- Export brinjal. Will contamination affect the export demand for Indian brinjal?
- Non-GM brinjal. Will the market for brinjal in India segment and will contamination affect the price of non-GM brinjal?
- Religiously significant brinjal. Will contamination compromise the sacredness of brinjal varieties with religious significance, such as *udipigulla*?
- Culturally significant brinjal. Will contamination compromise the cultural value of brinjal, such as the brinjal used in wedding ceremonies?

Needed Experiments and Observations

Field studies should be carried out to evaluate the risks of weediness and loss of genetic diversity (numbering is continued

Table 2. Host plants of BFSB, <i>Leucinodes orbonalis</i> , as reported in Waterhouse 1998	
Host Plant Species	Authority
<i>Solanum violaceum</i> Ortega	Menon 1962
<i>Solanum viarum</i> Dunel	Dhankhar et al. 1982
<i>Solanum myriacanthum</i> Dunal (shoots only)	Isahaque and Chaudhuri 1983
<i>Solanum virginianum</i> L.	Menon 1962
<i>Solanum nigrum</i> L.	Nair 1967; Das and Patnaik 1970; Isahaque and Chaudhuri 1983
<i>Solanum tuberosum</i> L. (shoots only)	Fletcher 1916; Mehto et al. 1980; Isahaque and Chaudhuri 1983
<i>Solanum lycopersicum</i> L.	Hargreaves 1937; Das and Patnaik 1970
Cape gooseberry	Pillai 1922
Green pea pods	Hussain 1925
Mango shoots	Hutson 1930
Cucumber, sweet potato, capsicum	Whittle and Ferguson 1987

from previous section).

8) Identify pests of wild relatives. Determine whether BFSB or other susceptible lepidopteran species feed on wild and weedy relatives of brinjal (*S. melongena*, *S. melongena* var. *insanum*, *S. incanum*, and other relevant species). Cohen et al. (2008) provide a sampling design for this type of study.

9) Fitness advantage of Bt transgene. If lepidopteran larvae are present at any stage of the life cycle of these wild or weedy relatives of brinjal, further studies should be carried out to determine whether a Bt transgene could enhance the survival or fecundity of wild/weedy plants under a variety of field conditions, thereby providing a fitness advantage. Snow et al. (2003) provide an experimental and sampling design for this type of study.

10) Quantification of fitness advantage. If this is the case, the Bt transgene is likely become more common in wild or weedy populations that hybridise with the crop. Further studies could be carried out to evaluate whether the fitness advantage is large enough to result in more problematic weed populations, whether the expected ubiquity of Bt toxins in wild or weedy populations could have unwanted effects on non-target lepidopteran insects, and whether the rapid spread of the Bt transgene (and linked crop genes) would negatively affect genetic diversity in wild relatives to a greater extent than ongoing gene flow from non-transgenic eggplant.

Risks to Biological Diversity

Conclusion 6. Most of the main kinds of possible adverse effects of Bt brinjal on biological species diversity and ecosystems have not been evaluated sufficiently. The scientific evidence does not support the EC-II conclusion (page 42), Bt brinjal event EE-1 “has no adverse impact on non-target organisms including beneficial organisms and soil microflora.”

The main potential adverse effects of a Bt crop to other species are:

- (A) Increased secondary pests, either through direct enhancement or through the reduction of natural enemy controls or other means;
- (B) Reduction in soil quality or health, adversely affecting crop production in either the short or long term;
- (C) Reduced value of non-crop economic activities (such as honey production or wild food harvesting);
- (D) Reduced cultural value by affecting a cultural icon or a species of cultural significance (e.g., Monarch butterfly in the United States);
- (E) Increased conservation concern, such as an adverse effect on an endangered species;
- (F) Reduced environmental quality through an effect on an ecosystem service such as pollination;
- (G) Increased human disease via environmental change.

For Bt brinjal, (G) is unlikely, so a good risk assessment will focus on (A) through (F), allocating the most effort to address the most important issues. The conclusion follows from the following scientific considerations.

Finding 13. The over-reliance of the GEAC risk assessment on the specificity of the Cry1Ac protein (EC-II, page 37) is not scientifically justified, and the risk assessment is considerably more uncertain than presently indicated.

The GEAC risk assessment bases its analysis of effects on other species on an assumption about the specificity of Cry1Ac, which is stated in EC-II (page 37) and the Dossier (volume 1, page 38) (detailed below). There are three critical weaknesses to this specificity hypothesis that renders it a poor assumption on which to base the assessment.

First, the EC-II assessment states: “... non-target insects lack receptors for the proteins on the surface of their gut cells.” The actual evidence that all non-target species lack receptors for Cry1Ac on the midgut epithelium is quite poor. Toxicity in arthropods is incompletely known, but present understanding involves interaction of the Cry toxins with at least two gene products produced by the organism. Genome sequencing work to date has shown that vertebrate lineages do not have any

homologous loci, and therefore cannot be affected by known toxicity pathways. All arthropod lineages have homologous loci and therefore can possibly be adversely affected by Cry toxins. The toxicity of Cry1Ac has been tested on <100 arthropod species. There are more than 120,000 species of Lepidoptera alone (Southwood 1975) and over 60% of the world's biological diversity comprises arthropod species. A recent review of the literature on the effects of Cry toxins on arthropod natural enemies in laboratory studies (a small segment of the possible biological diversity that can be affected) has found that Cry toxins have direct effects on some of these species even though the toxicity mechanism is not fully known (Lövei et al. 2009, Shelton et al. 2009a, b, Andow et al. 2009, Andow and Lövei 2010).

Consequently, the scientifically accurate statement is “non-target insects probably lack receptors for the proteins on the surface of their gut cells.” The uncertainty inherent in this more scientifically accurate statement should suggest that it is an assumption and a poor basis on which to build conclusions in an environmental risk assessment.

Would a company that is developing a Bt product be willing to do so because they presume the presence of the necessary receptors in the target organism, or would they feel compelled to demonstrate the occurrence of those receptors before investing money? This is the analogous situation to basing a conclusion of safety on the presumed absence of receptors.

Second, while we know that one mechanism of toxicity of Cry toxins to arthropods involves binding to receptors in the midgut epithelium, followed by other reactions that lead to perforation of the midgut and death, we cannot be sure that this is the only mechanism by which Cry toxins can affect biological species. Because the specificity hypothesis is based on this one toxicity mechanism, it prejudices the risk assessment to consideration of a narrower range of possibilities than should be appropriate.

Third, the assessment (Dossier) states “The Cry1Ac protein expressed in Bt brinjal shows strict host-range sensitivity for lepidopteran insects and has no deleterious effects on non-target organisms (volume 1, page 38).” “Strict host-range specificity” must be considered a hypothesis and “has no deleterious effects” is an overstatement. Because host range studies of Cry1Ac are based on relatively few taxa, we know the Cry1Ac is toxic to a small range of Lepidoptera (although not all of them, and even some closely related species have widely varying sensitivity), but we do not know that it is not toxic to all other arthropods.

The consequence is that the assessment should not rely on toxin specificity as a key pillar to its argument, as it presently does (EC-II, page 37, and Dossier, Volume 1, page 38). The assessment can and should use the specificity hypothesis in a weight of evidence argument, but this is not done in the present assessment. As a result, the risk assessment in EC-II is considerably more uncertain than it presently indicates.

Finding 14. Only 1 of 7 species tested in the laboratory to assess environmental risk occur in brinjal fields in India. These laboratory tests provide little relevant information about the potential impact of Bt brinjal on species in India.

Seven species were tested in the laboratory using toxicology tests with native Cry1Ac toxin. Of these species, only *A. mellifera* occurs in brinjal fields in India, and could be of economic significance itself. The remaining 6 species are not associated with brinjal in India or it cannot be determined. For example, the species identity of the ‘green lacewing’ used in the tests is uncertain, because it is called *Chrysopa carnea*. This taxon has been reclassified and split into multiple species. If the tests were actually done on the species that now occurs in India, then it cannot be *Chrysoperla carnea*, and the locality of the population used should be provided. The other five species are surrogate species, and do not occur in brinjal fields. For example, *Nasonia vitripennis* parasitises houseflies and has no association with brinjal. Although the surrogate species approach remains widely used for pesticide testing, their use has been severely criticised as unreliable since the late 1980s (e.g., Suter 2007). Extensive research has shown that every species responds in its own way, and no species is a good representative for any other species other than itself.

These tests do not help determine whether or not Bt brinjal has an effect on non-target species. First, it was not clear what adverse environmental effects these studies were designed to assess (among the possibilities A-F described above), so it is not

clear what inferences about environmental safety were intended to be drawn from the studies. Second, the Dossier did not contain any of the experimental details for these studies, so it was not possible to assess independently the reliability of the summarised results.

Third, these laboratory studies used native Cry1Ac toxin, not the chimeric Ccry1A toxin that is found in Bt brinjal. There is no need to use native Cry1Ac when Ccry1A could be produced in heterologous systems (See transgene characterisation chapter). EC-II (page 78) states that “the [native Cry1Ac] protein ... has been demonstrated to be biochemically and functionally similar to the one produced in Bt brinjal event EE-1 through a series of tests.” Biochemically, the native Cry1Ac is not identical to Ccry1A in Bt brinjal, because it is recognised as different (EC-II, page 66). None of the functional tests are described and none of the data are provided in EC-II or the Dossier, so the claim cannot be evaluated. At the bare minimum, these functional tests should demonstrate similar uptake, metabolism and degradation *in vivo* under relevant environmental conditions, which are the minimum standards for proving functional similarity for generic drugs. In addition, similarity should be assessed using statistical equivalence tests. Functional tests should also include competitive equilibrium and non-equilibrium binding studies to brush-border membrane vesicles with Cry1Ac, Cry1Ab, or Cry1Aa receptors, competitive absorption and release studies on substrates such as clay minerals and fractionation columns, and proteinase digestion studies examining the rate of degradation and the rate of end-product formation for several different proteinases found in insects and other species.

It is essential to identify appropriate indicator species that occur in Indian brinjal fields and correspond to one or more of the kinds of possible adverse effects (A-F above) that need to be assessed, and then to assess impacts on these species in ecologically relevant ways. One approach is provided by Hilbeck et al. (2008).

Finding 15. EC-II examines the risk of potential non target pests, but overstates conclusions based on limited and highly variable data. The limited field trial data suggest that phytophagous mites will not become secondary pests of Bt brinjal, but the data are insufficient to draw conclusions about whether the other important non-target pests will or will not become secondary pests.

The most useful non-target data used in the EC-II assessment is from the series of limited field trials conducted at 19 sites (Multi-site trials, Dossier, volume 6). These studies address potential adverse effect (A) increased secondary pests, either through direct enhancement or through the reduction of natural enemy controls. This risk is one of the most important to consider for brinjal producers, because a secondary pest outbreak on Bt brinjal could cause crop failure, excessive debt, and loss of livelihood, or increased insecticide or miticide use. For example, mealy bugs have become a serious pest of Bt cotton in India. While it is not clear that the cause is related to the Bt transgene or to the genetic background of the Bt cotton varieties, this has had serious detrimental consequences to farmers.

Based on examination of the results reported in the Dossier (volume 6), the following species and groups of species had sufficient data to interpret: aphids (primarily *Aphis gossypii*), leafhoppers (primarily *Amrasca devastans*), thrips (*Thrips* sp. and *Frankliniella schultzei*), white fly (primarily *Bemisia tabaci*), and mites (*Tetranychus* spp.). All other species reported have insufficient data to make a sound conclusion about secondary pest status. The five species are probably the most important arthropod pests of brinjal. The first four of these (aphids, leafhoppers, thrips and white fly) typically have high inter-annual variation in population density. Because this variation makes it more difficult to detect population trends over a short period of time, a few more years of study will be necessary before reliable inference can be made about secondary pest status of these insects. Mites, however, typically have more uniform population densities from year to year. Consequently, the results from the limited field trials suggest that mites will not be significant secondary pests in Bt brinjal.

There are several important diseases of brinjal that should also be evaluated, including little leaf wilt and *Fusarium* wilt. However, the disease data are too sparse to draw any meaningful inferences. There was one observation of an increased frequency of little leaf wilt on Bt brinjal at Coimbatore during 2005 that might indicate future troubles and would merit additional study.

Root-knot nematodes (*Meloidogyne* spp.) are serious pests of brinjal in many areas in India. These were not considered in EC-II, the Dossier, or the Supplemental Materials. If these pests became more severe on Bt brinjal, it would have significant negative consequences on brinjal farmers.

The data are insufficient for evaluating whether Bt brinjal disrupts natural biological control (farmer's friends), and thereby causes a secondary pest outbreak. The natural enemies were not very abundant in the study, and the data do not distinguish among species. When a species is rare, it is very difficult to determine the effect of an experimental treatment. Differentiation of the data to identify species, rather than species groups, is necessary to draw conclusions about the disruption of biological control.

Finding 16. The soil studies were not designed for evaluating effects on soil health, brinjal productivity, or the productivity of other crops grown by brinjal farmers, and therefore, is not helpful for evaluating environmental safety.

The soil experiments are supposed to address potential adverse effect (B) "reduction in soil quality or health, adversely affecting crop production in either the short or long term." The experiments in the Dossier (volume 5) and the Supplemental Materials focus on the five response variables: total culturable bacteria, total culturable fungi, total nematodes, total collembola, and total earthworms. There are three significant sources of uncertainty associated with these studies that make the studies incapable of evaluating environmental safety.

First, the response variables do not relate to soil quality associated with brinjal production in India, or if they relate, it is only through tenuous indirect connections. The responses have been used in Bt maize in the temperate zone, but even in this circumstance, they have been of supplementary value, to other more critical measures. EC-II has not provided any justification for how these measures relate to Indian soils under brinjal. For example, if total culturable bacteria were higher in Bt brinjal, would this mean that soil quality is better or worse? Culturable bacteria represent only a few percent of all soil species, so environmental meaning of this measure is dubious. If the response does not allow an inference regarding soil quality or health or crop production, then the response cannot be interpreted in the context of environmental risk.

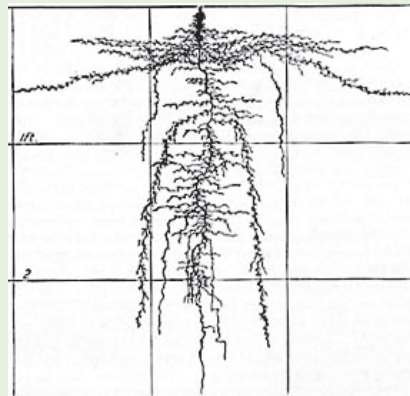
A better study design would include direct associations between measured responses and environmental effects. If, for example, nutrient or micronutrient depletion were one of the indicators of long term soil health for brinjal or vegetable

BOX 7

The Root Rhizosphere

The influence of a living plant on the soil is related to the proximity of the soil to the living root. Living plants interact with the soil through their roots. Plant roots are leaky and plant compounds leave the root into the surrounding soil. Soil organisms, including bacteria and fungi can seek out the resources leaked from roots and establish complex ecological communities near the root. Because of the importance of living roots in the soil, the "root rhizosphere" is defined as the couple of centimetres from the main plant roots.

Bt brinjal, like other GM crops, is expected to have its greatest influence on the soil in the root rhizosphere, if it has any influence at all. Similar to many plants, brinjal has a complex root structure that changes daily (see Brinjal Root, figure below, <http://www.soilandhealth.org/01aglibrary/010137veg.roots/010137ch27.html>). Brinjal has a mail tap root, and does not have a fibrous root structure. Therefore to evaluate the effect of Bt brinjal on soils, the rhizosphere soil should be sampled.



The most common way to collect rhizosphere soil is to dig up the root system, shake off the loose soil, and collect soil that is still adhering to the root mass (Icoz et al., 2008, see figure on right below). The method used in the experiment was to take "root zone" soil from as far away as 20 cm from the plant stem (figure on right below; blue area has a radius of 20cm). This method would include a substantial amount of non-rhizosphere soil in the samples, which would dilute any effects that could have occurred in the rhizosphere. The sampling method makes it very difficult to detect any effects of Bt brinjal.

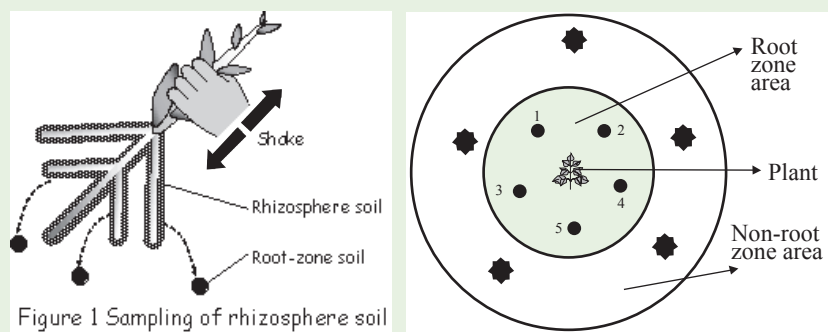


Figure 1 Sampling of rhizosphere soil

production in India, then the level of appropriately selected nutrients and/or micronutrients should be examined. If build-up of refractory soil organic matter (SOM) were an indicator of long-term soil health (it improves water holding capacity and improves soil aggregate distribution and soil structure), then the production and degradation of refractory SOM should be examined. If the use of a Bt crop were to create a soil “toxicity” or suppressiveness to subsequent crops, then this should be directly evaluated, along with feasible ways that suppressiveness could build up in the soil.

Second, the collection of the soil samples may be faulty (Box 7). By sampling soil from as far away as 20cm from the main brinjal stem, actual effects of Bt brinjal could have been diluted to the background levels, resulting in the insignificant results. Thus dilution of rhizosphere soil is a possible alternative explanation for the reported no observed effects, and the true meaning of the results is not known.

Third, it is essential to know species identification as much as possible, especially for the collembola and earthworms. This is because community responses of groups of species may mask important species-specific responses.

Some final points about the scientific validity of and reporting of data in the studies need to be made (Box 8). The results reported in the two reports on soil biology in the Supplementary Materials are based on unreplicated trials. Because of the lack of replication, these data should not be admitted as evidence in a risk assessment. That these two studies even appear as Supplementary Materials to EC-II indicates a standard well beneath minimal scientific norms. Replication is absolutely essential for any scientific study, and replication is equally important for studies supporting environmental risk assessment.

Finding 17. The EC-II (page 41) statement “there is no accumulation of the [Bt] protein in the soil associated with production of Bt brinjal” was not supported by any scientific data.

The statement (EC-II, page 41) cites results from Bt cotton, and EC-II provides no justification that the results from cotton are at all relevant for Bt brinjal. Results from Bt maize demonstrate that Cry toxins persist for up to three years in soils (Zwahlen and Andow 2005). EC-II could have used maize data and come to the opposite conclusion. Actually, neither cotton nor maize is a good model for understanding degradation and accumulation in brinjal. The relevant data would be for brinjal itself.

In general, degradation depends on the initial concentration the Bt protein (Cry1A toxin) and exposure to factors that degrade the toxin. These in turn will depend on the structure of the brinjal plant (e.g., stem diameter, leaf to stem ratio, etc), the tillage systems used after brinjal production, and the season of production of the brinjal. Thus, the three varieties of Bt brinjal

BOX 8

Scientific Validity

There are numerous factors that contribute to the scientific validity of a scientific study, but two are absolutely essential: replication and reporting sufficient statistics.

Replication is absolutely essential for any scientific study, and replication is equally important for studies supporting environmental risk assessment. An unreplicated study is merely an anecdote, and should not be permitted as evidence in an environmental risk assessment. Replication is essential because it gives the risk assessor a certain confidence that the reported results are not merely a one-off chance happenstance. The greater is the replication, the more confidence can be placed in the results.

That two studies on soil biology even appear as Supplementary Materials to EC-II indicates a standard well beneath minimal scientific norms. Such studies should not have been accepted.

A second critical and often poorly understood factor essential to scientific validity is the reporting of sufficient statistics. This is a minimal standard for reporting scientific results and has been violated numerous times in the Dossier and Supplemental Materials. In short, all studies should report all treatment means, some measure of variance around the mean, and the sample size contributing to the mean. None of the soil studies reported a measure of variance for any of the response variables. Even more astounding, treatment means are not reported for some of the response variables. These oversights also occur in most of the other studies. Without this minimal information, it is impossible to evaluate the quality of the data and the soundness of the conclusions.

that are considered are likely to have different degradation rates, and studies directly on Bt brinjal should be conducted. These studies should mimic normal brinjal residue management practices by brinjal farmers rather than rely on artificial experimental methods.

The Supplemental Materials have two reports on soil biology. As just noted, both of these studies were unreplicated and the quantitative values of the results are not reported. Thus, the data provided in EC-II, the Dossier, and the Supplemental Materials are insufficient to conclude anything about degradation or accumulation of Ccry1A in the soil.

Finding 18. The assessment of effects on species of conservation concern, species of cultural concern, other economically significant species (such as silkworm), and other ecosystem services is incomplete or insufficient.

Relatively little care was given to evaluating potential adverse effect (C): reduced value of non-crop economic activities (such as sericulture, honey production, or wild food harvesting). Only European honey bee was considered in a pair of toxicity trials using native Cry1Ac toxin. Other species of concern include Asian honey bee (*Apis cerana*), and silkworm (*Bombyx mori*) which is grown by many brinjal producers. Indeed, in many places, mulberry, the food of silkworm, and brinjal are grown side-by-side. In addition, some consideration should be given to potential impacts on other economic activities that occur near brinjal fields, including effects on fish (singara), water chestnut, hilsa (Bengal salmon), and surrounding crops (climbing French bean, other climbing varieties of beans, and leafy greens, such as amaranth and spinach).

There is little consideration given to potential adverse effect (D): reduced cultural value by affecting a cultural icon or a species of cultural significance (e.g., Monarch butterfly in the United States). Some obvious cultural icons in India are cows (*Bos taurus*) and bhodi tree (*Ficus religiosa*), but additional consideration should be given to identifying species that are culturally significant to the many ethnic and religious groups in India. Some assessment of effects of Bt brinjal on cows is given under the human and animal health assessment section. Indeed, in some cases, brinjal itself is a cultural icon, important in weddings, religious ceremonies, and culturally significant foods. Threats to cultural identity are of considerable significance to most peoples, and this issue should not be dismissed or glossed over.

Potential adverse effect (E): increased conservation concern, such as an adverse effect on an endangered species, is addressed in one paragraph in the Dossier (volume 1), which states, “There are no listed endangered species of Lepidoptera in India that will be exposed to the Cry1Ac protein produced in Bt brinjal. The Cry1Ac protein is contained within the tissues of the Bt brinjal plant; therefore only insects that feed on brinjal will be exposed to the Cry1Ac protein.” It would be more useful and a substantially better environmental risk assessment to list all known endangered species that live near brinjal fields (or the ones closest to brinjal) and then provide reasons for why each may not be at risk. Endangered species may be affected indirectly by other affected species, in addition to the direct effects from consuming Bt brinjal as referenced by the Dossier.

Finally more consideration should be given to considering effects on ecosystem functions and services. Although pollination is considered in EC-II, effects on Asian species of bumblebees need to be evaluated. In addition, effects on soil nutrients and micronutrients, soil organic matter, and possibly other functions and services should be considered.

Needed Experiments and Observations

Although this aspect of the environmental risk assessment may fulfil the requirements of the GEAC, in actual fact, the GEAC has never made clear what concrete environmental values are at stake. One of the main goals of risk assessment is to identify specific potential adverse effects and estimate the probability that a specific adverse effect of a given size would occur via some feasible and concrete pathway. When judged against this standard, EC-II is substantially inadequate, either because the risk assessment model that was used is inadequate or because the EC-II did not apply the risk assessment model properly. It seems quite possible that risk assessment model is inadequate, and to the end of providing an adequate model, many of the following are needed.

1) Kinds of risks to biological diversity. Determine the kinds of risks to biological diversity that are important for India in the use of Bt brinjal. The kinds of possible risks associated with a Bt crop are:

- Increased secondary pests, either through direct enhancement or through the reduction of natural enemy controls. EC-II examined this cursorily, but given the common occurrence of secondary pests on Bt crops around the world (e.g., Greene

et al. 2006, Lu et al. 2010) and the often dire financial straits of Indian brinjal farmers, a more thorough evaluation is merited.

Imagine if the government claims there are no risks of secondary pests, and then a secondary pest arises. The government might be perceived as favouring GM seed companies at the expense of small-scale farmers.

- Reduction in soil quality or health, adversely affecting crop production in either the short or long term. EC-II examined this problem cursorily. If India considers this a serious potential risk, then it needs to be examined more concretely, considering what crops follow brinjal and the soil factors that limit productivity of these crops. This approach to risk assessment is akin to fault-tree analysis (Hayes 2002), and can more rapidly identify effects that people care about than the approach used in EC-II.
- Reduced value of non-crop economic activities (such as honey production or wild food harvesting). The potential non-crop activities that could be at risk in India need to be determined. This may include silkworm production and wild honeybees (e.g., *Apis cerana*). India might be wise to consider an expanded list of potential adverse effects.
- Reduced cultural value by affecting a culturally iconic species or a species of cultural significance (e.g., Monarch butterfly and bald eagle in the United States). India should first consider if there are any such species (cows and Bhodi trees may be such species), and then, as discussed below, consider how great a risk Bt brinjal poses to these species.
- Increased conservation concern, such as an adverse effect on an endangered species. India should first consider if there are any such species associated with brinjal. If there are none, the endangered species that occur closest to brinjal should be considered, and then, as discussed below, consider how great a risk Bt brinjal poses to these species.
- Reduced environmental quality through an effect on an ecosystem service such as pollination. Of the possible ecosystem services to be assessed, India apparently considers only pollination, although there is no assessment of effects on native pollinators and pollination services of plants other than brinjal. India should consider if there are additional ecosystem services (MEA 2005) that need to be assessed for Bt brinjal.

2) Specify risk hypotheses. Specify scenarios (also known as risk hypotheses) by which the kinds of risks listed in 1 could occur in India. This includes specifying a concrete potential adverse effect and an assessment endpoint that can be measured. These scenarios are testable hypotheses about how adverse effects could possibly arise. They are statements about possibilities not necessarily realities. This also includes specification of the people who may be affected. For this analysis, organic brinjal farmers need not be considered, because these farmers will not be using Bt brinjal. Details on the construction of risk hypotheses can be found in USEPA (1998) and Hilbeck et al. (2008).

3) Test risk hypotheses. Test the risk hypotheses either to falsify them (and conclude that there is insignificant risk) or estimate the risk (qualitatively or quantitatively). In some cases, laboratory toxicity tests can be useful on appropriately chosen indicator species and in some cases small-scale field experiments can be useful, but in all cases careful thought must be given to find the simplest ways to meaningfully assess the risks.

4) Secondary pests. It is not possible to state exactly what experiments must be done until India decides which of kinds of risk listed in 1 are important enough to assess. It seems clear, however, that India considers secondary pests to be a serious potential risk that needs to be evaluated. EC-II has already specified a concrete potential risk (loss of yield and income to Bt brinjal farmers), and has also identified a list of potential assessment endpoints. These are:

- aphids (primarily *Aphis gossypii*),
- leafhoppers (primarily *Amrasca devastans*),
- thrips (*Thrips* sp. and *Frankliniella schultzei*),
- white fly (primarily *Bemisia tabaci*),
- mites (*Tetranychus* spp.),
- *Helicoverpa armigera*,
- stem borer (*Euzophera perticella*),

- grey weevil,
- little leaf wilt,
- *Fusarium* wilt, and
- root-knot nematodes (*Meloidogyne* spp.).

The Dossier (volume 6) reports on limited field trials where these potential secondary pests were counted. As discussed in the text above, these limited trials provide little useful information about potential secondary pest status. To generate more useful data, the list of potential secondary pests should be prioritised so that the most serious potential secondary pests are identified. This can be done using multi-criteria decision analysis (MCDA; Fenton and Neil 2001, Dodgson et al. 2009), as demonstrated in Nguyn et al. (2008). Several factors are probably involved in releasing secondary pests, including release from insecticides, release from competition, and so on. These factors can be evaluated for each of the high priority species identified in the MDCA.

A similar approach can be used to identify potentially significant biological control agents (farmer's friends) that could be destabilised by Bt brinjal. This analysis, however, may be unlikely to identify many potentially significant candidates because brinjal fields are sprayed with insecticides so many times that few biological control agents are likely to persist in brinjal fields. This fact, however, indicates that a significant biological control agent might be one that colonises brinjal rapidly from sources outside of brinjal fields.

Resistance Risk in Target Species

Conclusion 7. The evolution of resistance in BFSB (*Leucinodes orbonalis*) to overcome Bt brinjal is a real risk that must be managed. EC-II does not acknowledge this risk and the Dossier does not propose effective means to manage it.

The evolution of resistance is a real risk that is widely acknowledged worldwide (Gould 1998, Tabashnik et al. 2008), including in developing countries (Fitt et al. 2008) and India (Hanur 2008). Every regulatory authority that can claim scope over this issue has chosen to regulate this risk, typically by imposing post-commercialisation restrictions on the use of Bt crops to manage the evolution resistance in BFSB. Resistance can cause many kinds of adverse effects (Box 9). For Bt brinjal, these adverse effects may be (a) loss of whatever benefits Bt brinjal provides by controlling BFSB, leaving society to bear only the risks associated with Bt brinjal. (2) Harm to brinjal farmers who use *Bacillus thuringiensis*-based insecticides to control BFSB. Presently, microbial applications of *B. thuringiensis* are being encouraged for use in pest control in India because this is much safer for human health than the synthetic insecticides being used. Resistance could make farmers more reliant on synthetic chemical insecticides. In addition, some of the local insecticide technologies may incorporate *B. thuringiensis*. If resistance to Bt brinjal occurs, these other farmers may find it more difficult to control BFSB, increasing their control costs or reducing their brinjal yields.

In the following we evaluate potential ways to manage this risk. Some of the steps that have been used by other regulatory authorities to determine how to manage resistance include: determining the "dose" of the transgene, estimating the frequency of resistance alleles in the target population prior

Table 3. Possible methods for delaying the evolution of resistance to a Bt crop in target pests

Method	Goal	Means
Refuge	Delays resistance	Ensures survival of some susceptible pests
High dose	Delays resistance	Kills heterozygous resistant pests
Seed mixtures of Bt and non - Bt seed	Delays resistance	Ensures survival of some susceptible pests
Gene pyramiding	Can delay resistance	May kill homozygous resistant pests
IPM	Can delay resistance	Suppresses homozygous resistant pest populations
Replace with new toxin genes	Respond to resistance failure	Provides new mode of toxicity

BOX 9

Adverse Consequences of Resistance and Benefits of Managing Resistance

Resistance in insects to pest control is a serious problem worldwide. Although resistance problems have been known for nearly 100 years, resistance became a significant agricultural problem after World War II, when modern, intensive agricultural technologies proliferated. Resistance has occurred quickly when there has been strong, uniform selection on a pest population for sufficiently long periods of time over spatially extensive areas. Modern intensive agriculture, with its reliance on pesticides, monoculture and uniform production practices has provided these conditions, and resistance has proliferated. It took most of the 20th century before an entomological consensus was reached about the seriousness of the problem (NRC, 1986). Resistance to Bt toxins has been documented in 17 insect species (Tabashnik, 1994; Huang et al., 1999), so it is now widely assumed that resistance to transgenic insecticidal crops, such as Bt brinjal can occur.

- **Insecticide use.** In the USA alone, resistance costs about US\$133 million/ year in extra insecticide applications, measured in 1980 dollars (Pimentel et al., 1980). Unexpected yield losses from resistance have not been estimated, but are likely to be a similar amount. In northeastern Mexico and the Lower Rio Grande of Texas, resistance to insecticides caused about 700,000 acres of cotton to be lost (Adkisson, 1971; 1972), devastating many local communities, some of which have never recovered.
- **Farm income.** Effective management of resistance will allow farmers to benefit from a transgenic insecticidal crop for a long period of time. For example, Bt maize can increase net income to farmers of US\$10-60/ha (Rice and Pilcher, 1998). Resistance would mean loss of this income which would have significant detrimental effects on farm families. Bt maize, however, can also result in no yield increase, and a farmer can lose US\$35/ha.
- **Seed company.** Resistance will harm companies that sell transgenic insecticidal crops. However, if profits of seed companies were the only reason for managing resistance, there would be no need for society to intervene. However, seed companies are only one of the many stakeholders, and their concerns do not match the concerns of the other important stakeholders, including consumers and farmers, hence the need to regulate resistance.
- **Other farmers.** Resistance may harm other farmers who depend on Bt-based insecticides and do not or will not use transgenic insecticidal Bt crops.
- **Pesticide regulation.** Resistance destabilizes pesticide regulation. For example, the USA Environmental Protection Agency (EPA) normally registers pesticides only after in-depth risk assessment and review. A large loophole is that unregistered pesticides can be used under an emergency exemption with very little review. During 1991-1994, about 30 % of all emergency exemptions requests were made because of resistance (Matten et al., 1996). With effective resistance management, the need for emergency exemptions could be significantly reduced.

to commercialisation, characterise the genetic structure of the target population, measure the distances adults will move before mating, develop and test practicable management practices that farmers can implement, and so on.

Several approaches have been suggested to delay the evolution of resistance (Table 3). Some are known to delay resistance, while others can delay resistance when the circumstances are right (Table 3). Replacing an old toxin with a new one is a response to resistance failure, and is not a method to delay resistance. Other approaches that are not listed here are speculative, and it is not known if they can delay resistance evolution. Of the methods that are known to delay resistance, the high dose/refuge strategy has been highly successful. Seed mixtures are better than no strategy, but not as effect as a refuge strategy. Rotation of different Bt varieties is not feasible because two different Bt transgenes are not even being considered. Rotation with other varieties containing EE-1 does not delay resistance at all. Tissue-specific expression and temporary expression of a Bt transgene have not even been developed, so they are not practical approaches to resistance management.

The proposed insect resistance management (IRM) plan for Bt brinjal and BtFSB is described in Vol. 8 of the Dossier. When evaluating an IRM plan, it is critical to determine if the IRM plan will delay the evolution of resistance and the onset

of control failures, or merely document its occurrence. The proposed IRM plan involves the following seven elements:

- A. Establish baseline susceptibility in BFSB using an F1 phenotypic screen on larvae.
- B. Monitor susceptibility using the F1 phenotypic screen on larvae.
- C. Evaluate control efficacy of Bt brinjal using bioassays on field-collected larvae.
- D. Suggest to farmers that they should plant 5% of their brinjal to non-Bt brinjal as a refuge.
- E. Remedial action if control failures occur. This could involve spraying the field with insecticides or suspending sales of Bt brinjal.
- F. Encourage the use of Bt brinjal within an integrated pest management system.
- G. Educate farmers about resistance risk and the dangers of planting saved F2 seed from their Bt production fields.

Rather few of the elements of this strategy will function to delay resistance evolution. A, B, and C will help anticipate and document control failures. E suggests actions that may be taken if control failures occur. F normalises the use of Bt brinjal in brinjal production systems, but has no effect on the rate of resistance evolution. D and G could affect the rate of resistance evolution. As discussed below, the use of non-Bt brinjal refuges (D) can help delay resistance evolution. The replanting of saved seed may either speed up or slow down resistance

evolution. Saved seed will be a mixture of cross-pollinated and self-pollinated seed, and will probably be a mixture of about 50% Bt and 50% non-Bt plants. If the expression level of Ccry1A in the saved Bt plants is reduced, the saved seed will speed up resistance. However, if the expression level is reduced substantially, resistance may be delayed. If the expression level of Ccry1A in the saved Bt plants is not affected, then resistance will be delayed. The effects of seed saving are complex (Glaum et al, unpublished). No data is provided to enable determination of which of these cases is likely for EE-1 Bt brinjal.

The case of Bt cotton in India is informative for deliberations about Bt brinjal. Although, as discussed below, there remains uncertainty over the status of reported resistance failures (See Box 10) due to pink bollworm in Gujarat, the mere fact that there has been a reported resistance failure should give everyone pause to consider the ephemerality of the Bt technology in transgenic plants. Resistance failures of Bt crops are still unusual, and appear to be associated with poorly implemented IRM plans (Bt maize in South Africa) and a low dose target pest (Bt maize in Puerto Rico and in South Africa). The Bt cotton in India is high dose against pink bollworm, but the necessary non-Bt cotton refuges were probably not planted.

Monsanto stated that resistance failures had occurred in four districts in Gujarat due to resistant pink bollworm in Bt cotton², Bagla 2010). The four districts are Amreli, Bhavnagar, Junagarh and Rajkot, which are parts of the Saurashtra region. Together, these four districts comprise a substantial part of Gujarat. During 2009, Monsanto and Mahyco scientists detected unusual survival of pink bollworm. Using established and verified protocols, they confirmed that control failures in those

²(Monsanto.com)

BOX 10

Definition of Resistance

Resistance is caused by genes in the target pest insect that reduce susceptibility to a toxin, and is a trait of an individual. Resistance is defined as a phenotype of an individual that can survive on the transgenic insecticidal plant from egg to adult and produce viable offspring. There is much confusion in the scientific literature over the definition of resistance. However, from a genetic or an evolutionary perspective, it is essential to define resistance as a trait of an individual. A consequence of this definition is that if only one individual in a population is resistant, the population contains resistance.

Some researchers will use the term “tolerance” instead of resistance. There are several reasonable definitions of tolerance, but some of them overlap strongly with the definition of resistance and lead to confusion. A “tolerant” individual is one that is not resistant, but has the ability to grow on toxin concentrations that are higher than that possible for a typical individual.

Much of the confusion with the term “resistance” stems from the incorrect use to describe a characteristic of a population of pests. Specifically, “**resistance failure**” describes a field population with enough resistant individuals to cause economic damage to the target crop. This is also called control failure from resistance (aka field resistance). There are several reasonable operational definitions. For example, a control failure could be defined as occurring when the pest causes detectable economic damage to the crop, when the pest causes economic damage that is similar to that caused by susceptible insects on a non-resistant crop variety, or when the economic damage is considered unacceptable to the grower.

districts were due to pink bollworm resistance. The substantive basis for Monsanto's determination has not been released to the public, so it is not possible to verify independently this conclusion.

Monsanto's conclusion has been vociferously criticised by Dr. Kranthi (Director Central Institute of Cotton Research (CICR), Nagpur) who stated that the methodology followed by Monsanto was unscientific and therefore unacceptable on the following grounds:

1. The unusual survival of the pink bollworm in Saurashtra region of Gujarat was due to weather conditions that favoured the pest survival and not because of resistance as stated by Monsanto.
2. There were unusually high numbers of pink bollworm males during 2009 in Saurashtra. CICR data³, All India Coordinated Cotton Improvement Project (AICCIP) reports (2009-10) demonstrate that during 2009, there were unusually high catches of pink bollworm moth in the pheromone traps installed in Junagarh at an average of about 700 moths per week all through mid-November until the end of January. This occurred only in Saurashtra, only in 2009. At the other 12 centres where monitoring was carried out, trap catches were less than 100 moths per trap per week.
3. India is the only country in the world that cultivates hybrids and also that the bolls on F1 hybrid plants possess 25% non-Bt seeds. This enables high survival of pink bollworm larvae that can feed on the developing non-Bt seed.
4. The Monsanto bioassay data were derived from 33 pink bollworm larval population samples collected from Bt cotton fields in all the four districts of Saurashtra, instead of collecting on conventional cotton as per the standard methodology. Therefore, their inferences on resistance were not correct.
5. Further, thus far there has been no scientific evidence anywhere in the world including India to indicate that the pink bollworms had developed resistance to Cry1Ac under field conditions.

Although none of the points raised by Dr. Kranthi either singly or together refute Monsanto's conclusion, some of the points raise sufficient doubt in the conclusion. Kranthi's point 5, whether true or not, is irrelevant to India. Just because resistance failures have not occurred elsewhere, does not mean that India cannot be the first place they occur. Point 4 does not throw the Monsanto conclusion into question. It simply means that they have discovered resistance associated with Bt cotton. Kranthi's point would indicate that resistance may have not yet spread to all fields in the districts, but his point does not deny that resistance occurred. Kranthi's point 3 probably undercuts his argument more than it supports it. While the non-Bt seed may contribute to a higher pink bollworm population, it is also likely that it led to faster resistance evolution because RS heterozygous larvae probably had a higher than normal survival rate. Points 1 and 2 also do not disprove Monsanto's conclusion, but they do raise alternative possibilities that together lead to doubt about the validity of the conclusion. Kranthi's points 1 and 2 essentially say that the environmental conditions in the Saurashtra region were substantially different from normal. If these unusual environmental conditions also reduced expression of Cry1Ac toxin in the Bt cotton varieties, then resistance failures may not have occurred. Instead, the alternative explanation is that environmental conditions caused failure of the Bt cotton product to perform as expected. Thus, Monsanto's conclusion cannot be accepted as fact. If the details of the Monsanto studies were released, then it would be possible to determine if Monsanto's conclusion is sound.

Finding 19. The aspects of the IRM plan for EE-1 Bt brinjal that could delay resistance evolution are all purely voluntary and contain no incentives for farmers. Only the non-Bt refuge is likely to delay resistance evolution, but compliance to the refuge strategy is likely to be low. The education program is aimed both to ensure sales of Bt brinjal seed and affect the risk of resistance.

Of the seven elements of the Bt brinjal IRM plan, A, B, C, and G are compulsory, and Mahyco has the responsibility to carry them out. However, A, B, and C will not affect the rate of resistance evolution, and the only compulsory aspect about G is that educational programs will be conducted for farmers. The remaining elements of the IRM plan are voluntary. The use of non-Bt refuges will delay resistance; however, their use is voluntary. If Bt brinjal is effective at controlling BFB on actual farms and the losses to BFB are severe, then there will be incentives for farmers to plant most of their brinjal as Bt brinjal. There are no incentives to plant non-Bt brinjal, and no disincentives to planting too much Bt brinjal. Thus compliance to the refuge strategy is likely to be low (however, see discussion below). If Bt brinjal is not that effective at controlling BFB or losses from

³(www.cicr.org.in)

BFSB are not severe, then adoption of Bt brinjal is likely to be low, and there is a much lower risk of resistance. The proposed education program will be designed to inform farmers that saving Bt brinjal seed will result in faster resistance evolution, and consequently they should purchase new seed each year. It is not clear that saving seed will speed up resistance, but it is clear that the education program will help increase seed sales.

There are several approaches to resistance management in small-scale production systems in Vietnam that are discussed in Fitt et al. (2008). These approaches should be considered in the context of Bt brinjal in India. One possibility is the use of unstructured refuges, and another approach is to develop community-wide tactics.

Finding 20. Event EE-1 Bt brinjal is a low dose event, which will require extra effort to manage effectively.

Dose reflects the level of expression of the Bt transgene in the Bt plant and is either high or low (Box 11). The dose is not the same as the efficacy or kill-rate of the Bt plant, but it has an operational definition related to efficacy (Box 11). EC-II, the Dossier and the Supplemental Materials make it clear that the present population of BFSB is susceptible to Bt brinjal. Examination of the results from the small-scale field trials (Dossier, volume 6), indicates that efficacy of Bt brinjal varies from 38% to 97% kill of the BFSB population, with an average of 73% kill and a standard deviation of 21%. This is far less than the concentration needed for a high dose event. Indeed, even the highest observed kill rate (97% kill) is less than a high dose event. Consequently, event EE-1 is a low dose event. To be clear, the data provided in the Dossier (volume 6) contradict the statement in the Dossier (volume 7) that Bt brinjal kills all BFSB larvae.

Finding 21. Without any management of resistance evolution, Bt brinjal is projected to fail in 4-12 years. Effective use of 20% non-Bt refuges can extend this time by 25% or more.

For the purpose of providing a rough analysis of resistance risk and IRM, we simulate the evolutionary process to project possible evolutionary futures. To do this, we make the following simplifying assumptions:

- a. BFSB is found feeding only on brinjal.

As discussed above, BFSB may feed on wild and weedy relatives of brinjal, and if it does and they are spatially intermixed with brinjal, these could provide a natural refuge, and the time to failure would be longer than projected here. In addition,

BOX 11

High Dose and Low Dose

The dose of the insecticidal toxin in a Bt crop is a major factor determining the level of resistance risk. Dose depends on both the concentration of the toxin in the Bt plant and the genetic characteristics of the target pest. Dose is a measure of the relative mortality of the three possible genotypes associated with resistance evolution. These genotypes are the RR homozygotes (with two resistance, R, alleles), the SS homozygotes (with two susceptibility, S, alleles), and the RS heterozygotes (with one of each kind of allele).

A “high-dose” is defined as one that kills a high proportion (>95 %) of heterozygous resistance genotypes, so that the RS heterozygotes have a similar mortality as the homozygous susceptible genotypes (Georghiou and Taylor 1977; Roush, 1997; Gould, 1998). In this case, resistance is said to be recessive, and resistance evolution can be slow and can be delayed substantially.

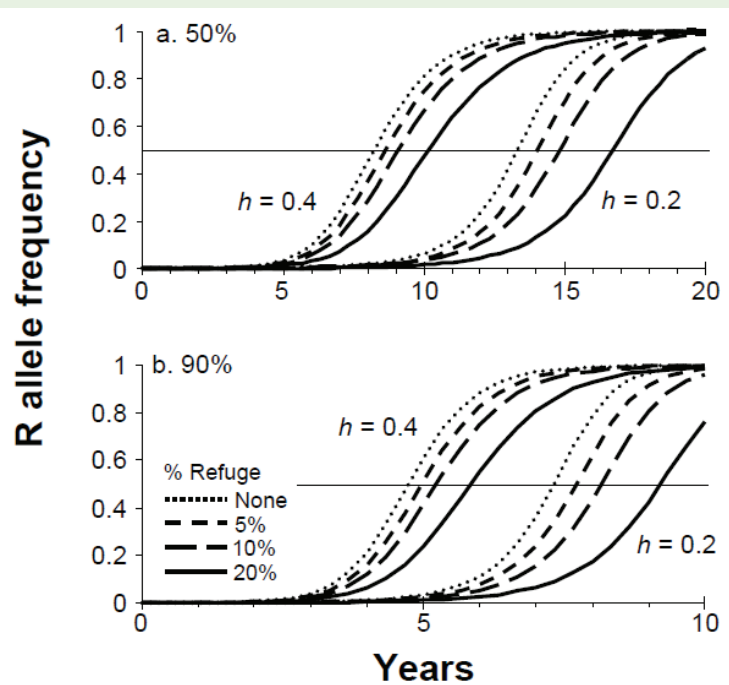
A “low-dose” is anything that is not a high-dose. If the mortality of the RS heterozygote is similar to the RR homozygote, resistance is said to be dominant, and resistance evolution can be extremely fast. If the mortality of the RS heterozygote is between the SS homozygote and the RR homozygote, resistance is said to be intermediate, and resistance evolution can be fast.

A provisional operational definition of “high-dose” is: a Bt plant that expresses Cry toxin at a concentration that is 25 times the concentration to kill 99% of the target pest (Gould and Tabashnik, 1998). An alternative is at least 99.99 % mortality of homozygote susceptibles relative to a non-Bt control (ILSI, 1999). Caprio et al. (2000) found that dominance and SS survival were correlated, such that higher SS survival was associated with higher dominance (low-dose). They showed that a concentration of Cry toxin that is 50 times the concentration to kill 99% of the target pest is high enough that all known resistance alleles would be functionally recessive (= high-dose).

By any of these criteria, EE-1 Bt brinjal is a low dose event.

- non-Bt brinjal refuges will cause a greater delay in resistance than projected here.
- BFSB is the only species at risk of resistance evolution on Bt brinjal. If there are other species at risk, they should be analysed as well to determine if they could be a more serious resistance risk.
 - BFSB has evolved resistance to insecticides in India. The scientific documentation of this is thin, although it is widely stated. If BFSB has evolved resistance to insecticides then we can conclude that it has a population structure that would allow evolution to Bt brinjal as well.
 - BFSB larvae can move from plant to plant, and fruit to fruit. This would imply that seed mixtures may speed up the rate of resistance evolution, and this IRM tactic might best be avoided.
 - BFSB adults can move a few hundred meters and males and females are similarly dispersive. No published papers on adult movement could be found. The level of dispersal that is assumed is low relative to other species of Crambidae that are pests in other crops. Given the small size of brinjal fields, this assumption means that most adults will leave the field that they were born in (their natal field), but they will have little trouble finding another field (or returning to their natal field). This leads us to assume that the proportion of adult males and females that leave their natal field is 0.9 ($r = 0.9$). If r were lower, evolution would be delayed, but if r were too low, evolution will speed up.
 - All Bt brinjal have the same level of control of BFSB (including any non-official seed). Heterogeneity in expression of *Ccry1A* could create hot spots where evolution occurs very rapidly and spreads to other areas. This would result in faster resistance failures. Based on the survival rates reported in the Dossier (volume 6), we calculated the average survival on Bt brinjal to be 0.27, so $K = 0.27$.
 - There is no fitness cost of resistance in BFSB ($L = 1$). A fitness cost of resistance is defined as when the resistance gene has a lower fitness than the susceptibility gene in the absence of selection for resistance. Thus, the resistance allele would be favoured on Bt brinjal, but the susceptibility allele would be favoured on any non-Bt host plant, such as the non-Bt refuge. Actually, a fitness cost is expected. Gassman et al. (2009) showed that nearly all Bt resistance genes had some level of fitness cost, however, it is not possible to predict the fitness cost until resistance is discovered. Because the fitness cost varies from nil, which would have no influence on resistance evolution, to large, which would greatly delay resistance if a refuge were used, we modelled no fitness cost. This is a worst-case assumption. If it turns out that BFSB resistance has a significant fitness cost, then the time to control failures would take longer to reach. More significantly, however, the use of effective 20% refuges would extend the time to resistance by much more than 25%.
 - BFSB has as many as 8 generations a year (Alam et al 2003), but only three of them will be associated with Bt brinjal.

Figure 2. Time to failure of Bt brinjal for a. 50% adoption rate of Bt brinjal and b. 90% adoption rate. For each adoption rate, time to failure is shown for heterozygosity (h) = 0.2 and 0.4, and for each of these heterozygosity values, time to failure is shown for four refuge options, no refuge (or no compliance to the refuge), 5%, 10% and 20% refuge (assuming 100% compliance). Failure time is the time until the R allele frequency reaches 0.5. Simulations are based on the model of Alstad and Andow (1996) with $r = 0.9$, $s = 1$, $F = 100$, $a = 6$, $b = 0.8$, $p_0 = 0.0001$, $K = 0.27$, $L = 1$, and 3 generations of BFSB on Bt brinjal per year. Other generations of BFSB are assumed to be on non-Bt plants during the rest of the year.



In some areas of India brinjal is cultivated once a year during the rainy season, and BFSB can complete up to three generations. In other regions brinjal is cultivated throughout the year, and BFSB can complete 8 or more generations. We assume that wherever brinjal is grown, Bt brinjal will be grown during only one time of the year, so that only three generations of BFSB will be exposed to Bt brinjal a year. This is probably satisfactory for those regions with single cropping. For regions where brinjal is grown throughout the year, Bt brinjal could be grown during two or more seasons. In these cases, the time to resistance failure would be projected to occur much sooner. If two crops are grown resistance failure would occur twice as fast, and if three crops are grown, it would occur three times as fast as what we project here.

- i. Based on the results from the small-scale field trials (Dossier, volume 6) and the published literature (Caprio et al. 2000, Meihls et al. 2008, Anilkumar et al. 2008) on other low dose Bt events, we suggest that when resistance is found in BFSB, that it will be partially recessive with heterozygosity (h) between 0.2 and 0.4.
- j. We also assume that there is strong density dependent population growth. This is because larvae are forced to interact in brinjal fruits. This results in $a = 6$ and $b = 0.8$. the stronger the density dependence the longer the time to resistance failure. However, this effect is minor except in some special cases.
- k. We assume that per capita fecundity (F) is 100 offspring per female. Although most moths can produce several hundred eggs, this value is used because it counts only one of the two sexes (the females), and includes egg mortality and other forms of density-independent mortality.
- l. Finally, we assume that the frequency of resistance alleles (p_0) in the natural BFSB populations is low, less than one in 10,000. This value is lower than most observed initial R allele frequencies for Bt resistance. Time to resistance failure is longer for lower values of initial resistance frequency.

Using these assumptions, we can simulate evolution for two levels of adoption of Bt brinjal (50% adoption and 90% adoption), the two values of heterozygosity, and several refuge assumptions. Adoption rate is not for the entire country. Because evolution will occur locally, adoption is probably related to the district level within states, and possibly at finer spatial scales. In general, heterozygosity has large effects on resistance evolution, so we simulated the extreme values that are expected for BFSB and EE-1 Bt brinjal. We simulated no refuge (or 0% compliance), and 5, 10, and 20% non-Bt refuge with 100% compliance. Intermediate levels of compliance will produce results intermediate between 0% refuge and the recommended % refuge. The simulations show that resistance is projected to occur rapidly, sometime between 4 and 17 years (Fig. 2). The precise values can be disputed, but a robust conclusion is that under some conditions, resistance failures can occur rapidly (less than 4 years), and under other conditions, it may take many years for resistance failures to show up. Thus, efforts should be taken to ensure that India is more likely to experience the latter conditions than the former conditions.

This could be difficult. Local adoption rate has the largest effect on resistance evolution (Fig. 2). If adoption stays below 50% and heterozygosity is closer to 0.2 than 0.4, then Bt brinjal may last for many years. However, if Bt brinjal is truly as economically beneficial as projected in EEII and the Dossier, local adoption rates are likely to be much higher than 50%, and the resistance risk is very high. The resistance risk is greatest where adoption is highest, so India should pay attention to adoption rate if Bt brinjal is commercialised.

The level of heterozygosity has a large effect on the rate of resistance evolution. Unfortunately, it is not possible to know the value for heterozygosity until a resistance allele is discovered in BFSB. Thus, it should be a high research priority to discover resistance alleles to EE-1 Bt brinjal in BFSB (see below).

A 5% refuge has little effect on the rate of resistance evolution (Fig. 2). If there is a cost to resistance a 5% refuge will have a larger effect at delaying evolution, but this is still likely to be small quantitatively. A larger percent refuge will be better at slowing resistance evolution. A 20% refuge will increase the time to resistance failure by 25% (Fig. 2). If there is a cost to resistance, the efficacy of the refuge in delaying resistance is likely to increase substantially. If India is going to manage resistance a refuge larger than 5% seems necessary.

The assumptions that we have made in these simulations are not worst-case assumptions. Indeed, BFSB may be exposed

to selection more than 3 generations a year, heterozygosity may be higher than 0.4, and the initial frequency of resistance alleles may be higher than 0.0001. If any of these were true, Bt brinjal would fail even faster. On worst case assumption is the lack of a cost of resistance. If an efficacious refuge were used, the cost of resistance could extend the time to resistance failure substantially. These projections can be improved by estimating the values of some of the key parameters. Given the possibility of rapid failure, many of these experiments should be conducted prior to commercialisation.

Finding 22. Resistance monitoring in BFSB should begin as soon as possible, especially in the peri-urban large-scale commercial production regions, and should expand to include rural areas at risk.

Because Bt brinjal is projected to fail quickly unless resistance is managed, it is essential that the frequency of resistance in BFSB populations is monitored to enable the government, industry and farmers to respond to the increased risk of resistance failures. The presently proposed monitoring plan (elements A, B and C above) will not provide information on the frequency of resistance. They are designed to monitor for control failures due to resistance. Monitoring the frequency of resistance will provide an early warning, so that farmers are not subjected to yield failures associated with control failures. Monitoring for control failures will result in some farmers losing brinjal yield without warning. The social good is better served by monitoring for the frequency of resistance.

This does not deny that there are advantages to monitoring for control failure. For any technology, including agricultural technologies, it is useful to be able to know when the technology no longer works as expected. This is what monitoring for control failures accomplishes. This information has little present value for farmers, because by the time the information is available, control failures have occurred. It can help them choose appropriate brinjal varieties for planting in the future. The information may be of use to government, which can modify policies related to GM crops based on the information. The information is useful to the technology providers, in this case Mahyco, because they can adjust their future sales, variety development programs, and educational materials to farmers in ways to retain their share of the brinjal seed market.

There are many monitoring methods available, but pedigreed screens (Andow and Bentur 2010), such as F_2 and F_1 screens, are widely used and are probably appropriate to the BFSB. The only technical concern in using pedigreed screens is that individual pedigrees can be distinguished and maintained during testing. Because resistance is assumed to be only partially recessive and possibly almost co-dominant ($h = 0.2$ or 0.4), phenotypic screens may be the most cost-effective. A pedigreed F_1 screen differs subtly, but in extremely important ways, from the proposed F_1 screen (element B above). A pedigreed F_1 screen maintains the identity of the parents for each F_1 offspring tested, while the proposed F_1 screen combines all of the offspring prior to testing. If offspring are combined, it is no longer possible to know how many parents had offspring or how many parents carried resistance alleles. In addition, random sampling breaks down, because some parents contribute more offspring than others. A pedigreed F_1 screen should be required for monitoring. Of course, if resistance is found to be nearly completely recessive, then even this approach will not work, and an F_2 screen would be more cost-effective.

Locations that are monitored should be distributed over the geographic range where Bt brinjal is used, but efforts should be concentrated in locations with the highest use of Bt brinjal. The Indian government should track the use of Bt brinjal to identify the locations where it is used most commonly. There are no data that indicate the spatial scale over which locations should be sampled. As a first approximation, Bt brinjal use should be tracked at the District level, but it is likely that smaller areas than this may require monitoring. The peri-urban, large-scale commercial growers may be the initial users of Bt brinjal, so some monitoring should be initiated in these areas as soon as possible.

Needed Experiments and Observations

Several rather significant issues need to be addressed. Points 3, 4, 5, and 7 can be delayed until early during commercial use, but if they are delayed, then IRM must be based on worst-case assumptions, and the registrant should be granted a conditional registration that will automatically expire unless they provide the information by a specified date.

1) Develop resistance monitoring method. Develop and implement a pedigreed F_1 screen. This should not take undue effort, but the method should be verified, repeating much of the work that has already been reported for baseline susceptibility using a discriminating dose, such as Bt brinjal plant material in laboratory assays. By doing so, a more accurate and meaningful

baseline susceptibility can be established.

2) Prioritise resistance management tactics. Evaluate alternative IRM tactics using mathematical models. The mathematical model underlying Figure 2 should be elaborated to assess the efficacy of all possible IRM tactics so that an appropriate strategy can be developed.

3) Determine scale of monitoring. Determine appropriate spatial scale for monitoring. This should be established through the ecology of BFSB, by examining the spatial correlation in population dynamics, spatial clustering of brinjal production areas, and average movement of adult BFSB. This should not be based on deme size estimates using F-statistics or analogous approaches. These experiments do not require the use of Bt brinjal.

4) Find resistance. Discover resistance alleles in BFSB. This can be done through the pedigreed F_1 screen, mass selection in the laboratory on field-collected populations of BFSB, and other methods. Once a resistant colony of BFSB is established, it is essential to estimate key genetic parameters, including heterozygosity, cross-resistance, and cost of resistance. Bt brinjal must be used in this work, however, it can be grown in a greenhouse.

5) Parameter estimation. Estimate biological parameters relevant to resistance evolution. These include plant-to-plant movement of larvae, movement of male and female adults, density-dependent larval mortality, and average fecundity. All of these experiments can be conducted without using Bt brinjal, and will be useful for developing other IPM tactics to control BFSB.

6) Farmer acceptance of tactics. Determine if brinjal farmers are willing to plant a non-Bt refuge on their farms. This can only be determined using stated preference (SP) descriptive surveys or experiments. An experimental approach would be preferred. Results from SP observations and experiments typically overestimate farmers' willingness to plant refuges, so these results should be interpreted as the most optimistic use of refuges possible.

7) Monitor compliance. Because of the uncertainty in SP observations and experiments, physical surveys of farmers using Bt brinjal should be conducted, mapping the planting of Bt brinjal and refuges by sampling plants and testing them with lateral flow sticks. Farmers' statements about refuge use are often too unreliable.

Socioeconomic Analysis

There are several broad socioeconomic issues that were not considered by EC-II or the Dossier. These risks are simultaneously both socioeconomic and environmental risks, and they are unique to the conditions of India.

Socioeconomic analyses of new technologies vary considerably in scope, ranging from narrow utilitarian analysis to ones considering a wider variety of human values and concerns. A narrow utilitarian analysis may focus on the average profitability of the farm – how the technology affects net average income. A slightly broader analysis might further consider how the new technology affects the economic security of the farm. Yet broader approaches would consider how the new technology affects supply and price in relation to projected demand. These are all more or less standard approaches to utilitarian economic analyses of new technologies. To address a wider variety of human values and concerns, the relevant issues must be stated and considered explicitly. This analysis is initiated herein, but before it can be completed, larger policy issues will need to be resolved. Sociological aspects can be added to the analyses by considering how a technology differentially affects the poor and may otherwise drive changes in social structure. Sociological changes may result in other costs or benefits that can influence government policy.

The socioeconomic analyses in this section start with a narrow analysis, which is narrow even by utilitarian standards, opens to consider aggregate supply, demand and price, and finishes with a sketch of an analysis that addresses a more diverse set of values, including equity and fairness whilst considering the sociological changes that Bt brinjal might induce.

Profitability – Utilitarian Analysis

Conclusion 8. Hybrid Bt brinjal may increase profitability for large-scale, commercial brinjal producers by at most Rs.23,439/ha. For these producers, a reduction of 6.5 insecticide applications may also occur, but this potential benefit may be unrealised if secondary pests arise. Small-scale resource-poor farmers may improve profit by Rs.3,250/ha, and many may see no benefit from hybrid Bt brinjal. Adoption of brinjal IPM will certainly increase profitability, perhaps by Rs.66,794/ha. Farmers are expected to retain only 10% of the economic surplus from hybrid Bt brinjal, but are expected to retain 63% of the economic surplus from brinjal IPM.

The narrow utilitarian analysis used by the GEAC in EC-II and the Dossier assumes that the only relevant human value is related to money, and the best social option is the one that produces the most money. Human flourishing is reduced to whatever money can buy, and issues of equity and fairness are irrelevant to this way of thinking. A wider utilitarian approach is not used; such an approach might consider individual happiness, desire or preference to be a more ultimate value than money.

To conduct the narrow utilitarian analysis suggested by GEAC and EC-II, it is only necessary to have information on the predicted average profitability of Bt brinjal. A key to the analysis, however, is that profitability is measured realistically for all of the farmers of interest.

Profitability can be estimated from data on agronomic performance and efficacy of Bt brinjal. However, data on agronomic performance and efficacy should be critically evaluated before being used to predict the affect of Bt brinjal on Indian farmers. There is first a question of whether all Indian farmers are represented by experimental approaches used. As will be elaborated below, this is not likely. Second, is a question of whether the experimentally estimated yields accurately represent farmer yield. The extensive literature on yield gaps suggests strongly that the reported yield advantage of Bt brinjal is substantially overstated. Third, is a question of how Bt brinjal actually fits into farmer production practices and other technological innovations in pest

control for brinjal. This is a complex question that will require some effort to gain clarity.

Finding 23. The agronomic performance and efficacy experiments for hybrid Bt brinjal are designed for large-scale commercial brinjal production systems, and do not reflect the production systems used by small-scale resource-poor farmers. The data are probably appropriate for about 4% of brinjal production in India.

The multi-site trials (MST, Dossier, volume 6) and the large-scale trials (LST, Supplemental Materials) are not designed to reflect the diversity of production systems in India. Specifically, these experiments mimic the production systems of large-scale commercial brinjal producers who already purchase hybrid seed and do not reflect the production systems used by small-scale resource-poor farmers.

A. No trials were conducted where the most brinjal is grown. West Bengal, Orissa, and Bihar produce 61% of the brinjal in India. Bt brinjal has been banned from these states, but because agronomic performance varies with geographic locality, the results from the MST and LST can apply at most to 39% of the brinjal production in India.

B. Comparisons are made among a Bt brinjal hybrid, a genetically similar non-Bt brinjal hybrid, a commercial hybrid with similar fruit morphology, and a local variety with similar fruit morphology (in year 2 of the MST). The local variety used in the MST experiments is not necessarily the most common or preferred local variety. Thus, the brinjal varieties are often not suitable for estimating profitability of small-scale resource poor farmers, who grow local varieties.

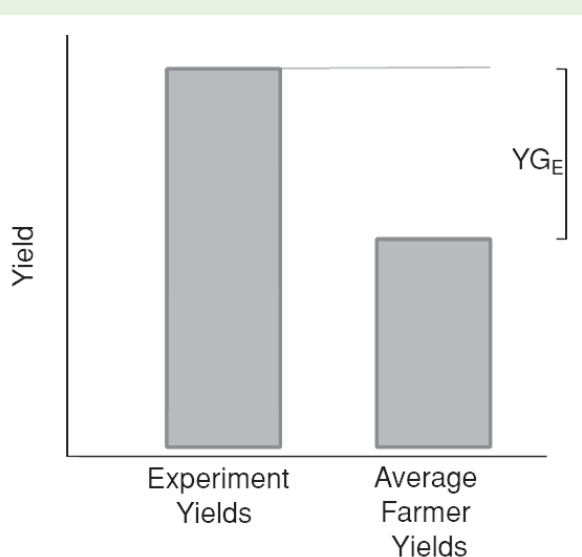
C. Agricultural inputs in the MST and LST are those recommended for large-scale commercial production. Small-scale resource-poor farmers typically use saved seed, manures and composts for fertilisers and locally produced insecticides and hand-rouging for pest control. Thus, the production system used are appropriate for large-scale commercial farmers, but not for small-scale resource-poor farmers. On average, about 10% of the brinjal producers outside of West Bengal, Orissa and Bihar are large-scale commercial producers (APEDA 2009). So the MST and LST data are appropriate only for about 4% of the brinjal producers in India; these are producers that are outside these states and produce on large-scale (100 x 0.39 x 0.10).

D. The LST field trials were probably conducted on soils representative of intensive, large-scale, commercial production. Availability of potash, a critical macro nutrient for brinjal depends on many factors. Under intensive, large-scale, commercial brinjal production, soil organic matter content (SOM) can drop to 0.3-0.4%. When SOM is this low, brinjal yield potential depends primarily on the levels of external input. SOM for many small-scale resource-poor farmers is typically much higher than this, and under these conditions, brinjal yield is a complicated function of soil quality and external inputs. Consequently, the agronomic yield data probably do not represent production systems of small-scale resource-poor farmers. Although soil pH and SOM content are not reported for the LST field trials, the trials were conducted on experimental farms that were likely to reflect standard, commercial production systems with low SOM.

E. Brinjal yield loss from BFSB is different for large-scale commercial producers and small-scale resource-poor farmers (Table 1 in Context and Need section). The MST and LST assess yield loss the way large-scale commercial producers would assess it. Small-scale resource-poor farmers sell partially infested fruits on the market and consume the unsold remainder. Less damaged fruits can be marketed at a good price because BFSB damage symptoms indicate to some consumers that reduced amounts of pesticides have been used.

F. A critical economic value of small-scale resource-poor farmers is not considered. For these farmers, brinjal shelf life is critical for determining the economic value of the brinjal crop. Under small-scale production practices, brinjal typically has a much longer shelf life than brinjal produced under large-scale production practices. The longer shelf life means brinjal retains its

Figure 3. Measure of yield gap between yields observed in experimental trials and average yields on farm.



economic value for a longer period of time, and small-scale resource-poor farmers may distribute their supply over time to obtain higher and/or steadier income. In this way, brinjal is essential for the economic security of small-scale resource-poor farmers.

Large-scale commercial growers comprise about 10% of all brinjal producers outside of the main production areas in West Bengal, Orissa, and Bihar. Therefore, the profitability analysis presented by EC-II is appropriate for only about 4% of all the brinjal producers in India ($0.1 \times (1 - 0.61)$).

Despite all of the limitations, a narrow utilitarian may still suggest that the MST and LST data can be used to estimate profitability of Bt brinjal for farmers in India. This is a widely-shared perspective and is not as simple to refute as the previous argument might make evident. For example, limitation A is already taken into account in the calculation in the previous paragraph, and Table 1 (Context and Need section) indicates that limitation E might be addressed by discounting the MST and LST results by 2/3rds. In a similar way, it could be argued that limitations B, C, and D can be addressed by discounting the MST and LST by some additional unknown X% to take into account those data limitations. For lack of a better value, a narrow utilitarian might perhaps suggest that 50% would suffice. Therefore, the profitability for small-scale resource-poor farmers might be 1/6th that estimated by EC-II. However, this calculation does not take into account limitation F or the issue of yield gaps, which is covered next.

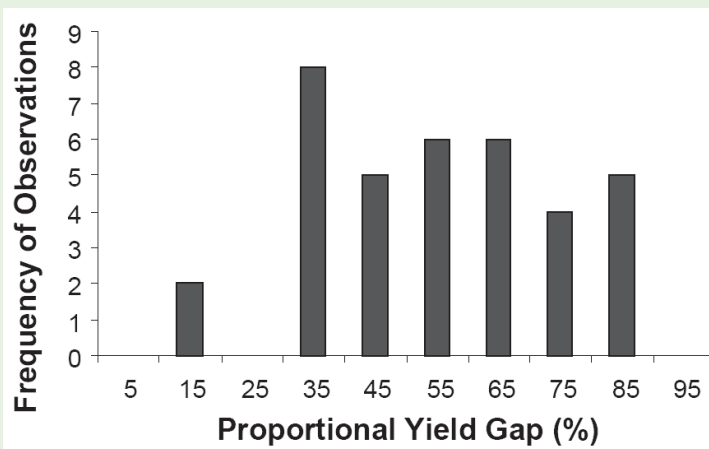
Finding 24. Yield gaps are prevalent between experimentally estimated yield and average farmer yield. The yield benefit of hybrid Bt brinjal estimated from the controlled MST and LST experiments should be multiplied by 0.54 to estimate the yield benefit for the average large-scale commercial farmer. This also reduces the estimated benefit to small-scale resource-poor framers.

Box 12

Brinjal Yield Gap: Overestimation of Yield Benefit of Bt Brinjal

Because of the existence of yield gaps between yields measured under experimental conditions at agricultural colleges and experiment stations versus the yields obtained on farmer fields (Fig. 3), the yield benefit of Bt brinjal has been overestimated. This can be demonstrated as follows. Suppose Y_{Bt} is experimental Bt brinjal yield and Y_{nBt} is the experimental non-Bt brinjal yield. The yield benefit of Bt brinjal is calculated in the MST and LST as $Y_{Bt} - Y_{nBt}$. Because yields determined from experiments overestimate average farmer yields by the proportional yield gap, PYG_E , both Y_{Bt} and Y_{nBt} should be multiplied by PYG_E to estimate the average farmer yields for the same experimental treatments. Thus, $PYG_E \times Y_{Bt}$ and $PYG_E \times Y_{nBt}$ estimate average farmer yields for the two experimental treatments. The yield benefit of Bt brinjal for the average farmer is consequently $PYG_E (Y_{Bt} - Y_{nBt})$, which can be rewritten as $PYG_E (Y_{Bt} - Y_{nBt})$. Thus, the yield benefit for Bt brinjal was overestimated in the MST and LST by the proportion given by PYG_E .

The proportional yield gap for rice and maize can be estimated from data published in Lobell et al. (2009). The majority of data on rice is from India. The data on maize is mostly from the US, Latin America and Africa. The distribution of estimated proportional yield gaps is shown in the figure below.



The average proportional yield gap for these data is 54%.

Considerable research efforts are expended to reduce the yield gap (= bring the PYG up to 1.0) and bring average farmer yields up to the levels obtainable on experiment stations. To do this, it is essential to understand the underlying causes of the yield gaps, which are many and varied. The proportional yield gaps for US maize in Nebraska, the heart of the Corn Belt were 40% and 56%. These values are very close to the mean for all countries, including many developing countries, so the high technology used in the US does not eliminate yield gaps; likewise, Bt brinjal is unlikely to substantially reduce the brinjal yield gap. Probably the most significant factors contributing to yield gaps are a class of factors related to local variation or field-specific factors (Lobell et al. 2009). These include farmer variation in management and weather and soil conditions. Considerable effort must be expended to reduce yield gaps (= raise proportional yield gaps to 1.0). This implies for Bt brinjal that simply making the technology available to farmers will not result in the dramatic yield increases promised in EC-II. Instead, Mahyco and the Indian government must invest considerable amounts in outreach efforts so any yield benefits can be realized. Such an investment, of course, reduces the social economic surplus that Bt brinjal might generate and thereby reducing its social value.

A yield gap is the difference between the average yield obtained by farmers and the “potential” yield for a given agricultural technology, and can be estimated in several ways. One of the most common ways to measure potential yield of an agricultural technology is through experimental yield trials, such as those conducted in the MST and LST for Bt brinjal. Typically there is a substantial yield gap between the experimentally obtained yields and those that the average farmer obtains (Fig. 3). This is calculated as the yield gap, $YG_E = Y_E - Y_F$, where Y_E is the yield estimated in the experiment and Y_F is the average farmer yield. The proportional yield gap for experiments (PYG_E) can be calculated as $PYG_E = Y_F/Y_E$. This can be used to adjust data obtained from controlled experimental conditions to average farmer conditions for a given agricultural technology, such as Bt brinjal.

The data in the MST and LST were obtained from experiments conducted under controlled conditions at experiment stations and agricultural colleges. These data will overestimate yields that would be obtained by farmers according to an estimated proportional yield gap, PYG_E . This implies that the yield benefit from Bt brinjal will have been overestimated in the MST and LST (Box 12). If PYG_E can be estimated, it is possible to use the MST and LST data to estimate the true expected benefits to the average Indian brinjal farmer (presumably a large-scale producer), subject to the limitations mentioned in the previous section.

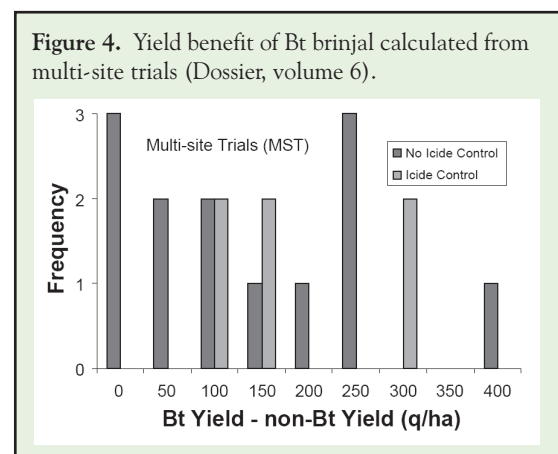
Yield gaps for brinjal have not been summarised in the literature, so there is no ready value that can be used for $PYGE$ in this case. However, all reported yield gaps are positive (Lobell et al. 2009), meaning that the yield estimated in an experimental trial of an agricultural technology is always larger than the average farmer yield with the same technology. Moreover, given the difficulty growing vegetable crops compared to grains, one might expect that the yield gap for a vegetable crop would be as large as or larger than the yield gap for staple grains. Armed with these two assumptions, it is possible to use published data on yield gaps in grain crops (Box 12). These considerations give a yield gap of 54%, meaning that the estimated benefit of Bt brinjal provided by the MST and LST must be multiplied by 0.54 to give an estimate of the yield benefit to the average large-scale, commercial producer.

Finding 25. The expected maximum potential yield benefit from hybrid Bt brinjal is probably ≤ 43.7 q/ha for large-scale commercial farmers and ≤ 7.2 q/ha for small-scale resource-poor farmers; about 16 % of the time hybrid Bt brinjal is not expected to out-yield non-Bt brinjal.

A maximum potential yield benefit from Bt brinjal for the average large-scale commercial farmer can be calculated from the MST and LST data provided in the Dossier (volume 6) and the Supplemental Materials. This is a maximum potential yield benefit because it is estimated under well-controlled experimental conditions, and the yield gap is expected to reduce the actual benefit that could be obtained by the average large-scale commercial farmer.

The MST data are aggregated across all three years of the studies. At each site, an experiment to compare Bt brinjal with non-Bt brinjal was replicated four times in small plots of 27m². This is smaller than the average field size even for small-scale resource-poor farmers, but is larger than some fields. Experiments were conducted under controlled conditions using standard commercial brinjal production practices. During the first two years of MST, no insecticides for the control of BFSB were applied to the control treatment, non-Bt brinjal (Fig. 4, No Insect Control). During the final year of MST, insecticides were applied to the non-Bt brinjal to control BFSB as needed (Fig. 4, Insect Control). It should be expected that the yield benefit of Bt brinjal should be higher when compared to a no-insecticide for BFSB treatment than when compared to a treatment where BFSB is controlled with insecticides. However, the MST data does not meet this expectation. The average yield benefit with a no-insecticide control treatment is 139 q/ha, while it is 192 q/ha with an insecticide control treatment. There are many possible reasons for such a difference, but no explanation can be independently verified. Therefore, all of the MST data are pooled.

The results from each of the 19 MST experiments are shown in Fig. 4. One trial had a very large yield advantage for Bt brinjal of 420 q/ha, while three trials (16% of total trials) had almost no yield benefit. On



average, the Bt brinjal varieties yielded 156 q/ha more undamaged brinjal than in the non-Bt control with no control for BFSB. The accuracy of this estimate is uncertain. In its favour, all of the MST experiments are well-replicated and statistically valid. Against this is the small MST plot size, which always leads to overestimates of yield, typically because plants near the edges of plots suffer less competition for nutrients and light and have higher yields. On balance, MST results probably overestimate the true yield benefit.

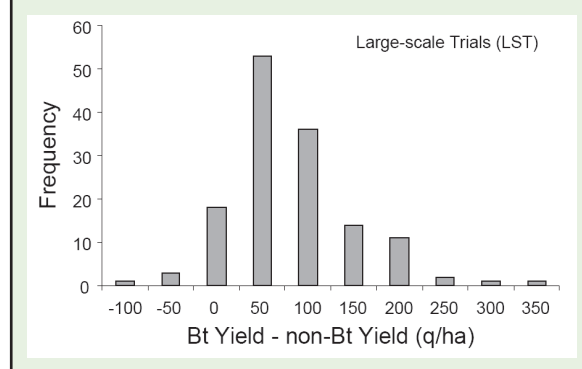
The LST data are aggregated across all sites and the two years of study. At each of 11 sites, seven different varieties of Bt brinjal were tested against an appropriate non-Bt control variety, which had insecticides applied to control BFSB as needed. Plots were about 108m², but none of the plots were replicated within a site. Thus, from a technical statistical perspective this experiment is either unreplicated and therefore without scientific value, or sites are replications, and between site differences must be ignored as noise. In this second interpretation, it must be assumed that the chosen sites are representative of the broader range of Indian environments with large-scale commercial brinjal production. Experiments were conducted under controlled conditions using standard commercial brinjal production practices.

The results from all of the 11 sites over two years are shown in Fig. 5. One variety-site had a very large yield advantage for Bt brinjal of 330 q/ha, while 22 variety-sites (16% of total variety-site comparisons) had almost no yield benefit or a yield loss. On average, the Bt brinjal varieties yielded 81 q/ha more undamaged brinjal than in the non-Bt control with control for BFSB. This is about half that obtained from the multi-site trials. The accuracy of the LST estimate is uncertain. In its favour, the plot sizes are large enough to provide meaningful yield estimates. In addition, a large number of sites are tested for multiple years; both factors lend greater confidence to the result. Against these is the lack of replication at any site. However, if sites and years are treated as replicates, the LST estimate is probably more accurate than the MST estimate.

Because the LST experiments are not replicated within any site, it is not sound science to examine the interaction of site and variety. Typically, however, large-scale crop variety experiments are designed to determine which variety performs best at which locations (i.e., a site by variety interaction). Unfortunately, the LST is not appropriately designed. This means that the LST cannot reliably inform anyone (including farmers, Mahyco, or the Indian government) about which variety does best at which location. However, it is possible that this critical limitation to the data will be ignored. If this limitation is ignored, a foolhardy or unscrupulous person might try to recommend which Bt varieties performed best at which location. Taking the foolhardy approach, the two highest yielding Bt brinjal varieties at each of the 9 sites where the experiment was conducted both years were identified. The average yield benefit for these “best” varieties was 125 q/ha for Bt brinjal. This does not exceed the average for the MSTs, which merely emphasises that yield gaps occur due to spatial scale even under highly controlled conditions. Objectively, however, this value should not be used to calculate the yield benefit of hybrid Bt brinjal.

All of the information in this lengthy discussion of utilitarian value can be combined to estimate the average yield benefit from Bt brinjal for farmers in India (Table 4). Farmers in West Bengal, Orissa, and Bihar cannot use Bt brinjal because it is not allowed to be grown in those states by state law. Therefore, their expected maximum yield benefit from Bt brinjal is 0. The remaining 39% of Indian brinjal production may be able to use Bt brinjal, and if they do, Bt brinjal is expected to increase brinjal yield for both large-scale commercial farmers and small-scale resource-poor farmers (Table 4). The approximately 4% large-scale commercial producers outside of West Bengal, Orissa, and Bihar may expect that hybrid Bt brinjal will yield between 43.7-84.2 quintal/ha more than non-Bt brinjal, with the true value probably closer to the lower value. The approximately 35% small-scale resource-poor farmers may expect only 7.2-13.9 quintal/ha more from hybrid Bt brinjal. Again the lower value is more likely than the higher value. Taking the weighted average over the entire country, Indian farmers may expect EE-1 hybrid Bt brinjal to yield only 4.3-8.2 quintal/ha more if everyone who could adopt it did do so (assuming EE-1 is approved for commercial use). If, in addition, all of the brinjal farmers in West Bengal, Orissa, and Bihar were allowed to use Bt brinjal and they did use it, Bt brinjal would be expected to yield 11.0-21.1 q/ha more.

Figure 5. Yield benefit of Bt brinjal calculated from large-scale trials (Supplemental Materials).



These values for the yield benefit of Bt brinjal are significantly less than those suggested by Mahyco and EC-II.

The main reasons for this are that (1) Mahyco and EC-II did not consider how EE-1 Bt brinjal would be compatible with present brinjal production systems, and (2) they assumed that yields obtained under controlled experimental conditions accurately estimated yields on farm and ignored the scientific literature on yield gaps. Moreover, they did not adjust their estimates to reflect their own data. Their estimates are based on the MST data. The newer LST data shows significantly lower potential yield benefits from EE-1 Bt brinjal, yet their estimates have not been adjusted downward.

Table 4. Estimated expected maximum yield benefit from hybrid Bt brinjal to farmers in India based on a comparison to a similar non-Bt brinjal hybrid without any control of BFSB, using data from the MST and LST, yield gaps, and other factors related to Indian production systems described above.				
Location	Size of farm	Proportion of farmers	Maximum potential yield benefit (q/ha)	
			MST	LST
West Bengal, Orissa, Bihar	Mostly small-scale resource-poor	0.61	0	0
Rest of India	Large-scale commercial	0.04	84.2	43.7
Rest of India	Small-scale resource-poor	0.35	13.9	7.2

Even so, the low estimates presented here are likely to overestimate the actual yield benefits to India as a whole because it is unlikely that all farmers will adopt EE-1 hybrid Bt brinjal technology. There are three factors that may limit adoption. As discussed above, hybrid Bt brinjal may not fit well with some of the large-scale commercial production systems that presently rely on open-pollinated varieties (OPVs), and is unlikely to fit into small-scale resource-poor production. In addition, hybrid Bt brinjal may be too costly for some farmers to afford to use. Lastly, the yield risk of using hybrid Bt brinjal may deter many farmers from adopting it. About 16% of the field trials showed hybrid Bt brinjal having no yield advantage over non-Bt brinjal even when non-Bt brinjal had no control of BFSB. Many farmers are risk-averse and may not adopt hybrid Bt brinjal if the risk of no benefit is too high.

In addition, the estimates are likely to be overestimates because the relevant control comparison for farmers is the Bt variety against the best non-Bt variety being used in a region. The control comparison used in the MST and LST was the Bt variety against a genetically similar non-Bt variety. The best non-Bt variety in a region is expected to have higher yields than a genetically similar non-Bt control variety used in the MST and LST experiments, and this also will reduce the estimated yield benefit of hybrid Bt brinjal. This serves to reiterate previous points about the limitations of the data from the MST and LST experiments. One should expect a large yield gap and refrain from making any inferences about which hybrid Bt brinjal variety is best in a region.

The MST and LST were designed to answer the narrow question: how much does the EE-1 Bt transgene, by itself and all other things being equal (the *ceteris paribus* assumption), improve agronomic performance. This is not the relevant question for a farmer. A farmer is interested in whether hybrid Bt brinjal will “improve” things compared to what the farmer is presently doing. For farmers, all things are not equal, and the concern is a comparison of brinjal varieties (often under unequal conditions), not merely the effect of the transgene. The narrow framing of the MST and LST limits the broader applicability of these data to problems of interest to the farmer or the Indian government.

Finding 26. Insecticide use might decline in large-scale commercial Bt brinjal production systems by an average of 6.5 applications. However, other factors may modulate this substantially, and new secondary pests will result in more insecticide use. It is not possible to estimate how insecticide use might change if Bt brinjal were used by small-scale resource-poor farmers.

Another expected advantage of Bt brinjal is a reduction in insecticide use. A reduction in the need for insecticide

applications on brinjal would be welcomed by many farmers. The vast majority of Indian farmers recognise that insecticides have adverse effects on their personal health and the health of their families (Alam et al. 2006, Barel et al. 2006).

Brinjal farmers typically overuse insecticides (see Context and Need section above). This is true for both large-scale commercial farmers and small-scale resource-poor farmers. Small-scale resource-poor farmers probably apply insecticides much more frequently than large-scale commercial farmers.

Although EC-II and the Dossier imply that Bt brinjal will address the overuse of insecticides, the LST experiments are designed to determine how much Bt brinjal can reduce insecticide use compared to optimal use of insecticides to control BFSB. Optimal control is based on applying insecticides when BFSB damage exceeds some threshold. Threshold-based application of insecticides is a fundamental cornerstone of Integrated Pest Management (IPM).

The results for the 11 sites and two years of LST experiments are shown in Fig. 6. At one site, two of the Bt brinjal varieties eliminated a whopping 16 applications of insecticides (2.4% of observations). However, in 6.4% of the observations Bt brinjal did not eliminate any applications of insecticides. The variation is quite large (Fig. 6), and it may be a bimodal distribution, with some Bt brinjal varieties in some sites in some years eliminating >10 insecticide applications and another group eliminating <6 applications. On average Bt brinjal eliminated **6.5 applications of insecticides**, which is substantial, but much smaller than projected by EC-II and the Dossier. Looking only at the two best Bt varieties at a site (which as discussed above is not a legitimate use of the data), Bt brinjal eliminated 6.9 applications of insecticides. The accuracy of these estimates is not known. Spillover effects due to insects moving from one plot to another may lead to underestimating or overestimating the number of applications eliminated by Bt brinjal. Without additional information on the ecology and phenology of BFSB, it is not possible to know if there is systematic bias in this estimate.

In any event, this estimate may apply to large-scale commercial brinjal farmers but cannot apply to small-scale resource-poor brinjal farmers. The experimental conditions of the LST are similar to large-scale commercial production. Unlike yield, it is not clear how to adjust these estimates of insecticide application to the production systems of small-scale resource-poor farmers. In addition, the concept of yield gap does not transfer to cover pest control.

The emergence of secondary pests of Bt brinjal would increase insecticide application. As discussed under the Biodiversity section above, EC-II has not carefully evaluated the potential for a secondary pest. Some new secondary pests have been anticipated and insecticides are automatically applied to Bt maize in the US for control of corn rootworms. Others have not been anticipated, and have resulted in increased insecticide use on Bt crops relative to the projected need for insecticides. The appearance of secondary pests of Bt crops has been the rule rather than the exception (A. Zeilinger, personal communication), so the estimated reduction of 6.5 applications of insecticide will probably be an overestimate.

Finding 27. Hybrid Bt brinjal may improve net returns of large-scale commercial farmers by at most Rs.23,439/ha and of small-scale resource-poor farmers by at most Rs.3,250/ha. In comparison, brinjal IPM has improved net returns of small-scale resource-poor farmers by Rs.66,794/ha.

The marginal value of Bt brinjal can be calculated using farm budgeting methods. It would be instructive to compare the value of Bt brinjal with other technologies for controlling BFSB to enable policy makers to sound decisions. The main technologies that can be used to improve control for BFSB are Bt brinjal, IPM, traditional methods (CIKS 2010b), and organic production. Unfortunately, data are incomplete for traditional methods and organic production, but a detailed comparison of Bt brinjal and IPM can be made. This is unfortunate, because only organic production is truly an alternative to Bt brinjal because Bt brinjal cannot be used in organic production systems in India. IPM and Bt brinjal are not true alternatives, because it is possible to use both, perhaps with an added value. However, in accounting for the marginal value of a new technology, it is

Figure 6. Number of insecticide applications eliminated by Bt brinjal compared to the non-Bt control based on the large-scale trials (Supplemental Materials).

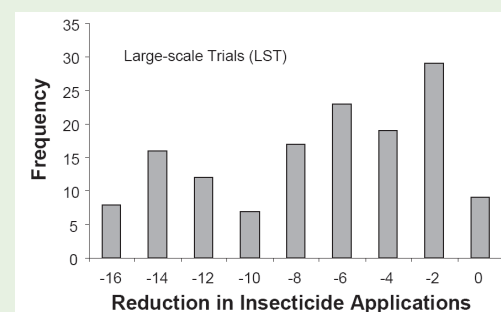


Table 5. Net returns on variable costs of brinjal production for two agricultural technologies for managing BFSB, IPM for brinjal and hybrid Bt brinjal. – indicates returns without the technology and + indicates returns with the technology. Diff. is the difference between + and –.

Location and Technology	Source of Data	Farm-scale	Net Return on Variable Costs (/ha)		
			–	+	Diff.
West Bengal (Rs.)					
Brinjal IPM (Baral et al. 2006)	On-farm	Small-scale	170,500	205,075	34,575
Bt brinjal (Krishna & Qiam 2008)	Experimental	All*	24,248	68,322	[44,074]
Bt brinjal (here)	Adjusted Expt.	Small-scale			[0]
Bt brinjal (here)	Adjusted Expt.	Large-scale			[23,439]
West Gujarat (Rs.)					
Brinjal IPM (Alam et al. 2006)	On-farm	Small-scale	141,037	224,000	82,963
Andhra Pradesh & Karnataka (Rs.)					
Bt brinjal (Krishna & Qiam 2008)	Experimental	All*	66,158	106,433	40,276
Bt brinjal (here)	Adjusted Expt.	Small-scale			3,250
Bt brinjal (here)	Adjusted Expt.	Large-scale			23,439
Bangladesh (Tk.)					
Brinjal IPM (Alam et al. 2006)	On-farm, <i>rabi</i>	Small-scale	151,518	234,217	82,699
Brinjal IPM (Alam et al. 2006)	On-farm, <i>kharif</i>	Small-scale	100,965	242,724	141,759

* See discussion. It is unlikely that these estimates apply to both small-scale resource-poor farmers and large-scale commercial producers.

critical to specify the existing technology carefully or else come double counting may result.

Several works allow total or partial farm budgeting to estimate the marginal value of Bt brinjal and Brinjal IPM. Full cost accounting is done only for IPM in West Bengal (Alam et al. 2006). Total variable costs are evaluated for IPM (Alam et al. 2006 (Gugarat), Baral et al. 2006) and Bt brinjal (Krishna and Qiam 2008). Partial accounting is done here using the information presented above.

Net return on variable costs is reported in Table 5. The IPM results are the most accurate ones in the table. They are calculated on-farm, based on what farmers actually do and what farmers actually obtained while growing brinjal. All of the Bt values are probably overestimates of the true value of the technology. Krishna and Qiam (2008) base their results on the Mahyco MST experiments, which greatly overestimate the value of Bt brinjal (as detailed above). However, Krishna and Qiam (2008) recognise that the yield values reported in the MST experiments are unrealistically high, so they reduce these yield values arbitrarily. Specifically, they halved the yield advantage of hybrid Bt brinjal reported in the MST (although they state that they use 40%, the actual values reported in their table 2 belies this statement) for use in their economic model. This is more reasonable than the approach used by EC-II and the Dossier to discuss yield advantages of hybrid Bt brinjal. However, as noted in Table 4 above, this is not enough of a reduction and the predicted yield benefit is still substantially less than that used by Krishna and Qiam (2008).

In addition, Krishna and Qiam (2008) assumed a 75% reduction in insecticide use against BFSB, which is considerably larger than that estimated above from the LST experiments above and double-counts some of the gains possible using IPM. Thus, Krishna and Qiam (2008) have overestimated the net return for Bt brinjal. Although the actual net returns cannot truly be known until after Bt brinjal has been approved for commercial use, the results estimated above for the yield benefit from Bt brinjal (Table 4) and the average reduction of 6.5 sprays in large-scale commercial brinjal production can be used to generate a more accurate estimate of net return for Bt brinjal. Even this value is probably overestimated, as discussed above. To estimate net return, brinjal prices reported in Krishna and Qiam (2008) were used, Rs.462/q in East India and Rs.421/q elsewhere. In addition, Rs.500/ha was used as the cost of a single application of insecticide, which is the average value calculated from data in Alam et al. (2006). The net returns for Bt brinjal in West Bengal are placed in brackets because Bt brinjal is not allowed in this region (Table 5).

These data (Table 5) bear the conclusion that Krishna and Qiam (2008) have over-estimated the value of hybrid Bt brinjal by about 2-fold. West Bengal presently forbids the commercial use of Bt brinjal. Krishna and Qiam (2008) presume that this will be overturned. Their estimate of an increase in net return of Rs.44,074/ha for Bt brinjal assumes that small-scale resource-poor farmers will adopt hybrid Bt brinjal at a similar rate as large-scale commercial producers (Krishna and Qiam 2007). The previous discussion in this report provides many reasons to expect that small-scale resource-poor farmers will adopt at a lower rate than large-scale commercial farmers. Kolady and Lesser (2005) show to the contrary that small-scale resource-poor farmers are predicted to adopt hybrid Bt brinjal at a lower rate than large-scale commercial producers. Finally, Krishna and Qiam (2007, 2008) rely on farmer's "stated preferences" for hybrid Bt brinjal after being given expected benefits of hybrid Bt brinjal based on the Mahyco MST experiments. These inflated benefits may have induced many small-scale resource-poor farmers to state that they would use hybrid Bt brinjal. In addition, it is well known that a stated preference is higher than a realised preference. In other words, people say they will do something at a much higher rate than they actually do it. Thus, it is unlikely that Krishna and Qiam's (2008) results for West Bengal, Andhra Pradesh and Karnataka are accurate for small-scale resource-poor farmers, and may only apply to large-scale commercial producers. Even considering only large-scale commercial producers, however, Krishna and Qiam's (2008) estimates for net return are probably twice the realistic return for hybrid Bt brinjal (compare their Rs.44,074 and Rs.40,276 to the more accurate Rs.23,439). Overall, it is more likely that hybrid Bt brinjal will increase net returns for large-scale commercial producers by **at most Rs.23,439/ha** and for small-scale resource-poor producers by **at most Rs.3,250/ha**; these estimates do not double count potential reductions in insecticide use.

These data (Table 5) also show that brinjal IPM has been vastly more profitable than projections for hybrid Bt brinjal. Brinjal IPM has been about 3-times more profitable than Bt brinjal is projected, and has directly improved profitability of small-scale resource-poor farmers. Converting 1.5Tk. = 1.0Rs., IPM has returned Rs.34,575 – Rs.94,506/ha in eastern India and Bangladesh and Rs.82,963/ha in Gujarat. Averaging across these estimates, brinjal IPM has increased profitability for the small-scale resource-poor farmers who have used it by **Rs.66,794/ha**. Moreover, as will be discussed below, farmers will retain more of the benefit from IPM than they would retain from Bt brinjal.

Clearly, compared to hybrid Bt brinjal, a national policy that promotes the proven technology of brinjal IPM (such as ICAR 2006) would provide greater financial benefits to the millions of small-scale resource-poor farmers in India at reduced risk to the farmers and the environment. Brinjal IPM is also likely to improve profitability of large-scale commercial producers. While hybrid Bt brinjal may improve profitability of large-scale commercial producers over and above the benefits from brinjal IPM, it is not likely to improve profitability of small-scale resource-poor farmers significantly. To set a rational national policy on brinjal pest control, economic information on traditional BFSB control practices, organic production, and integrated use of neem-based insecticides is also needed. GEAC should consider the need for hybrid Bt brinjal in the context of other comparable agricultural technologies.

Finding 28. The estimated economic surplus for brinjal IPM is significantly larger than for hybrid Bt brinjal. Farmers are expected to receive 63% of the surplus from brinjal IPM but only 10% of the surplus from hybrid Bt brinjal. Increased public investment, greater promotion, and strengthened public policy for brinjal IPM relative to those for hybrid Bt brinjal will result in greater social benefits in India and a major increase in profitability for small-scale resource-poor farmers.

A technology that causes a large improvement in crop yield and is adopted by many farmers will increase the supply of the crop thereby depressing its price. At the depressed price, farmers will benefit less from having used the technology, but consumers will benefit from the reduced cost of food. The economic surplus is an estimate of the total social value of the technology and is based on projected technology adoption rates, changes in crop supply, and changes in crop price to the

consumer. It is typically divided into a producer surplus (related to the increased supply times the reduced price compared to the present supply times the present nominal price) and a consumer surplus (related to the increased supply times the present nominal price compared to the increased supply times the reduced price). The reduced price is related both to the projected increased supply and a projected change in demand. In India, demand for brinjal is expected to grow as the Indian population increases. In addition, the producer surplus must be divided between the farmer and the producer of the new technology. This is related to the price of the new technology and the quantity of the technology that will be used to generate the increased supply.

Economic surplus for hybrid Bt brinjal has been estimated by Krishna and Qiam (2008) for all of India, using data only from West Bengal, Andhra Pradesh and Karnataka. Unfortunately, this estimate relies on several assumptions that all lead to an overestimate of the economic surplus. These include a) hybrid Bt brinjal increases yield by 40% over hybrid non-Bt brinjal and by 60% over OPV non-Bt brinjal; b) Bt brinjal reduces insecticide costs by 75%; c) small-scale resource-poor farmers will adopt hybrid Bt brinjal at the same rate as large-scale commercial farmers; and d) stated preference for hybrid Bt brinjal accurately estimates the future choice to use hybrid Bt brinjal. Overall these assumptions may also overestimate the proportion of the total economic surplus that accrues to consumers. Although three technology scenarios are presented (Krishna and Qiam 2008), Scenario II is the most likely projected near-time future for hybrid Bt brinjal, if it is commercialised. It assumes that only hybrid Bt brinjal varieties are available and that only 2% of farmers in eastern India are likely to adopt hybrid Bt brinjal. Because West Bengal, Orissa, and Bihar comprise nearly all of the production in eastern India, and all three forbid the commercial use of Bt brinjal, Scenario II is the most likely future scenario.

Economic surplus for brinjal IPM has been estimated by Alam et al. (2006) for the Birbhum District of West Bengal. They do not attempt to estimate economic surplus for all of India. Because the data are based on actual farmer behaviour, the estimate of economic surplus does not suffer the same failings as the estimate for Bt brinjal.

The economic surplus for brinjal IPM is estimated at Rs.326 million for one district of West Bengal (Table 6). In contrast, the economic surplus for Bt brinjal is estimated at Rs.1,008 million for all of India, and this is considerably overestimated. Brinjal IPM will provide significantly greater social benefits to India than hybrid Bt brinjal. Although these two technologies are not mutually exclusive (they can be used together), a significantly larger public investment in IPM technology is merited.

The consumer surplus is estimated to be 37% and 41% for the two technologies (Table 6). The similarity in the estimates suggests that similar price elasticities of supply and demand were used in both calculations, which is reassuring.

More interesting is the farmer surplus. Farmers receive most of the benefits from brinjal IPM (63%). Company surplus is estimated to be 0%, because brinjal IPM requires mostly hand rouging and intermittent insecticide application. If pheromone use becomes a key component of brinjal IPM, some of the producer surplus will go to companies providing pheromone. Farmers are projected to receive only 10% of the economic surplus from hybrid Bt brinjal, even under the optimistic case described by Krishna and Qiam (2008). Almost half of the economic surplus is projected to go to Mahyco. The division between farmers and Mahyco is based on Krishna and Qiam's (2007, 2008) estimate of the willingness-to-pay for hybrid Bt brinjal by Indian farmers, which is the optimal price for Mahyco to charge. If Mahyco were to charge less for hybrid Bt brinjal seed then farmers would receive a larger proportion of the economic surplus.

It is remarkable that farmers may receive relatively little of the potential economic benefit from hybrid Bt brinjal even though they will be assuming most of the risk of producing it.

	Brinjal IPM ¹	Hybrid Bt Brinjal ²
Economic Surplus (Rs.M)	326 ³	1,008 ⁴
Consumer surplus (%)	37	41
Farmer surplus (%)	63	10
Company surplus (%)	0	48
1 Results from Alam et al. 2006 2 Results from Krishna and Qiam 2008 3 For the Birbhum District of West Bengal only 4 For all of India		

These results amplify the need for increased public investment, greater promotion, and strengthened public policy commitment in brinjal IPM relative to those in hybrid Bt brinjal. ICAR (2006) has endorsed the promotion and use of IPM in India, but a coordinated public-sector approach to management of BFB in brinjal is not readily apparent.

Economic Security – Utilitarian Analysis

Conclusion 9. No analysis of the effects of Bt brinjal on the economic security of Indian farmers has been conducted or cited. Small-scale resource-poor farmers using hybrid Bt brinjal may have increased risk of economic failure. An analysis of the effect of hybrid Bt brinjal on the economic security of brinjal farmers should be conducted.

Economic security is important for farmers to enable them to continue with their livelihood from year to year and also to meet financial obligations throughout the year. Both are related to debt. Year-to-year security is ensured if the farmer can afford to reduce debt annually. This can be met if annual profitability is consistent and not subject to large swings in value. Short term security is ensured if the farmer has sufficient cash reserves to meet short-term debt obligations. This can be met if there is sufficient cash flow during the growing season when capital is tied up in the growing crops.

Economic security is a major focus of concern for Indian farmers. The high suicide rate among Indian farmers is an indication of the nearly-criminal lack of economic security provided by society. As discussed above (Context and Need), brinjal farmers in India have a quite low suicide rate, because brinjal helps cash flow and buffers variation in other crops. A socioeconomic analysis of all new agricultural technology should include an evaluation of effects on economic security.

No analysis of the effects of Bt brinjal on the economic security of Indian farmers has been conducted or cited by EC-II or the Dossier. Any changes to policy on the patenting of plants could have significant implications for the economic security of Indian farmers, but this is outside the scope of this report.

An analysis of year-to-year security would rely on estimates of inter-annual variation in net returns to Bt brinjal compared to present production practices. Factors that can affect this include: a) yield variation, b) variation in insecticide use, c) debt associated with insecticide and seed purchase, and d) proportion of the total economic surplus returning to the farmer. There is no data on a) or b). Debt associated with seed purchase is likely to be greater for hybrid Bt brinjal than for other seed because Bt brinjal is likely to be more expensive. However, debt associated with purchase of synthetic insecticides may be reduced. The proportion of the economic surplus returning to farmers may be as low as 10% (Krishna and Qiam 2008). The ratio of increased debt to surplus returned may give one estimate of inter-annual economic security.

Hybrid Bt brinjal may increase farmer suicide rates. If we assume that hybrid Bt brinjal is adopted by small-scale resource-poor farmers, then these farmers will be foregoing their ability to independently source their brinjal seed, and over time they may become dependent for seed from Mahyco. This will jeopardise their economic security, because for small-scale resource-poor farmers money saved on inputs is money earned. Presently, brinjal farmers have among the lowest rate of suicides among farmers in India, so jeopardising economic security is likely to raise suicide rates. In addition, once hybrid Bt brinjal seed is purchased at high cost, it may require other crop management practices (fertiliser use and insecticide applications) to reduce the risk of seed failure. This would generate still higher cash costs to the small-scale resource-poor farmer. At the same time, local natural agricultural resources (seeds, manures, local insecticides) may decline or vanish, as they did under Green Revolution rice production. Such changes would further jeopardise economic security.

An analysis of cash flow would be complicated because it requires a detailed examination of debt and income during the growing season, and there may be many ways a farmer meets these cash flow demands, including taking out additional debt. Moreover, there are biological factors that matter. Even if Bt brinjal does yield significantly more for small-scale resource-poor farmers, its shelf life may be shorter than the local variety, and consequently some of the yield increase may rot before it can be sold. In addition, a farmer using Bt brinjal may have to abandon the niche market afforded by the local variety, and may receive a lower price because of competition with other hybrid brinjal varieties.

Health, Religion and Culture

Conclusion 10. There are many important human values outside of utilitarian values that are important for economic development. Hybrid Bt brinjal may adversely effect human health, religion and culture. These potential concerns should be assessed.

Nobel prize winner, Amartya Sen, has eloquently argued that utilitarian values are too narrow a guide for economic development (Sen 1999). He suggests that other factors associated with human freedom are essential for humans to flourish under economic development. These factors can be analysed under a more general consequentialist paradigm, which is much broader than utilitarianism. Three such elements have emerged in the environmental assessment of Bt brinjal. None of these have been considered ex ante as a potential effect of Bt brinjal.

Health. Brinjal is an essential vegetable in the health of all peoples of India. It provides food, some essential nutrients and has significant medicinal values. Hybrid Bt brinjal may reduce brinjal varietal diversity, similar to the way that Green Revolution rice eliminated many of the local rice varieties. Now the red varieties of rice, formerly common in India have become rare, and despite the recent demand for these varieties, the market has not responded. Losses of brinjal varietal diversity may also lead to induced shortages of presently undervalued brinjal varieties.

It is not the purpose here to address the direct human health effects of Bt brinjal, but to raise the issue that adopting hybrid Bt brinjal may alter the food security of farmers and their families. Hybrid Bt brinjal may have different or lower food or nutritional quality than the variety it replaces, and this may alter the health of the farm family. Hybrid Bt brinjal could reduce the price of brinjal, resulting in poorer nutrition among poor farm families because of temporary cash flow shortages.

Religion. Several local brinjal varieties are used in religious ceremonies because of their special properties. As mentioned previously udipigulla is important in certain religious ceremonies in Karnataka. Would gene flow from Bt brinjal to these local varieties compromise their ceremonial value? Inquiries to the appropriate religious leaders may provide guidance on whether this is a real concern.

Culture. Many more local brinjal varieties serve important cultural functions in addition to their role as food. These include roles in special foods made for auspicious occasions such as weddings. Would gene flow from Bt brinjal to these local varieties compromise their cultural value? Surveys and interviews of people in these regions may provide guidance on whether this issue is a real concern.

Large – Scale Indirect Socioeconomic Effects

Conclusion 11. Conclusion 10. There are many important human values outside of utilitarian values that are important for economic development. Hybrid Bt brinjal may adversely effect human health, religion and culture. These potential concerns should be assessed.

New agricultural technologies may have large-scale indirect adverse effects of socioeconomic concern. Although these are not typically addressed as a part of the environment, they are nevertheless a part of the environment just as humans and human activities are part of the environment.

Bt brinjal is not a panacea and like Bt cotton (Nguyen et al. 2008), will require new and additional scientific information to maintain its benefits for society over the relatively short time periods of 5-10 years. This implies that India will need a geographically extensive extension service that is prepared to provide timely information about new demands for pest control as Bt brinjal is commercialised. Without this support, many of the potential social benefits of Bt brinjal will likely be lost soon after commercialisation. Lacking such support should make the government pause to consider the wisdom of its investment in Bt brinjal.

Second, Bt brinjal may lead to the loss of many local brinjal varieties through indirect means. If hybrid Bt brinjal is adopted by small-scale resource-poor farmers, then other crop management practices (fertiliser use and insecticide applications) may change to reduce the risk of seed failure. This would lock farmers into a Bt brinjal technology package, which would probably lead to a reduction in local natural agricultural resources, especially local brinjal varietal diversity. The loss of local brinjal varietal diversity may be considerable, and this threat must be seriously considered, and if needed, actions taken to avoid it.

Third, the internal supply of brinjal is expected to increase, driving the wholesale price down (Krishna and Qiam 2008). Although Bt brinjal farmers may receive only a small portion of the benefits stemming from increased supply (discussed above), the predicted price decline will transfer greater economic risks to both small-scale resource-poor and large-scale commercial non-Bt brinjal farmers. This is not fair.

Fourth, commercialisation of Bt brinjal may interfere with government plans to develop a strong vegetable export market. Affluent importing countries in Europe and Asia may question their desire for Indian brinjal because of an aversion to genetically engineered foods. The commercialisation of Bt brinjal may lead to suspicions about other Indian vegetable exports, which would slow down the growth of the export market.

Needed Experiments and Observations

1. Brinjal yield gap. Compile scientific literature on yield gaps for brinjal. A brinjal yield gap can be substituted for the grain-based yield gap used in this report.
2. Returns to alternatives. The Indian government should promote research to estimate net economic returns from traditional, local, and organic practices to limit damage from BFSB. Once these values are known, the government can adjust its policies to promote the technologies that are most likely to help Indian farmers and consumers.
3. Economic security. Assess the effect of hybrid Bt brinjal on the short- and long-term economic security of large-scale commercial farmers and small-scale resource-poor farmers.
4. Non-utilitarian values. Assess the indirect effects of Bt brinjal on human health, religion and culture.
5. Large-scale indirect effects. Assess risks associated with indirect loss of brinjal biological diversity, and government plans to expand the vegetable export market
6. Rapid response capacity. Evaluate the capacity of the Indian extension service to respond quickly to production demands that will appear soon after hybrid Bt brinjal is commercialised.

Sequencing the Needed Risk Assessment Activities

Although the GEAC and Mahyco have already invested considerable time and effort into environmental risk assessment (ERA) for EE-1 hybrid Bt brinjal, much of the effort was misdirected and did not assess actual adverse environmental consequences in India. The needs for ERA that are outlined here are developed with an eye towards improving oversight of genetically engineered crops in India, and redirecting efforts to issues that actually matter to India.

Addressing these needs will provide a sound scientific basis for assessing these more serious issues. The needs are compiled here in abbreviated form from the separate sections above, listed according to section and preserving the original numbering for easier cross-reference. An expanded description including some rationale is provided in the previous sections, which the interested reader may consult.

Characterisation of Transgene – Inserted Transgene

1. Probe sensitivity. Provide the size and sequence of the Bt probe and identify the part of the transgene to which it is expected to bind.
2. Transgene number. Use additional probes to explore for incorporated transgene fragments associated with other parts of the brinjal genome.
3. Transgene sequence. Sequence the inserted transgene and any other inserted fragments identified in 1) and 2).
4. Flanking regions. Sequence the regions flanking the inserted transgene (and transgene fragments).
5. Gene disruption. Identify the brinjal DNA in the flanking regions and determine if it is associated with a potentially functioning brinjal gene.

Characterisation of Transgene – Transgene Expression

1. Amino acid sequence. Provide the amino acid sequence of the expressed Ccry1A protein.
2. Ccry1A expression. Quantify expression levels of Ccry1A in pollen.
3. Marker expression. Quantify expression levels of antibiotic marker genes.
4. Heterologous system. Develop a heterologous expression system to produce sufficient quantities of Ccry1A that can be used for risk assessment research.

Environmental Risk Assessment – Gene Flow and Its Consequences

1. Fitness of intraspecific hybrids and backcrosses. Quantify the relative fitness of hybrids and backcrosses between Bt brinjal and brinjal landraces, and between Bt brinjal and intraspecific wild relatives, such as *S. melongena insanum*.
2. Identification of wild relatives. Determine if there are additional species of *Leptostemonum* that occur in India.
3. Hybridisation. Determine which identified wild relatives are known not to hybridise with brinjal.
4. Overlap. For the species that remain from 3), determine overlap of flowering time and geographic distribution with brinjal.
5. Cross-compatibility. For species with potential overlap, experimentally evaluate cross-compatibility and explaining experimentally any discrepancies with previously published results.
6. Fitness of interspecific hybrids and backcrosses. For cross-compatible species, quantify the relative fitness of hybrids and backcrosses between Bt brinjal and these species.
7. Gene flow rates. Additional gene flow experiments are not recommended.
8. Identify pests of wild relatives. Determine whether brinjal fruit and shoot borer (BFSB) or other susceptible lepidopteran species feed on wild or weedy relatives of brinjal.

9. Fitness advantage of Bt transgene. If lepidopteran larvae are on these wild or weedy relatives, determine whether a Bt transgene could enhance the survival or fecundity of wild/weedy plants.
10. Quantification of fitness advantage. If there is a fitness advantage of the Bt transgene, determine if it is enough to increase weediness, reduce genetic diversity of wild relatives, or have unwanted effects on non-target lepidopteran insects.

Environmental Risk Assessment – Risks to Biological Diversity

1. Kinds of risks to biological diversity. Determine the kinds of risks to biological diversity that are important for India in the use of Bt brinjal. Possibilities are: increased secondary pests; reduced soil quality or health; reduced value of non-crop economic activities; reduced cultural value; increased conservation concern; and reduced environmental quality.
2. Specify risk hypotheses. Specify scenarios (aka risk hypotheses) by which the kinds of risks listed in 1 could occur in India.
3. Test risk hypotheses. Test the risk hypotheses either to falsify them (and conclude that there is insignificant risk) or estimate the risk (qualitatively or quantitatively).
4. Secondary pests. Prioritise key secondary pests and natural enemies and conduct experiments to determine the risk of secondary pest outbreaks.

Environmental Risk Assessment – Resistance Risk in Target Species

1. Develop resistance monitoring method. Develop and implement a pedigreed F1 screen.
2. Prioritise resistance management tactics. Evaluate and prioritise alternative resistance management tactics using mathematical models.
3. Determine scale of monitoring. Determine appropriate spatial scale for monitoring.
4. Find resistance. Discover resistance alleles in BFSB.
5. Parameter estimation. Estimate biological parameters relevant to resistance evolution. These include plant-to-plant movement of larvae, movement of male and female adults, density-dependent larval mortality, and average fecundity.
6. Farmer acceptance of tactics. Determine if brinjal farmers are willing to plant a non-Bt refuge on their farms or implement other tactics.
7. Monitor compliance. Physical surveys of farmers using Bt brinjal should be conducted to determine if they are using the resistance management tactics.

Socioeconomic Analysis

1. Brinjal yield gap. Compile scientific literature on yield gaps for brinjal.
2. Returns to alternatives. Estimate net economic returns from traditional, local, and organic practices to limit damage from BFSB.
3. Economic security. Assess the effect of hybrid Bt brinjal on the short- and long-term economic security of large-scale commercial farmers and small-scale resource-poor farmers.
4. Non-utilitarian values. Assess the indirect effects of Bt brinjal on human health, religion and culture.
5. Large-scale indirect effects. Assess risks associated with indirect loss of brinjal biological diversity, and government plans to expand the vegetable export market.
6. Rapid response capacity. Evaluate the capacity of the Indian extension service to respond quickly to production demands that will appear soon after hybrid Bt brinjal is commercialised.

This may seem a daunting list of needs for an adequate ERA. However, if these issues had been addressed in a timely fashion, the actual workload at any one time is not that great. In addition, if some of the ERA issues had been considered earlier, they could have influenced how a developer of a genetically engineered plant would have proceeded.

The needs are analysed according to three qualitative criteria. First, in what kind of environment can the work be done? Environments vary from very artificial to realistic of brinjal production. Four environments are distinguished for this report:

desk, laboratory and growth chambers, greenhouse, and field. The “desk” environment does not require working with any living material. The realism of the environment increases from the laboratory to the field. Second, does the work require use of Bt brinjal? In many cases, use of Bt brinjal would improve the experiment, but satisfactory conclusions could be reached if a non-Bt improved hybrid were used instead. For example, hybridisation and cross-compatibility studies would best be done with Bt brinjal, but sufficiently useful data could be derived from experiments using a non-Bt improved hybrid. In these cases, Bt brinjal is not required. Third, during what stage of development of the genetically engineered crop could the ERA be initiated? We use the following stages of development of a genetically engineered plant (Andow et al. 2006) and associate ERA activities with these stages. This association is not required, but it is the earliest time in the development process that it makes sense for an ERA activity to be initiated.

The stages are:

- A. Design of plant transformation
- B. Initial screening of transformants
- C. Characterising focal events in the laboratory and growth chambers
- D. Characterising focal events in the green house
- E. Small-scale field testing
- F. Large-scale field testing
- G. Commercial use and post-commercialisation activities

The results of this analysis are shown in Table 7. Several patterns can be perceived. Twenty-six ERA needs can be initiated as desk work or in the laboratory, which obviates the need for field releases of Bt brinjal to assess risk. Seventeen of these needs can only be initiated in the field. Of these 17, less than half require the use of Bt brinjal, so most of the risk assessment activities can be done without releasing any Bt brinjal into the environment.

Second, about half of the ERA needs require the use of Bt brinjal; the other half can be accomplished without using Bt brinjal plants, although two of these would provide more useful data if Bt brinjal was used.

Third, 28 of these ERA needs can be initiated prior to field release. Only 8 must be initiated after field release. Because this report does not focus on post-commercial monitoring, only one ERA need identified here must be initiated after commercial release. Thus, most of the ERA needs can be met prior to field release of Bt brinjal.

Some of the needs requiring Bt brinjal release in the field are conditioned on certain results being found in experiments conducted in the greenhouse or laboratory. All of the needs evaluating fitness of hybrids and backcrosses of Bt brinjal and a wild relative require Bt brinjal in the field (as a control). However, these experiments are contingent on previous findings that there is cross-compatibility and sufficient temporal and spatial overlap, or that there are lepidopterous pests on the wild relative.

Thus, there is little need for additional exposure of Bt brinjal to the environment to complete an ERA that evaluates serious concerns for India.

Post-commercialisation monitoring (aka post-market monitoring) is a controversial topic and probably deserves an analysis similar in depth as this report on the ERA process. Although the reasons for development and implementation of post-commercial marketing are many and varied (see lengthy discussion in NRC 2002), two reasons stand out. First, there is a need for monitoring of specific anticipated adverse effects with significant risk, such as monitoring for resistance to Ccry1A in Bt brinjal. This kind of monitoring must be set up prior to commercialisation. Because the monitoring goals are already established, long-term funding must be secured, a system of reporting established, and remedial responses and response triggers clarified.

Second, monitoring is needed when knowledge about risk is too uncertain. In this way, monitoring can substitute for certainty in an ERA. This second approach, however, carries with itself substantial risk. Monitoring is expensive and, over time, the costs of monitoring take on a higher perceived value, especially if the benefits from monitoring are hard to understand. One benefit of monitoring is not finding or avoiding an adverse effect. Absence of a problem is difficult for many people to perceive as a benefit. Consequently, over time, pressure increases to reduce or stop monitoring. This means that monitoring is a poor substitute for

certainty in an ERA because there will always be pressure to reduce or eliminate this kind of monitoring. An in-depth analysis of post-commercialisation monitoring would be a welcome addition to the literature on ERA for genetically engineered organisms.

Table 7. Analysis of timing of needed ERA activities.			
Need	Environment of Study	Requires Bt brinjal?	Stage of Product Development ¹
Inserted Transgene			
1) Probe sensitivity	Laboratory	Yes	C
2) Transgene number	Laboratory	Yes	D
3) Transgene sequence	Laboratory	Yes	D
4) Flanking regions	Laboratory	Yes	D
5) Gene disruption	Laboratory	Yes	D
Transgene Expression			
1) Amino acid sequence	Laboratory	Yes	D
2) Ccry1A expression	Greenhouse	Yes	D
3) Marker expression	Greenhouse	Yes	D
4) Heterologous system	Laboratory	No	D
Gene Flow and Its Consequences			
1) Fitness of intraspecific hybrids and backcrosses	Field	Yes	E
2) Identification of wild relatives	Desk and Field	No	A
3) Hybridization	Desk	No ²	A
4) Overlap	Desk and Field	No	A
5) Cross-compatibility	Greenhouse	No ²	C
6) Fitness of interspecific hybrids and backcrosses	Field	Yes	E
7) Gene flow rates	None	No	
8) Identify pests of wild relatives	Desk and Field	No	A
9) Fitness advantage of Bt transgene	Greenhouse or Field	Yes	D
10) Quantification of fitness advantage	Field	Yes	E
Risks to Biological Diversity			
1) Kinds of risks to biological diversity	Desk	No	A
2) Specify risk hypotheses	Desk	No	C
3) Test risk hypotheses	Laboratory, Greenhouse, Field	Yes	C
4) Secondary pests	Desk and Field	No and Yes	A and F ³
Resistance Risk in Target Species			
1) Develop resistance monitoring method	Laboratory	Yes	D
2) Prioritize resistance management tactics	Desk	No	A
3) Determine scale of monitoring	Laboratory and Field	No	C
4) Find resistance	Laboratory	Yes	D
5) Parameter estimation	Laboratory and Field	No and Yes	B and E ³
6) Farmer acceptance of tactics	Field	No	D
7) Monitor compliance	Field	Yes	G
Socioeconomic Analysis			
1) Brinjal yield gap	Desk	No	C
2) Returns to alternatives	Desk and Field	No	A
3) Economic security	Desk and Field	No	E
4) Non-utilitarian values	Desk and Field	No	C
5) Large-scale indirect effects	Desk and Field	No	E
6) Rapid response capacity	Desk	No	D

¹A. Design of plant transformation; B. Initial screening of transformants; C. Characterizing focal events in the laboratory and growth chambers; D. Characterizing focal events in the green house; E. Small-scale field testing; F. Large-scale field testing; G. Commercial use and post-commercialisation activities.

²Bt brinjal not necessary to obtain useful data for ERA, but results would be more directly useful if Bt injal were used.

³There are multiple parts to this need that will be initiated at different times.

About the Author

ANDOW, DAVID A.

232 Hodson Hall
Department of Entomology
University of Minnesota
St. Paul, MN 55108

Tel: 612-624-5323
FAX: 612-625-5299
E-Mail: dandow@umn.edu

Education

Brown University, Providence, Rhode Island, B.S. 1977. Biology, magna cum laude
Cornell University, Ithaca, New York, Ph.D. 1982. Ecology

Academic Employment (from 1990)

Graduate Faculty, Field of Conservation Biology, University of Minnesota 1990-date
Faculty, Center for Natural Resources Policy & Management, University of Minnesota 1991-date
Graduate Faculty, Field of Sustainable Agriculture, University of Minnesota 1995-date
Professor, Department of Entomology, University of Minnesota 1998-2005
Distinguished McKnight University Professor, Department of Entomology, University of Minnesota 2005-date
Graduate Faculty, Field of Risk Analysis for Invasive Species and Genotypes, University of Minnesota 2007-date
Graduate Faculty, Field of Natural Resources Science & Management, University of Minnesota, Affiliate Senior Member, 2009-date

Scientific Memberships

Ecological Society of America
Entomological Society of America
Japanese Society of Applied Entomology and Zoology
New World Agriculture and Ecology Group
Sigma Xi Scientific Society
Society of Population Ecology

Honors

Japanese American Citizens League Scholarship - 1973
Sigma Xi - 1977
National Science Foundation Graduate Fellow
King/Chavez/Parks Visiting Professor, University of Michigan, 1990
Best Publication in *Landscape Ecology*, 1991 (International Association for Landscape Ecology)
McMaster Fellow (Commonwealth Scientific & Industrial Research Organization, Australia), 2002
JSPS Fellow (Japanese Society for the Promotion of Science), 1991, 2004
EMBRAPA consultancy, 2004, 2010
Distinguished McKnight University Professor, 2005
Elected International delegate to the Brazilian Entomological Society (2008)

Selected Professional Activities and Committees

WHO (World Health Organization)

Technical Consultation on Genetically Modified Mosquitoes, 4-6 May 2009, Geneva

FAO (Food and Agriculture Organization)

Consultation on “Biosafety within a *Biosecurity* framework contributing to sustainable agriculture and food production” (2006)

International Assessment of Agricultural Science and Technology for Development (IAASTD) – World Bank

Lead Author, Chapter 5, North America/Europe (2005-2007)

WTO (World Trade Organization)

Technical and scientific expert to the Dispute Resolution Panel on *European Communities – Measures Affecting the Approval and Marketing of Biotech Products (DS291, DS292, DS293)* (2004-2005)

NAFTA – Commission for Environmental Cooperation (CEC)

Maize Advisory Group Article 13 Report on Potential Effects of Transgenic Maize on Traditional Maize Varieties in Mexico (2002-2004). Consensus report: CEC 2004. Maize and biodiversity: The effects of transgenic maize in Mexico: Key findings and recommendations. Commission for Environmental Cooperation, Montreal. 35pp.

IPGRI (International Plant Genetic Resources Institute)

Workshop on “Technical issues associated with the development of CGIAR policies to address the possibility of adventitious presence of transgenes in CGIAR *ex situ* collections” (2004). Consensus report: IPGRI 2004. Final Report on technical issues associated with the development of CGIAR policies to address the possibility of adventitious presence of transgenes in CGIAR *ex situ* collections. IPGRI, Rome. 25pp.

Convention on Biological Diversity (CBD)

Cartagena Protocol, Liaison Group (2002)

Canada-Norway Expert Workshop on Risk Assessment for Emerging Applications of Living Modified Organisms, 4 - 6 June 2007, Montreal, Canada

African Regional Workshop on Capacity-Building and Exchange of Experiences on Risk Assessment and Risk Management of LMOs, 23 - 25 August 2007 - Addis Ababa, Ethiopia

US National Academy of Sciences – US National Research Council (NRC)

Workshop on Ecological Monitoring of Genetically Modified Crops (2000-2001). Consensus report: NRC 2001. Ecological monitoring of genetically modified crops. National Academy Press, Washington, DC. 45pp.

Subcommittee on Environmental Impacts Associated with Commercialization of Transgenic Crops: Issues and Approaches to Monitoring (2000-2002). Consensus report: NRC 2002. Environmental effects of transgenic plants: The scope and adequacy of regulation. National Academy Press, Washington, DC. 320pp.

Workshop on Exploring Genetic Modification of Plants: New Approaches and Implications for Definitions (2002). NRC. 14-15 August 2002.

Standing Committee on Agricultural Biotechnology, Health, and the Environment (2000-2006)

Workshop on International Aspects of Biotechnology (2004) Global Challenges and Directions for Agricultural Biotechnology: Mapping the Course. NRC. 25-26 Oct 2004.

United States Department of Agriculture (USDA)

NC 205 Impact of Integrated Crop Management Practices on European Corn Borer and Related Stalk Boring Insects (1984-date)

NCR 157 Evaluating Sustainable Agricultural Production and Marketing Systems (1988-1993)

Office of the Secretary

Agricultural Biotechnology Research Advisory Committee (ABRAC) (1990-1994)

ABRAC, Risk Assessment Subcommittee (1992-1994) Chair (1992-1994)

United States – Environmental Protection Agency (EPA)

Science Advisory Board, Biotechnology Subcommittee (1987-1996)

Biotechnology Science Advisory Committee (BSAC). Subcommittee on Deliberate Release (1987)

BSAC, Subcommittee to Review Application for Deliberate Release (1988)

Science Advisory Panel (SAP), Preliminary Risk & Benefit Assessments for Bt Plant-Pesticides (2000)

Office of Research and Development, Four Workshops for IRM for Bt corn (2001)

SAP, Preliminary Non-target Assessment for Mon 863 (2002)

SAP, Preliminary Resistance Management Plan for Mon 863 (2002)

United States Department of Interior, Fish and Wildlife Service

Karner Blue Butterfly Recovery Planning Team, Chair (1994-2003)

Karner Blue Butterfly Recovery Team, Chair (2003-2007)

Editor *Ecological Applications*, 1996-2002 (2 terms)

Environmental Biosafety Research, 2000-date, co-editor-in-chief, 2000-2005

Applied Entomology and Zoology, 1999-date (3 terms)

Entomological Science (Japan), 1999-date (3 terms)

Acta Entomologica Sinica, 2002-date (2 terms)

Ecology Letters, 2004-2006

Biological Invasions, 2004-2008

Population Ecology, 2008-date

Selected Publications

Refereed Journals (123 total; plus 87 book chapters)

1. Andow, D.A., A.G. Nicholson, H.C. Wien, and H.R. Willson, 1986. Insect populations on cabbage grown with living mulches. **Environmental Entomology** 15: 293-299.
2. Vandermeer, J., and D.A. Andow. 1986. Prophylactic and responsive components of an integrated pest management program. **Journal of Economic Entomology** 79: 299-302.
3. Andow, D.A., Teng, P.S., Johnson, K.B., and Snapp, S.S. 1989. Simulating the effects of bioengineered non-ice nucleating bacteria on potato yields. **Agricultural Systems** 29: 81-92.
4. Andow, D.A. and Hidaka, K. 1989. Experimental natural history of sustainable agriculture: Syndromes of production. **Agriculture, Ecosystems and Environment** 27: 447-462.
5. Andow, D.A., Kareiva, P.M., Levin, S.A. and Okubo, A. 1990. Spread of invading organisms. **Landscape Ecology** 4: 177-188.
6. Andow, D.A. and Ostlie K.R. 1990. First generation European corn borer response to three conservation tillage systems in Minnesota. **Journal of Economic Entomology** 83: 2455-2461.
7. Andow, D.A. 1991. Yield loss to arthropods in vegetationally diverse agroecosystems. **Environmental Entomology** 20: 1228-1235.

8. Davis, D. W., Andow, D. A. and Hutchison, W. D. 1993. Registration of three European corn borer resistant sweet corn germplasm lines: A684 *su*, A685 *su*, and A686 *su*. **Crop Science** 33: 1422-3.
9. Andow, D.A. 1993. Mustard as an antifeedant for *Epilachna varivestis* adults. **Coleopterists Bulletin** 47: 131-135.
10. Andow, D.A. 1994. Community response to transgenic plant release: Using mathematical theory to predict effects of transgenic plants. **Molecular Ecology** 3: 65-70.
11. Alstad, D.N. and D.A. Andow. 1995. Managing the evolution of insect resistance to transgenic plants. **Science** 268: 1894-1896.
12. Alstad, D.N. and D.A. Andow. 1996. Implementing management of insect resistance to transgenic crops. **AgBiotech News and Information** 8: 177-181. (Invited review).
13. Alstad, D. N. and D. A. Andow. 1996b. Evolution of insect resistance to *Bacillus thuringiensis*-transformed plants (reply to Technical Comment). **Science** 273: 1412-3.
14. Bolin, P.C., W.D. Hutchison, D.A. Andow and K.R. Ostlie. 1998. Monitoring for European corn borer (Lepidoptera: Crambidae) resistance to *Bacillus thuringiensis*: Logistical considerations when sampling larvae. **Journal of Agricultural Entomology** 15: 231-238.
15. Andow, D.A. and K. Hidaka. 1998. Yield loss in conventional and natural rice farming systems. **Agriculture, Ecosystems and Environment** 70: 151-158.
16. Andow, D.A., D.M. Olson, R.L. Hellmich, D.N. Alstad and W.D. Hutchison. 2000. Frequency of resistance alleles to *Bacillus thuringiensis* toxin in an Iowa population of European corn borer. **Journal of Economic Entomology** 93: 26-30.
17. Venette, R.C., W.D. Hutchison and D.A. Andow. 2000. An in-field screen for early detection and monitoring of insect resistance to *Bacillus thuringiensis* in transgenic crops. **Journal of Economic Entomology** 93: 1055-1064
18. Andow D.A. 2003. Negative and positive data, Statistical power, and confidence intervals. **Environmental Biosafety Research** 2: 75-80.
19. Haygood, R., A.R. Ives, and D.A. Andow. 2003. Consequences of recurrent gene flow from crops to wild relatives. **Proceedings of the Royal Society of London, Series B**, 270: 1879-1886.
20. Andow, D. A. 2003. UK farm-scale evaluations of transgenic herbicide-tolerant crops. **Nature Biotechnology** 21: 1453-4.
21. Andow, D. A. and A. Hilbeck. 2004. GE Crops: Balancing predictions of promise and peril. **Frontiers in Ecology and the Environment** (forum) 2:155-156.
22. Haygood, R., A. R. Ives and D. A. Andow. 2004. Population genetics of transgene containment. **Ecology Letters** 7: 213-220.
23. Andow, D. A. and A. Hilbeck. 2004. Science-based risk assessment for non-target effects of transgenic crops. **BioScience** 54: 637-649.
24. Snow, A. A., D.A. Andow, P. Gepts, E. M. Hallerman, A. Power, J.M. Tiedje, and L.L. Wolfenbarger. 2005. Genetically engineered organisms and the environment: current status and recommendations. **Ecological Applications** 15: 377-404.
25. Andow, D.A and C. Zwahlen. 2006. Assessing environmental risks of transgenic plants. **Ecology Letters** 9: 196-214.
26. Stodola, T. J., D. A. Andow, A. R. Hyden, J. L. Hinton, J. J. Roark, L. L. Buschman, P. Porter and G. B. Cronholm. 2006. Frequency of resistance to *Bacillus thuringiensis* toxin Cry1Ab in southern US Corn Belt population of European corn borer (Lepidoptera: Crambidae). **Journal of Economic Entomology** 99: 502-507.
27. Andow, D. A., G. L. Lövei and S. Arpaia. 2006. Ecological risk assessment for Bt crops. **Nature Biotechnology** 24: 749-751.
28. Lövei, G. L., D. A. Andow and S. Arpaia. 2009. Transgenic insecticidal crops and natural enemies: a detailed review of laboratory studies. **Environmental Entomology** 38(2): 293-306.
29. Nelson, K. C., D. A. Andow, and M. J. Banker. 2009. Problem formulation and option Assessment (PFOA) linking governance and environmental risk assessment for technologies: A methodology for problem analysis of nanotechnologies and genetically engineered organisms. **Journal of Law, Medicine, and Ethics** 37(4): 732-748.

Published Consensus Reports

1. NRC (National Research Council). 2001. Ecological monitoring of genetically modified crops. National Academy Press, Washington, DC. 45 pp.
2. ESA (Ecological Society of America). 2001. Position Statement: Genetically Modified Organisms. 1 p. <http://www.esa.org/pao/esaPositions/Statements/GeneticallyModifiedOrganisms.php>
3. NRC (National Research Council). 2002. Environmental effects of transgenic plants: The scope and adequacy of regulation. National Academy Press, Washington, DC. 320 pp.
4. IPGRI (International Plant Genetic Resources Institute). 2004. Final Report on technical issues associated with the development of CGIAR policies to address the possibility of adventitious presence of transgenes in CGIAR *ex situ* collections. International Plant Genetic Resources Institute, Rome. 25 pp.
5. CEC (Commission for Environmental Cooperation). 2004. Maize and biodiversity: The effects of transgenic maize in Mexico: Key findings and recommendations. Commission for Environmental Cooperation, Montreal. 35 pp.

References Cited

- Adkisson** PL 1971. Objective uses of insecticides in agriculture. In: *Agricultural Chemicals: Harmony or Discord for Food, People, and the Environment*. Swift JE (ed), Div. Agric. Sci., Univ. California, USA, pp 43-51.
- Adkisson** PL 1972. The integrated control of the insect pests of cotton. *Proceedings of the Tall Timbers Conference on the Ecology of Animal Control and Habitat Management* 4: 175-188.
- AGBIOS** (2003) Essential Biosafety, 2nd Edition. Merrickville, Ontario, Canada. <http://www.essentialbiosafety.com>. Accessed 25 November 2003.
- Alam** SN, MI Hossain, FMA Rouf, RC Jhala, MG Patel, LK Rath, A Sengupta, K Baral, SN Shylesha, S Satpathy, TM Shivalingaswamy, A Cork and NS Talekar. 2006. Implementation and promotion of an IPM strategy for control of eggplant fruit and shoot borer in South Asia. AVRDC Technical Bulletin 36.
- Alam**, S.N., Rashid, M.A., Rouf, F.M.A., Jhala, R.C., Patel, J.R., Satpathy, S., Shivalingaswamy, T.M., Rai, S., Wahundeniya, I., Cork, A., Ammaranan, C., and Talekar, N.S., 2003. Development of an integrated control strategy for eggplant fruit and shoot borer in South Asia. Shanhua, Taiwan: AVRDC-the World Vegetable Center. Technical Bulletin No. 28. AVRDC Publication No. 03-548. 56pp.
- Ali**, M. and K. Fujieda. 1989. Cross compatibility between eggplant (*Solanum melongena* L.) and wild relatives. *Journal of the Japanese Society for Horticultural Science (Engei Gakkai Zasshi)* 58:977-984.
- Andow** DA & Alstad DN (1998) F2 screen for rare resistance alleles. *Journal of Economic Entomology* 91: 572–578.
- Andow** DA, Alstad DN, Pang YH, Bolin PC & Hutchison WD (1998) Using an F2 screen to search for resistance alleles to *Bacillus thuringiensis* toxin in European corn borer (Lepidoptera: Crambidae). *Journal of Economic Entomology* 91: 579–584.
- Andow**, D. A. and J. S. Bentur. 2010. Pedigreed crosses to estimate recessive virulence allele frequencies in natural populations of gall midges. *Entomologia Experimentalis et Applicata* 135: 18-36.
- Andow**, D. A. and A. Hilbeck. 2004. Science-based risk assessment for non-target effects of transgenic crops. *BioScience* 54: 637-649.
- Andow**, D. A. and G. L. Lövei. 2010. Meta-analysis shows transgenic insecticidal plants have non-zero effects on natural enemies in the laboratory: A probative response to a polemic. *Environmental Entomology*, submitted.
- Andow**, D. A., G. L. Lövei, and S. Arpaia. 2009. Cry toxins and proteinase inhibitors in transgenic plants do have non-zero effects on natural enemies in the laboratory: Rebuttal to Shelton et al., 2009. *Envir. Entomol.* 38: 1528-1532.
- Andow**, D.A., P.A.V. Barroso, E.M.G. Fontes, M.F. Grossi-de-Sa, A. Hilbeck and G. P. Fitt. 2006. Improving the scientific basis for *environmental risk assessment through the case study of Bt cotton in Brazil*, pp. 1-20. In Hilbeck, A., D.A. Andow and E.M.G. Fontes (eds.), *Environmental Risk Assessment of Genetically Modified Organisms: Methodologies for Assessing Bt Cotton in Brazil*. CAB International, Wallingford, UK.
- Andreadis** SS, Álvarez-Alfageme F, Sánchez-Ramos I, Stodola TJ, Andow DA, Milonas PG, Savopoulou-Soultani M & Castánera P (2007) Frequency of resistance to *Bacillus thuringiensis* toxin Cry1Ab in Greek and Spanish population of *Sesamia nonagrioides* (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 100: 195-201.
- Anilkumar**, K. J., A. Rodrigo-Simon, J. Ferré, M. Pusztai-Carey, S. Sivasupramaniam, and W. J. Moar. 2008. Production and characterization of *Bacillus thuringiensis* Cry1Ac-resistant cotton bollworm *Helicoverpa zea* (Boddie). *Appl. Environ. Microbiol.* 74: 462-469.
- APEDA** (Agricultural and Processed Food Products Export Development Authority). 2009. APEDA report 2008-09- National Horticulture Board Data base. Government of India. http://apeda.com/apedawebsite/miscellaneous/new_publication.htm and http://apeda.com/TradeJunction/Statistics/Export_statistics/Product_description.aspx?hscode=07093000 and http://apeda.com/TradeJunction/India%20Production/Product_Detail.aspx?hscode=070

93000&hscode1=BRINJAL

- APOF (Association for the Promotion of Organic Farming). 2004. Operational Methodologies and Package of Practices in Organic Farming
- Bagla, P. 2010. Hardy cotton-munching pests are latest blow to GM crops. *Science* 327: 1439.
- Baral K, BC Roy, KMB Rahim, H Chatterjee, P Mondal, D Mondal, D Ghosh and NS Talekar. 2006. Socio-economic parameters of pesticide use and assessment of impact of an IPM strategy for the control of eggplant fruit and shoot borer in West Bengal, India. AVRDC Technical Bulletin 37.
- Behera, T. K. and N. Singh. 2002. Inter-specific crosses between eggplant (*Solanum melongena* L.) with related *Solanum* species. *Scientia Horticulturae* 95:165-172
- Cairns J Jr, Mount DI. 1990. Aquatic toxicology. *Environmental Science and Technology* 24: 154-161.
- Cairns J Jr. 1981. Sequential versus simultaneous testing for evaluating the hazards of chemicals to aquatic life. *Marine Environment Research* 4: 165-166.
- Campbell, L. G., A. A. Snow, and C. E. Ridley. 2006. Weed evolution after crop gene introgression: greater survival and fecundity of hybrids in a new environment. *Ecology Letters* 9:1198-1209.
- Capinpin, JM, M Lunde, JV Pancho. 1963. Cytogenetics of interspecific hybrid between *Solanum melongena* Linn. and *S. cumingii*. *Philip. J. Sci.* 92: 169-178.
- Caprio, M.A., Sumerford, D.V. and Simms, S.R. 2000. Evaluating transgenic plants for suitability in pest and resistance management programs. In L. Lacey and H. Kaya, eds., *Field manual of techniques in invertebrate pathology*, pp. 805-828. Kluwer, Boston.
- Chakraborti, S. 2001. A biorational approach for the management of brinjal fruit and shoot borer, *Leucinodes orbonalis* Guen. *J Entomological Research* 25 (1)
- Choudhury, B. 1995. Eggplant - Chapter 92, pp.464-465, In J. Smartt and N. W. Simmonds (eds.), *Evolution of crop plants*, second edition. New York: Wiley, 531 pp.
- CIKS (Centre for Indian Knowledge Systems). 2010a. Testing and propagation of methods based on Vrکشayurveda (traditional Indian plant science) for the cultivation of selected vegetables. Studies on disease resistance, growth enhancement and pest control for chili, tomato, brinjal and okra. <http://www.ciks.org/project-dst-vrk.htm> (last accessed 13 August 2010).
- CIKS (Centre for Indian Knowledge Systems). 2010b. Submission made to the Honorable Minister of State for Environment and Forests in connection with the national consultation on brinjal being held at Bangalore on 6th February 2010. <http://www.ciks.org/btbrinjal.html> (last accessed 13 August 2010).
- Codex Alimentarius Commission (CAC). 2003. Guideline for the conduct of food safety assessment of foods derived from recombinant-dna plants. *CAC/GL 45-2003*
- Cohen, M. B., A. A. Snow, S. Arpaiea, L. P. Lan, and L. M. Chau. 2008. Shared flowering phenology, insect pests, and pathogens among wild, weedy, and cultivated rice in the Mekong Delta, Vietnam: implications for transgenic rice. *Environmental Biosafety Research* 7:73-85.
- Cork A, Alam SN, Rouf FM, Talekar NS. 2003. Female sex pheromone of brinjal fruit and shoot borer, *Leucinodes orbonalis* (Lepidoptera: Pyralidae): trap optimization and application in IPM trials. *Bull Entomol Res.* 93(2):107-13.
- Das, M.S. and Patnaik, B.H. 1970. A new host of the brinjal shoot and fruit borer *Leucinodes orbonalis* Guen., and its biology. *Journal of the Bombay Natural History Society* 63: 601-603.
- Daunay, M.-C., R. N. Lester, and G. Ano. 2001. Eggplant. In: A. Charrier, M. Jacquot, S. Hamon, and D. Nicolas (eds.). *Tropical Plant Breeding*. Science Publishers, Inc., USA, and CIRAD, France, pp. 199-222.
- Deb, D. B. 1989. *Solanum melongena*, *S. incanum* versus *S. insanum* (Solanaceae). *Taxon* 38:138-139.
- Denolf, P., S. Jansens, M. Peferoen, D. Degheele and J. Van Rie. 1993. Two different *Bacillus thuringiensis* delta-endotoxin receptors in the midgut brush border membrane of the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae). *Appl Environ Microbiol.* 59(6): 1828-1837.
- DES (Directorate of Economics and Statistics). 2008. Govt. of India - 2007-2008.

- Dhamdhare, S, SV Dhamdhare and R Mathur.** 1995. Occurrence and succession of pests of brinjal, *Solanum melongena* Linn. At Gwalior (Madhya Pradesh) India. *J. Entomological Research* 19(1): 71-77.
- Dhankhar, B.S., Kishore, N., Sharma, D.R., Pandita, M.L.** 1982. Susceptibility of *Solanum khasianum* to brinjal shoot and fruit borer (*Leucinodes orbonalis* Guen.). *Haryana Agricultural University Journal of Research (India)* 12(3): 523-524.
- Dodgson, J.S., M. Spackman, A. Pearman and L.D. Phillips.** 2009. Multi-criteria analysis: a manual. Department for Communities and Local Government: London. Accessed at http://eprints.lse.ac.uk/12761/1/Multi-criteria_Analysis.pdf
- Doganlar, S., A. Frary, M.-C. Daunay, R. N. Lester, and S. D. Tanksley.** 2002. A comparative genetic linkage map of eggplant (*Solanum melongena*) and its implications for genome evolution in the Solanaceae. *Genetics* 161: 1697-1711.
- Elanchezhyan, K, BRK Muralis and DS Rajavel.** 2008. Influence of intercrops on incidence of major pests of brinjal and their natural enemies. *Annals of Plant Protection Science* 16(1).
- Ellstrand, N. C.** 2003. Current knowledge of gene flow in plants: implications for transgene flow. *Proceedings of the Royal Society [Biology]* 358:1163-1170.
- Elmegaard N, Jagers op Akkerhuis GAJM.** 2000. Safety Factors in Pesticide Risk Assessment: Differences in Species Sensitivity and Acute-Chronic Relations. Silkeborg (Denmark): National Environmental Research Institute. NERI Technical Report 325.
- Fenton, N. and M. Neil.** 2001. Making decisions: using Bayesian nets and MCDA. *Knowledge-Based Systems* 14(7): 307-325.
- Fitt, G.P., D.A. Andow, Nguyn Hu Huân, M. Caprio, C. Omoto, Nguyn Th, Nguyn Hng Sn, Bui Cách Tuyn.** 2008. Resistance risk assessment and management for Bt cotton in Vietnam, pp. 296-229. In D.A. Andow, A. Hillbeck and Nguyen Van Tuat (eds), *Environmental Risk Assessment of Genetically Modified Organisms: Challenges and Opportunities with Bt Cotton in Viet Nam*. CABI Publishing, Wallingford, UK.
- Fletcher, T.B.** 1916. One hundred notes on Indian insects. *Agricultural Research Institute Pusa, Bulletin* 59, No 27 (from Dhankar 1988).
- Forbes VE, Calow P.** 2002. Extrapolation in ecological risk assessment: Balancing pragmatism and precaution in chemical controls legislation. *BioScience* 52: 249-257.
- Forbes VE, Forbes TL.** 1994. *Ecotoxicology in Theory and Practice*. London: Chapman and Hall.
- Fukusawa, CA.** 1964. Genetics of clustered and solitary fruit segments from the interspecific cross between *Solanum melongena* and *Solanum cumingii*. *Aran. J. Agric.* 11: 5-75.
- Furini, A and J Wunder.** 2004. Analysis of eggplant (*Solanum melongena*)-related germplasm: morphological and AFLP data contribute to phylogenetic interpretations and germplasm utilization. *Theor. Appl. Genet.* 108: 197-208.
- Gassmann, A. J., Carrière, Y., and Tabashnik, B. E.** 2009. Fitness costs of insect resistance to *Bacillus thuringiensis*. *Annual Review of Entomology* 54:147-163.
- Georghiou GP & Taylor CE** 1977. Operational influences in the evolution of insecticide resistance, *Journal of Economic Entomology* 70: 653.
- Ghosh, S. K. and S. K. Senapati.** 2009. Seasonal fluctuation in the population of *Leucinodes orbonalis* Guen. in the sub-himalayan region of West Bengal, India and its control on eggplant (*Solanum melongena* L.). *Precision Agriculture* 10: 443-449.
- Gould F** (1998) Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. *Annual Review of Entomology* 43: 701-726.
- Gould F & Tabashnik BE** 1998. Bt-cotton resistance management. In: *Now or Never: Serious New Plans to Save Natural Pest Control*. Rissler J (ed), Union of Concerned Scientists, Cambridge MS, USA.
- Gowda, PHR, KT Shivashankar and S. Joshi.** 1990. Interspecific hybridization between *Solanum melongena* and *Solanum macrocarpon*: study of the F1 hybrids. *Euphytica* 48: 59-61.
- Greene, J. K., C. S. Bundy, P. M. Roberts, and B. R. Leonard.** 2006. Identification and management of common boll feeding bugs in cotton.
- Hanur, VS** 2008. Bt resistance and monophagous pests: Handling with prudence. *Current Science* 95(4): 449-451.

- Hargreaves, H. 1937. Some insects and their plants in Sierra Leone. *Bulletin of Entomological Research* 28: 513.
- Haseeb, M., D. K. Sharma and M. Qamar. 2009. Estimation of the losses caused by shoot and fruit borer, *Leucinodes orbonalis* Guen. (*Leptidoptera: Pyralidae*) in brinjal. *Trends in Biosciences* 2(1): 68-69.
- Hayes, K. R. 2002. Identifying hazards in complex ecological systems. Part 1. Fault tree analysis for biological invasions. *Biological Invasions* 4: 235-249.
- Haygood, R., A.R. Ives, and D.A. Andow. 2003. Consequences of recurrent gene flow from crops to wild relatives. *Proceedings of the Royal Society of London, Series B*, 270: 1879-1886.
- Hilbeck, A., S. Arpaia, A.N.E. Birch, Y. Chen, E.M.G. Fontes, A. Lang, Lê Th Thu Hng, G.L. Lövei, B. Manachini, Nguyn Thi Thu Cúc, Nguyn Vn Huhn, Nguyn Vn Tut, Phm Vn Lm, Pham Van Toan, C.S.S. Pires, E.R. Sujii, Trác Khng Lai, E. Underwood, R.E. Wheatley, L.J. Wilson, C. Zwahlen, and D.A. Andow. 2008. Non-target and biological diversity risk assessment, pp. 115-137. In D.A. Andow, A. Hilbeck and Nguyn Vn Tut (eds), *Environmental Risk Assessment of Genetically Modified Organisms: Challenges and Opportunities with Bt Cotton in Viet Nam*. CABI Publishing, Wallingford, UK.
- Huang F, Buschman LL, Higgins RA & McGaughey WH 1999. Inheritance of resistance to *Bacillus thuringiensis* toxin (Dipel ES) in the European corn borer. *Science* 284: 965-7. DOI: 10.1126/science.284.5416.965
- Huang F, Leonard BR & Andow DA (2007a) Sugarcane borer resistance to transgenic *Bacillus thuringiensis*-maize. *Journal of Economic Entomology* 100: 164-171.
- Hussain, M.L. 1925. Annual report of the Entomologist to Government of Punjab, Layallpur for the year ending 30 June 1924. Report of Department of Agriculture, Punjab 1923D24 (2) 1: 59-90 (from Dhankar 1988).
- Hutson, J.C. 1930. Report of insect pests in Ceylon during 1930. Peradeniya (from Dhankhar 1988).
- ICAR (Indian Council on Agricultural Research). 2006..Report on the meeting on integrated pest management (IPM) and biopesticides organized by the ICAR as the third meeting in the series of brainstorming sessions on topics suggested by the NKC where generation, discussion and use of knowledge in the agricultural sector would be important. New Delhi, 26 April 2006
- Icoz, I., Saxena, D., Andow, D. A., Zwahlen, C. and Stotzky, G. 2008. Microbial populations and enzyme activities in soil in situ under transgenic corn expressing Cry proteins from *Bacillus thuringiensis*. *Journal of Environmental Quality* 37: 647-662.
- IIHR (Indian Institute of Horticulture Research). 2007. Annual Report of All India Coordinated Project 2007 - Indian Institute of Horticulture Research Hesargatta, Bangalore
- IIHR (Indian Institute of Horticulture Research). 2008. Annual Report of All India Coordinated Project 2008 - Indian Institute of Horticulture Research Hesargatta, Bangalore
- IIVR (Indian Institute of Vegetable Research, Varanasi). 2007a. Assessment of crossability of *Solanum melongena* with other *Solanum* species during 2007. Supplemental Materials.
- IIVR (Indian Institute of Vegetable Research, Varanasi). 2007b. Assessment of crossability of Bt Brinjal (*Solanum melongena*) with *Solanum incanum* during 2007. Supplemental Materials.
- IIVR (Indian Institute of Vegetable Research, Varanasi). 2009. Pollen flow studies in Bt brinjal 2009. Supplemental Materials
- ILSI 1999. (International Life Sciences Institute, Washington DC USA) An evaluation of insect resistance management in Bt field corn: a science-based framework for risk assessment and risk management. Report of an Expert Panel. http://www.ilsilife.org/file/h5_IRM.pdf. Cited February 2008.
- Isahaque N.M.M. and Chaudhuri, R.P. 1983. A new alternate host plant of brinjal shoot and fruit borer, *Leucinodes orbonalis* Guen. in Assam. *Journal of Research, Assam Agricultural University* 4: 83-85.
- Isshiki, S., N Iwata and MMR Khan. 2008. ISSR variations in eggplant (*Solanum melongena* L.) and related *Solanum* species. *Sci. Hort.* 117: 186-190.
- Karihaloo, J. L., and L. D. Gottlieb. 1995. Allozyme variation in the eggplant, *Solanum melongena* L. (Solanaceae). *Theoretical and Applied Genetics* 90:578-583.
- Karihaloo, J. L., S. Brauner, and L. D. Gottlieb. 1995. Random amplified polymorphic DNA variation in the eggplant,

- Solanum melongena* L. (Solanaceae). Theoretical and Applied Genetics 90:767-770.
- Karnataka.** 2002. Report of the expert committee for study on farmers suicide in Karnataka. April 2002. Government of Karnataka, Department of Agriculture.
- Kashyap, V., S. Vinod Kumar, C. Collonnier, F. Fusari, R. Haicour, G. L. Rotino, D. Sihachakr and M. V. Rajam.** 2003. Biotechnology of eggplant. *Scientia Horticulturae* 97:1-25.
- Kolady, D and W Lesser.** 2005. Adoption of genetically modified eggplant in India:-An *ex ante* analysis. Paper prepared for presentation at the American Agricultural Economic Association Annual Meeting, Providence, Rhode Island, July 24-27, 2005.
- Kolady, D and W Lesser.** 2006. Is it feasible to provide genetically modified crops to small farmers on preferential terms?: An *ex ante* analysis of Bt eggplant in India. Paper prepared for presentation at the American Agricultural Economic Association Annual Meeting, Long Beach, California, July 23-26, 2006.
- Krishna V.V and M. Qaim.** 2007. Estimating the adoption of Bt eggplant in India: Who benefits from public-private partnership? *Food Policy* 32: 523-543.
- Krishna V.V and M. Qaim.** 2008. Potential impacts of Bt eggplant on economic surplus and farmers' health in India. *Agricultural Economics* 38: 167-180.
- Krishna, VV, U Pascual and D Zilberman.** 2010. Assessing the potential of labelling schemes for in situ landrace conservation: an example from India. *Environ. Devel. Econ.* 15: 127-151.
- Lall BS & S.Q Ahamad.** 1965. Biology and control of brinjal shoot and fruit borer. *Journal of Economic Entomology* 58: 448 - 451.
- Lester, R.N. and S. M. Z. Hasan.** 1991a. The distinction between *Solanum incanum* L. and *Solanum insanum* L. (Solanaceae). *Taxon* 39:521-523.
- Lester, R.N. and S. M. Z. Hasan.** 1991b. Origin and domestication of the brinjal egg-plant, *Solanum melongena*, from *S. incanum*, in Africa and Asia. In: J. G. Hawkes, R. N. Lester, M. Nees, and N. Estrada (eds). *Solanaceae III. Taxonomy, Chemistry, Evolution.* Royal Botanic Gardens, Kew, pp. 369-387.
- Levin, RA, NR Myers and L Bohs.** 2006. Phylogenetic relationships among the "spiny solanums" (*Solanum* subgenus *Leptostemonum*, Solanaceae). *Amer. J. Bot.* 93(1): 157-169.
- Lobell, DB, KG Cassman and CB Field.** 2009. Crop yield gaps: Their importance, magnitudes and causes. *Annu. Rev. Environ. Resource.* 34: 179-204.
- Lövei, G. L., D. A. Andow and S. Arpaia.** 2009. Transgenic insecticidal crops and natural enemies: a detailed review of laboratory studies. *Environmental Entomology* 38(2): 293-306.
- Lu, Y., K. Wu, Y. Jiang, B. Xia, P. Li, H. Feng, K. A. Wyckhuys, and Y. Guo.** 2010. Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of Bt cotton in China. *Science.*
- Mall, NP, RS Pundey, SV Singh and SK Singh.** 1992. Seasonal incidence of insect pest - estimation of the losses caused by shoot and fruit borer on brinjal. *Indian J. Entomology* 54: 241-247.
- Marçon, P. C. R. G., L. J. Young, K. L. Steffey, and B. D. Siegfried.** 1999. Baseline susceptibility of European corn borer (Lepidoptera: Crambidae) to *Bacillus thuringiensis* toxins. *Journal of Economic Entomology* 92(2): 279-285.
- Matten SR, Lewis PI, Tomimatsu G, Sutherland DWS, Anderson N, & Colvin-Snyder TL** 1996. The U.S. Environmental Protection Agency's role in pesticide resistance management. In: *Molecular Genetics and Evolution of Pesticide Resistance.* Brown TM (ed), American Chemical Society, Washington DC, USA. pp 243-253.
- McCabe, D.E. and Martinell, B.J.** 1993. Transformation of elite cotton cultivars via particle bombardment of meristems. *BioTechnology* 11, 596-598.
- MEA (Millennium Ecosystem Assessment).** 2005. Ecosystems and human well-being: Biodiversity synthesis. Washington (D. C.): World Resources Institute. 86 p.
- Mehto, D.N., Singh, K.M. and Singh, R.N.** 1980. Dispersion of *Leucinodes orbonalis* Guen. during different seasons. *Indian Journal of Entomology* 42: 539-540.
- Meihls, L. N., M. L. Higdon, B. D. Siegfried, N. J. Miller, T. W. Sappington, M. R. Ellersieck, T. A. Spencer, and B. E.**

- Hibbard. 2008. Increased survival of western corn rootworm on transgenic corn within three generations of on-plant greenhouse selection. *Proc. Natl. Acad. Sci. USA* 105:19177-19182.
- Meihls, L.N., Higdon, M.L., Siegfried, B.D., Miller, N.J., Sappington, T.W., Ellersieck, M.R., Spencer, T.A., Hibbard, B.E. 2008. Increased survival of western corn rootworm on transgenic corn within three generations of on-plant greenhouse selection. *Proceedings of the National Academy of Sciences*. 105: 19177-19182.
- Menon, P.P.V. 1962. *Leucinodes orbonalis* Guen. (Pyralidae: Lep.) new record on *Solanum indicum* Linn. in South India. *Madras Agricultural Journal* 49: 194
- Mikkelsen, T. R., B. Andersen and R. B. Joergensen. 1996. The risk of crop transgene spread. *Nature* 380: 31.
- Naik, VCB, PA Rao, PV Krishnayya and VS Rao. 2008. Seasonal incidence and management of *Leucinodes orbonalis* Guenee on brinjal. *Annals of Plant Protection Science* 16(2): 329-332.
- Nair, K.R. 1967. *Leucinodes orbonalis* Guen (Lepido: Pyralidae) as a serious pest of potato plants in Mysore. *Indian Journal of Entomology* 29: 96-97.
- Naitam, NR and SJ Markad. 2003. Evaluation of damage threshold based sprayings against brinjal shoot and fruit borer, *Leucinodes orbonalis* Guen. *Pest Management in Horticultural Ecosystems* 9(1): 63-65.
- Nguyen Th Thu Cúc, E.R. Sujii, L.J. Wilson, E. Underwood, D.A. Andow, Mai Vn Hào, B.P. Zhai, Ho Vn Chin. 2008. Potential effect of transgenic cotton on non-target herbivores in Vietnam, pp. 138-175. In D.A. Andow, A. Hilbeck and Nguyen Vn Tut (eds), *Environmental Risk Assessment of Genetically Modified Organisms: Challenges and Opportunities with Bt Cotton in Viet Nam*. CABI Publishing, Wallingford, UK.
- NPOP (National Programme for Organic Production). 2005. India Organic, National Standards for Organic Production, Section 3.2.1.3 and 3.2.5.7. Department of Commerce, Ministry of Commerce and Industry, New Delhi. <http://apeda.com/apedawebsite/organic>
- NRC (National Research Council). 1986. *Pesticide Resistance: Strategies and Tactics for Management*. National Research Council (NCR), National Academy Press, Washington DC, USA. http://www.nap.edu/catalog.php?record_id=619#toc
- NRC (National Research Council). 2000. *Genetically modified pest-protected plants: science and regulation*. National Academy Press, Washington, DC, USA.
- NRC (National Research Council). 2002. *Environmental effects of transgenic plants: the scope and adequacy of regulation*. National Academy Press, Washington, DC, USA.
- NRC (National Research Council). 2004. *Safety of genetically engineered foods: Approaches to assessing unintended health effects*. National Academy Press, Washington, DC, USA.
- Pareek, BL and MC Bhargava. 2003. Estimation of avoidable losses in vegetable crops caused by borer under semi-arid conditions of Rajasthan. *Insect Environment* 9(2): 59-60.
- Pasquet, R. S., A. Peltier, M. B. Hufford, E. Oudin, J. Saulnier, L. Paul, J. T. Knudsen, H. R. Herren, and P. Gepts. 2008. Long-distance pollen flow assessment through evaluation of pollinator foraging range suggests transgene escape distances. *PNAS* 105: 13456-13461.
- Patel, DA, PT Shukla and GC Jadeja. 2001. Morphological studies on interspecific hybrids between *Solanum indicum* L and *Solanum melongena* L. *Indian J Genet*. 61(2): 180-182.
- Patnaik, HP, DN Singh and S Mohapatra. 1997. Effect of NPK fertilizers on the incidence of shoot and fruit borer (*Leucinodes orbonalis* Guen.) in brinjal under insecticide protection. *Orissa Journal of Horticulture* 25: 18-21.
- Peswani, KM and R Lall. 1964. Estimation of losses of brinjal fruits caused by shoot and fruit borer, *Leucinodes orbonalis* Guenee. *Indian J Entomology* 26: 44-47.
- Pillai, R.M. 1922. *Developmental activities, Entomology*, TI. Department of Agriculture, Pretoria, South Africa (from Dhankar 1988).
- Pimentel D, Andow DA, Gallahan D, Schreiner I, Thompson T, Dyson-Hudson R, Jacobson S, Irish M, Kroop S, Moss A, Shepard M, & Vinzant B 1980. Environmental and social costs of pesticides: A preliminary assessment. *Oikos* 34: 126-140.
- Polignano, G, P. Ugenti, V. Bisignana and CD Gatta. 2010. Genetic divergence analysis in eggplant (*Solanum melongena* L.)

- and allied species. *Genet. Resour. Crop Evol.* 57: 171-181.
- Prabhu, M, D Veeraragavathatham, K Srinivasan and K Kannan.** 2005. Effect of nitrogen and phosphorus application on the incidence of fruit borer in hybrid brinjal COBH. *Agricultural Science Digest* 25(4).
- Raghuram, M.** 2010. Farmers guard Udupi brinjal's territory. *DNA Daily News* Aug 13, 2010. http://www.dnaindia.com/bangalore/report_farmers-guard-udupi-brinjal-s-territory_1422734-all (last accessed 16 August 2010)
- Ramirez, DA** 1959. Cytology of Philippine plants. II. *Solanum grandiflorum* Ruiz and Pav. *Philippine Agriculturalist* 43: 375/
- Rao N.** 1979. The barriers to hybridization between *Solanum melongena* and some other species of *Solanum*. In: J. G. Hawkes, R. N. Lester, and A. D. Skelding. (eds.). *The Biology and Taxonomy of the Solanaceae*, Academic Press, NY, pp. 605-614.
- Rice ME & Pilcher CD** 1998. Potential benefits and limitations of transgenic *Bt* corn for management of the European corn borer (Lepidoptera: Crambidae). *American Entomologist* 44: 75.
- Rieger, M. A., M. Lamond, C. Preston, S. B. Powles, and R. T. Roush.** 2002. Pollen-mediated movement of herbicide resistance between commercial canola fields. *Science* 296: 2386-2388.
- Roush RT** 1997. Managing resistance to transgenic crops. In: *Advances in Insect Control: the Role of Transgenic Plants*. Carozzi N & Koziel MG (eds), Taylor & Francis, London, UK. pp 271-294.
- Schaff, DA, CH Bayer and BL Pollack.** 1980. Interspecific hybridization of *Solanum melongena* x *S. macrocarpon* L. *Hort. Science* 15(3): 338-345.
- Sekara, A., S. Cebula and E. Kunicki.** 2007. Cultivated eggplants – origin, breeding objectives and genetic resources, a review. *Folia Horticulturae* 19(1): 97-114.
- Sen, A.K.** 1999. *Development as Freedom*. Alfred A Knopf: New York, 366pp.
- Shelton, A.M., S.E. Naranjo, J. Romeis, R.L Hellmich, J.D. Wolt, B.A. Federici, R. Albajes, F. Bigler, E.P.J. Burgess, G.P. Dively, A.M.R. Gatehouse, L.A. Malone, R. Roush, M. Sears, and F. Sehnal.** 2009a. Setting the record straight: a rebuttal to an erroneous analysis on transgenic insecticidal crops and natural enemies. *Transgen. Res.* 18: 317-322.
- Shelton, A.M., S.E. Naranjo, J. Romeis, R.L Hellmich, J.D. Wolt, B.A. Federici, R. Albajes, F. Bigler, E.P.J. Burgess, G.P. Dively, A.M.R. Gatehouse, L.A. Malone, R. Roush, M. Sears, F. Sehnal, N. Ferry and H.A. Bell.** 2009b. Appropriate analytical methods are necessary to assess nontarget effects of insecticidal proteins in GM crops through meta-analysis (Response to Andow et al. 2009). *Environ. Entomol.* 38: 1533-1538.
- Shukla, V and BS Prabhakar.** 1985?. Effect of plant density on yield and losses caused by fungus and insects in brinjal. *Agricultural Science Digest*.
- Singh, M.** 2009?. Centre of Origin, Inter-relationship, and Crossability in *Solanum melongena* (Brinjal). Supplemental Materials.
- Snow, A. A., D. Pilson, L. H. Rieseberg, M. Paulsen, N. Pleskac, M. R. Reagon, D. E. Wolf, and S. M. Selbo.** 2003. A *Bt* transgene reduces herbivory and enhances fecundity in wild sunflowers. *Ecological Applications* 13:279-286.
- SS (Solanaceae Source).** 2010. <http://www.nhm.ac.uk/research-curation/research/projects/solanaceaesource/> (last accessed 20 June 2010)
- St. Amand, P.C., D.Z. Skinner and R.N. Peaden.** 2000. Risk of alfalfa transgene dissemination and scale-dependent effects. *Theor. Appl. Genet.* 101: 107-114.
- Stark JD, Banks JE, Vargas R.** 2004. How risky is risk assessment: The role that life history strategies play in susceptibility of species to stress. *Proceedings of the National Academy of Sciences* 101: 732-736.
- Stodola, T.J. and D.A. Andow.** 2004. F2 screen variations and associated statistics. *Journal Economic Entomology* 97: 1756-1764.
- Suter, GW II.** 2007. *Ecological Risk Assessment*, Second Edition. CRC Press, Boca Raton, FL.
- Tabashnik BE** 1994. Delaying insect adaptation to transgenic plants: seed mixtures and refugia reconsidered. *Proceedings of the Royal Society B: Biological Sciences* 255: 7-12. DOI: 10.1098/rspb.1994.0002
- Tabashnik BE, Gassmann AJ, Crowder DW & Carrière Y.** 2008. Insect resistance to *Bt* crops: evidence versus theory. *Nature Biotechnology* 26: 199-202.

- Thanki, KV and JR Patel.** 1988. Seasonal incidence of shott and fruit borer, *Leucinodes orbonalis* Guen. on eggplant in Gujarat. *Indian J. Agricultural Science* 58: 867-868.
- Tudor, M and A. Tomescu.** 1995. Studies on the crossing compatibility of the species *Solanum sodomium* and *Solanum melongena* var. *Lucia*. In *Proceedings of the Eucarpia IX Meeting on Genetics and Breeding of Capsicum and Eggplant*, Budapest (Hungary), pp. 39-41.
- USEPA (US Environmental Protection Agency).** 1998. *Guidelines for Ecological Risk Assessment*. US Environmental Protection Agency, Washington, DC. EPA/630/R-95/002F
- USEPA (US Environmental Protection Agency).** 2000. *Preliminary Risk and Benefit Assessments for Bt Plant-Pesticides*, US Environmental Protection Agency, Washington, D.C., Briefing Document for the October 18-20, 2000 Science Advisory Panel Meeting.
- Viswanathan, T. V.** 1975. On the occurrence of natural hybridization between *Solanum incanum* L. and *Solanum melongena* L. *Current Science* 44:134.
- Warwick, S. I., A. Légère, M.-J. Simard and T. James.** 2007. Do escaped transgenes persist in nature? The case of an herbicide resistance transgene in a weedy *Brassica rapa* population. *Molecular Ecology*
- Waterhouse, D.F.** 1998, *Biological Control of Insect Pests: Southeast Asian Prospects*. ACIAR Monograph No. 51, 548 pp + viii, 1 fig. 16 maps.
- Whalen, MD.** 1984. Conspectus of species groups in *Solanum* subgenus *Leptostemonum*. *Gentes Herbarum* 12: 179-282.
- Whittle, K. and Ferguson, D.C.** 1987. Pests not known to occur in the United States or of limited distribution No 85: Eggplant fruit borer. APHIS 81.50. U.S. Department of Agriculture, Animal and Plant Health Inspection Service. Hyattsville, Maryland.
- Yue, B., F. Huang, B. R. Leonard, S. Moore, D. A. Andow, D. Cook, K. Emfinger and D. R. Lee.** 2009. Verifying an F1 screen for identification and quantification of rare *Bacillus thuringiensis* resistance alleles in field populations of sugarcane borer, *Diatraea saccharalis*. *Entomologia Experimentalis et Applicata* 129: 172-180.
- Zambryski, P.** 1992. Chronicles from the *Agrobacterium*-plant cell DNA transfer story. *Annual Review Plant Physiology and Plant Molecular Biology* 43, 465-490.
- Zwahlen, C. and D. A. Andow.** 2005. Field evidence for the exposure of ground beetles to Cry1Ab from transgenic corn. *Environmental Biosafety Research* 4: 113-117.

Sunray Harvesters
Bungalow 69
Mhow Cantt. MP 453 441, India
Email: arunarod@gmail.com

Design & Layout
Corporate Communication Services (India) Pvt Ltd.
43 Prospect Chambers Annex, 4th floor
Dr. D.N. Road
Mumbai 400 001, India

Printed at
Aegean Offset Printers
220- B, Udyog Kendra 1
Greater Noida
Uttar Pradesh - 201 306 (India)