Decisions taken in the 102nd Meeting of the Genetic Engineering Appraisal Committee (GEAC) held on 30.07.2010.

The 102nd meeting of the GEAC was held on 30.07.2010 in the Centre for Cellular and Molecular Biology (CCMB) at Hyderabad under the chairmanship of Shri M.F. Farooqui, Additional Secretary, MoEF and Chairman, GEAC.

The deliberations/decisions taken in the GEAC meeting in respect of Agenda items to 5 are as follows:

Agenda item No. 3 : Action taken report on the decision taken in the 101st GEAC meeting.

3.1 The Member Secretary, GEAC informed the Committee that decisions taken in the meeting held on 12.5.2010 have been communicated to the project proponents, concerned government departments and other agencies. Details of action taken were placed before the members and the following points were noted by the Committee.

3.2 Response to the show-cause notice issued by the Ministry on 16.06.2010 to the Project Director, Project Directorate on Poultry, Hyderabad and report of the sub-committee subsequent to the site visit on 19.6.2010 were placed for consideration of the GEAC. The Chairman invited Dr. Arjula Reddy, Co-chairman, GEAC to present the findings of the sub-committee. The following points were noted:

i. A pilot feasibility study was carried out solely as a research initiative to prove the available concept of transgenesis in chicken through sperm mediated method and not intended to produce chickens for human consumption and/or release into the environment including experimental field trials.
ii. For the study, the commercially available construct, "green fluorescent protein gene" of Stratagene, USDA make was procured from M/s Annapurna Scientific and Laboratories Equipments, Hyderabad and transferred using sperm mediated method into White Leghorn chicken. This green fluorescent protein is a proven product and is known to be non-pathogenic and non-toxic with no adverse effects on animals or humans as per available literature. The said gene is universally being used for standardizing the transgenic protocol in different species like mice, pig, goat, rabbit, chicken, etc.
iii. A total of 263 chicks were hatched for the experiment, out of which 16 were found positive for the protein through PCR assay. The remaining chicks were culled and disposed immediately by autoclaving and burying in a closed pit. Of the 16 birds that were positive by PCR, 7 had died by the time of the inspection by the sub-committee and were disposed off in a similar manner. Of the 9 remaining birds, 7 were males and two were females. All the tissues, biological samples, etc; used in the experiment including the eggs were destroyed by autoclaving and burying in a closed pit.
iv. The surviving chickens were properly secured and maintained in an isolated facility with all due care and labeling and no progeny was obtained from the flock. The sub-committee has ensured that all the surviving chickens (9 nos.) were destroyed and disposed by burning into ash in presence of the sub-committee of GEAC on 19.6.2010.
v. The institute has no Institutional Biosafety Committee (IBSC). However, the institute had written to DBT in September, 2008 requesting for a DBT nominee for their IBSC. All other members had been identified and their names have been to

communicated DBT. No meeting of the IBSC was held as there was no DBT nominee.

vi. The institute has given assurance that all activities in this regard have been totally stopped and all the regulations stipulated under the Environment (Protection) Act (EPA), 1986 will be strictly complied with.

vii. The sub-committee has recommended that the DBT should nominate a livestock geneticist or molecular biologist as their nominee at the earliest to monitor rDNA activities in the institute.

3.3 The Committee deliberated at length on the report of the sub-committee and concluded that the institute has violated the provisions of Rules 1989 and is therefore liable for prosecution as per the provisions of section 15 of EPA, 1986. The Committee also expressed deep concern to the fact that public sector institutions are not abiding by the law. It was decided that the matter needs to be brought to the notice of DG-ICAR. It was agreed that Chairman GEAC would write to DG-ICAR regarding this gross violation with a request to inquire how this has happened and initiate appropriate action on the matter. A final view on the matter will be taken by the GEAC on receipt of the action taken report.

3.4 During the discussion, Dr P M Bhargava, reported that the Sericulture Research Institute, Andhra Pradesh, Centre for DNA Fingerprinting and Diagnostics (CDFD) and Central Silk Board (CSB) under the Seri-Biotechnology Research Lab programme are developing a virus-resistant transgenic silkworm for the first time in the world. Member Secretary RCGM clarified that research on transgenic silkworm is being developed after obtaining all the statutory approvals under Rules 1989. It was decided to obtain a factual report from the institute on the status of research and details of approvals obtained so far for record.

Agenda item No. 4: Consideration of applications for confined field trials (Event selection, BRL-I and BRL-II) of transgenic crops expressing new genes as recommended by the RCGM.

4.1 Permission to conduct Biosafety Research Level-1 (BRL-1) trials on transgenic corn hybrids namely NK6240 containing *cry1Ab* gene (Event Bt11) at two locations during Kharif 2010 to evaluate efficacy of the Bt11 by M/s. Syngenta Biosciences Pvt. Ltd., Pune

4.1.1 The Committee considered the request from M/s Syngenta Biosciences (P) Ltd., to conduct Biosafety Research Level-I (BRL-I) trials on transgenic corn hybrids namely NK-6240 containing *cry1Ab* genes (event Bt11). The trials will be conducted at one location each within the institutional research farm of Maharanapratap University of Agriculture and Technology, Udaipur, and Banaras Hindu University, Varanasi, during Kharif 2010.

4.1.2 The main objective of the trial is to:

- evaluate efficacy of the Bt11 event against specific lepidopteron insect pests of corn;
- study the impact of the event on NTOs and soil ecosystem
- produce sufficient plant material for further biosafety research of the event;
- conduct protein expression studies of the transgenes in various plant parts at different time intervals.

4.1.3 It was noted that the transgenic corn event Bt11 Event contains the c*ry1Ab* coding sequence derived from *Bacillus thuringiensis* var *kurstaki* which is a common soil bacterium. The c*ry1Ab* gene encodes for the production of c*ry1Ab* protein, which is insecticidal. This event is primarily meant to protect the corn plant from lepidopteron insect pests of the crop.

4.1.4 This event also contains the marker gene *pat* derived from the soil bacterium *Streptomyces-viridochromogenes*. The *pat* coding sequence encodes for the production of phosphinothricin Acetyl-transferase (PAT) protein. This protein gives the plant tolerance to glufosinate ammonium, an active ingredient in herbicide.

4.1.5 The event was generated using protoplast transformation of cultured maize cells by a Not1 -restriction fragment of the plasmid pZO1502. This fragment contains only the following genetic sequences:

- c*ry1Ab* gene from Bacillus thuringiensis var.kurstaki
- *Pat* gene from the soil bacterium *Streptomyces viridochromogenes*.
- CaMV35S promoter from Cauliflower Mosaic Virus
- IVS6-ADH1 intron from maize
- *Nos terminator from Agrobacterium tumefaciens*

4.1.6 The map of the plasmid from which the Not1 restriction fragment was used for protoplast transformation has been submitted by the applicant.

4.1.7 The Expert members studied the construct map in detail and observed that the presence of marker gene expressing ampycillin is unlikely. However the applicant may be advised to confirm the same.

4.1.8 The event is approved in USA, Canada, Argentina, Colombia, Phillipines, Brazil and South Africa for cultivation and food / feed use. Many more countries like Japan, European Union, Netherlands, Switzerland and others have approved the product for use as food and feed.

4.1.9 The following study reports available and submitted

1. c*ry1Ab* (entrez database accession number AAA 72985): Assessment of amino acid sequence similarity to known or putative allergens
2. c*ry1Ab* (entrez database accession number AAA 72985): Assessment of amino acid sequence similarity to known or putative toxins
3. Equivalence of plant and microbial produced Bacillus turingiensis subsp Kurstaki HD-1 protein
4. Quantification of *Cry1Ab* protein in maize tissues and whole plants derived from - transformation event Bt11.
5. Quantification of *Cry1Ab* protein (Bt11 event) in insect protected sweet corn tissues, whole plants and processed products.
6. *PAT* (entrez database accession number AAU 00088): Assessment of amino acid sequence similarity to known or putative allergens
7. *PAT* (entrez database accession number AAU 00088): Assessment of amino acid sequence similarity to known or putative toxins
8. Acute oral toxicity study in mice using *PAT*

9. In vitro digestibility and inactivation of the marker gene product *PAT* under simulated mammalian gastric conditions
10. Determination of *PAT* in genetically transformed corn
11. Description of phenotype of Bt11 maize compared to non modified maize.
12. Molecular characterization of transgenic Bt11 Maize
13. Additional molecular characterization of event Bt11 maize by southern analysis
14. Southern analysis of event Bt11 maize to demonstrate generational stability
15. Bt11 flanking sequence blast analysis
16. Letter from Monsanto to cite studies in support of regulatory approval of transgenic maize, Bt11 developed by Syngenta seeds AG

4.1.10 The Committee noted that the Applicant has also submitted a report on Efficacy Testing of Insect Resistant Corn Expressing c*ry1Ab* Gene (Bt11 event) against targeted corn lepidopteron pests under green house conditions in Rabi 2009 to RCGM.

4.1.11 The Committee also noted that only F1 hybrid seed produced locally in greenhouse will be used for planting the BRL 1 trial. None of the imported seed would be used directly for planting BRL 1 trials.

4.1.12 It was further noted that IBSC and RCGM have recommended that BRL-I trials on transgenic corn hybrids namely NK 6240 containing c*ry1Ab* genes (event Bt11) at two locations during Kharif 2010 may be permitted.

4.1.13 In view of the above stated facts and taking into consideration the recommendations of the RCGM, the Committee approved the conduct of BRL-I trials at two locations as requested by the applicant subject to the following conditions:

i. Confirmation from the applicant that the event does not contain marker gene expressing ampicillin.

ii. Trials shall be conducted with an isolation distance of 300 m and physical barrier of 10 or 13 rows of African Tall Maize plants covering a distance of 6 to 7.8 meters all around the experimental plot area.

4.1.14 During the deliberations, the issue on whether the use of antibiotic marker gene in food crops and the type of antibiotic markers that can be allowed came up for discussion. The Committee requested Dr Ramesh Sonti, CCMB to prepare a note on the substantive issues which includes information on the type of selectable markers used in GM crops currently in the pipeline. It was agreed that the matter may be placed for discussion in the next GEAC meeting.

4.2 Permission to conduct second year Biosafety Research Level-1 (BRL-1) trials on two transgenic cotton (HXH) hybrids i.e. JKCH-1947 Bt EGII & JKCH-1050 Bt EGII containing *cry1Ac* (Event-1) and *cry1EC* (Event-24) at two locations in North zone during Kharif 2010 by M/s. J.K Agrigenetics.

4.2.1 The Committee considered the request from M/s. JK Agri Genetics Ltd. to conduct second year BRL-1 trials on two transgenic cotton (HXH) hybrids i.e. JKCH-1947 Bt EGII & JKCH-1050 Bt EGII containing *cry1Ac* (Event-1) and *cry1EC* (Event-24) at two locations in the North zone during Kharif 2010 at Bhatinda (Punjab) and Sriganganagar (Rajasthan) as per the SOPs guidelines.

4.2.2 The main objective of the trial is to:

- assess efficacy of genes in controlling the target pests and impact of genes on yield related traits and impact on non-target insect pests.
- level of protein expression at different intervals
- impact of transgenic cotton on growth and morphological traits of cotton and any other undesirable changes including germination, dormancy and weediness.

4.2.3 The Committee noted the GEAC had approved JKCH-1947 Bt EGII and JKCH-1050 Bt EGII containing *cry1Ac* (Event-1) and *cry1EC* (Event-24) for conduct of first year BRL-I trials in the North zone in its meeting held on 3.5.2009. The applicant has submitted the report on BRL-1 trials to the RCGM. The field trials were evaluated by the MEC on 4.11.2009 (Bhatinda) and 26.10.2009 (Sriganganagar) respectively.

4.2.4 The Committee also noted that IBSC has recommended the proposal in its 23rd meeting held on 10.4.2010. The proposal was also recommended by the RCGM in its 90th meeting held on 22.6.2010.

4.2.5 The Committee noted that the applicant has sought permission to conduct trials in the north zone during Kharif 2010. As the sowing season for the north zone is over, the Committee approved the conduct of second year BRL-I trials during Kharif 2011 at two locations as requested by the applicant and recommended by the RCGM.

4.3 Permission to conduct Biosafety Research Level-II (BRL-II) trials at 8 locations WideStrike™ cotton hybrids namely WS103 & WS106 containing *cry1F* (Event 281-24-236+ *cry1Ac* (Event 3006-210-23) in South zone during Kharif 2010 by M/s. Dow Agrosciences India Pvt. Ltd., Mumbai

4.3.1 The Committee considered the request from M/s. Dow Agrosciences India Pvt. Ltd., to conduct BRL-II with WideStrike™ cotton hybrids namely WS103 & WS106 containing *cry1F* (Event 281-24-236) + *cry1Ac* (Event 3006-210-23) in the south zone. The trials will be conducted at Hyderabad (Andhra Pradesh), Guntur (Andhra Pradesh), Jaggayapetta (Krishna Dist), Andhra Pradesh, Dharwad (Karnataka), Haveri (Karnataka), Coimbatore (Tamil Nadu), Salem (Tamil Nadu), Attur (Tami Nadu). The applicant has also sought approval for seed production of two hybrids at four locations each in 8 plots of 1.0 acre each (8 acre) in Attur, Salem District, Tamil Nadu.

4.3.2 The main objectives of BRL-II trials is to:

- study the bio-efficacy of WideStike trait on target lepidopteron pests and their impact on productivity in comparisons to non-transgenic hybrids and checks.
- establish biosafety of WideStike trait for non-target organisms like beneficial insects, soil micro-flora etc.
- generate data on protein expression levels in different plants parts at different crop stage from 30-150 days after sowing.

4.3.3 The Committee noted that the GEAC in its meetings held on 28.5.2008 and 10.6.2009 had approved the conduct of BRL-I with two Bt cotton hybrids expressing *cry1F* (Event 281-24-236) + *cry1Ac* (Event 3006-210-23) at two locations i.e. Andhra Pradesh (Ranga Reddy), Karnataka (Dharwad) in the south zone respectively for generating biosafety data.

4.3.4 The following documents have been submitted by the company:

a) Food, Feed Biosafety studies:

- Acute oral toxicity study in CD-1 mice.
- Protein Thermal Stability of Cry1F (synpro) and Cry1Ac (synpro).
- Pepsin Digestibility study of microbially- derived Cry1Ac (synpro) and Cry1F (synpro).
- Allergenicity Assessment of WideStrike proteins by sequence homology search.
- Compositional Equivalence of WideStrike in USA.
- Compositional Equivalence of WideStrike with i*ndigenously* grown cotton seeds in 2008 & 2009.
- Evaluation of the safety and nutritional equivalence of a genetically modified cotton seed meal in a 90-day dietary toxicity study in rats.(*published paper)*
- Nutritional Equivalence Study of Cry1F/Cry1Ac cotton seed meal: Poultry feeding study
- Safety of WideStrike transgenic cotton seeds in goats in India (2009-10).

b) Environmental Biosafety:

- Studies on micro-flora, earthworm and estimation of Cry1F and Cry1Ac protein concentration in soil from BRL-1 trials of Widestrike hybrids during Kharif 2008 and 2009.Conducted in (i) Institute of Microbial Technology, Chandigarh.(ii) SGS India Pvt Ltd, Ahmedabad.
- Study of pollen flow from WideStrike cotton in South Zone during Kharif 2008 season.
- Studies on germination, aggressiveness and weediness of WS hybrid conducted during Kharif 2008 and 2009.

c) Biosafety Research Trials and Associated studies :

- Biosafety Research Level-1 (BRL-1) trials in South Zone conducted during Kharif 2008 and 2009.
- Generation of baseline susceptibility data with detectable proteins on target pests.
- Bio-efficacy experiments with WideStrike hybrids.
- Quantification of Cry1F and Cry1Ac protein expression by WS hybrids in 2008 and 2009. Study conducted by Enzene BioSciences, Pvt. Ltd., Bangalore.
- DNA fingerprinting of WS cotton hybrids. Conducted at NBPGR, Pusa Campus, N. Delhi.

4.3.5 The Committee noted that the two seasons BRL-I trials conducted by the applicant in 2008 and 2009 have been evaluated by the MEC.

4.3.6 The Committee also noted that the applicant had made a detailed presentation to the RCGM in its meeting held on 23.6.2010 on the safety and efficacy of the product.

RCGM noted that the data submitted by the Company is in order and recommended to the GEAC for BRL-II trials.

4.3.7 After detailed deliberations, the Committee approved the conduct of BRL-II trials for two seasons with WideStrike^{TM} cotton hybrids namely WS103 & WS106 containing *cry1F* (Event 281-24-236) + *cry1Ac* (Event 3006-210-23) in the south zone as requested by the applicant and recommended by the RCGM at 3 locations under the direct supervision of Director CICR, Nagpur. The Committee also approved the request for seed production of two hybrids at 4 locations in 8 plots of 1.0 acre each in confined conditions.

4.4 Permission to conduct elite event selection trials on Glytol cotton (*Gossypium hirsutum*) hybrids during Kharif 2010 by M/s. Bayer Bioscience Pvt. Ltd. Gurgaon

4.4.1 The Committee considered the request from M/s. Bayer Bioscience Pvt. Ltd. Gurgaon elite event selection trials on Glytol cotton (*Gossypium hirsutum*) hybrids to evaluate the herbicide tolerant cotton hybrids by spraying Glyphosate herbicide. The 7 proposed hybrids namely SP499 G, SP503 G, SP7017 G, SP7140 G, SP7139 G, SP7152 G and SP7230 G, will be evaluated in Central zone (Aurangabad-Maharashtra) and South zone (Patancheru-AP) and 3 hybrids namely SP499 G, SP503 G and SP7017 G will be evaluated in North zone (Bhatinda-Punjab).

4.4.2 The Committee noted that decision on the above proposal was deferred in the GEAC meeting held on 9.6.2010 as the applicant had not submitted information on plasmid/construct/ transformation vector, etc. The DBT vide their letter dated 7.7.2010 has subsequently informed that the applicant had submitted the complete information including page 33 to 37 pertaining to plasmid/construct/ transformation vector details which was considered by the RCGM in its meeting held on 20.4.2010.

4.4.3 The Expert members studied the construct map in detail wherein it was opined that the applicant may be advised to submit the following information:

i. What is the region of homology between the intermediate cloning vector and the non-oncogenic Ti plasmid?

ii. What is the structure of the co-integrate plasmid?

iii. What is the structure of the T-DNA region in the co-integrate plasmid?

iv. What is the evidence that only the T-DNA region from the co-integrate plasmid is introduced into the cotton plants in which event selection trails will be conducted?

4.4.4 The Committee also gave an opportunity to the Company's representative to clarify the above points. The representative informed that the information will be submitted in due course. In view of the above stated facts, decision on the proposal was deferred.

Agenda item No. 5: Consideration of applications in respect of Pharmaceuticals

5.1 Permission to manufacture and marketing of Brucella abortus (strain 19) vaccine, Live, IP by M/s. Intervet India Pvt. Ltd.

5.1.1 The Committee considered the request from M/s. Intervet India Pvt.Ltd, for manufacture and marketing of Brucella abortus (strain 19) vaccine, a live attenuated and laboratory derived vaccine strain which will be used in the female calves of 6-8 months of age as a calf hood vaccination and would specifically target the infected pockets with vaccination of all the female calves.

5.1.2 The Committee noted that the product is not a recombinant product but this organism is classified under Risk Group III of schedule -1 of Rules 1989. Therefore approval of GEAC is mandatory. The containment facilities and infrastructure available in the manufacturing unit are as per the prescribed biosafety level –II and biosafety level-III for handling of material under Risk –III category.

5.1.3 It was further noted that the company has acquired ISO Certification for quality management System ISO 9001:2000, Environment Management System ISO 14001:2004 and for Occupational Health & Safety System OHSAS 18001:2007. They also possess a valid manufacturing license no PA/VACC/3 (valid till 29.9.2011). The product is commercially available in the market as a freeze dried vaccine from M/s Indian Immunological under the brand name of 'BRUVAX'. This product has been extensively used in the prevention and control of Bovine brucellosis.

5.1.4 In view of the above stated facts, the Committee approved the request for manufacture and marketing of Brucella abortus (strain 19) vaccine, Live, IP by M/s. Intervet India Pvt. Ltd.

5.2 Permission for Phase III clinical trials to conduct controlled study of the safety and immunologencity of Japanese Encephalitis Chimeric Virus Vaccine (JE-CV) by M/s Sanofi Pasteur India Pvt Ltd. New Delhi (former *Acambis Inc)*

5.2.1 The Committee considered the request from M/s Sanofi Pasteur India Pvt. Ltd. to conduct Phase III clinical trials controlled study of the safety and immununogencity of Japanese Encephalitis Chimeric Virus Vaccine (JE-CV). It was noted that the objective of the study is to asses JE-CV in phase III trials in pediatric populations in India. Subjects will be immunized with either a single dose administration of JE-CV at day 0 or with 3 doses of MBDV at day 0, day 7 and day 28, in accordance with the recommended regime of immunization for both vaccines.

5.2.2 The Committee noted that ICMR vide their letter dated 29.6.2010 has recommended phase III clinical trials to study the safety and immununogencity) of Japanese Encephalitis Chimeric Virus Vaccine (JE-CV). During the deliberations, members expressed concern that JE-CV, a chimeric *Flavivrus* vaccine has been derived from 17D strain of yellow fever vaccine and attenuated strain of SA-14-14-2 of the JE virus. As Flaviviruses are viable, non-homologous recombination between different Flavivirus species is a further possibility. Therefore environmental risk of dissemination of Flavivirus (live vaccine virus) from the blood of immunized subjects through mosquitoes is an area of concern that needs to be addressed.

5.2.3 After detailed deliberations, the Committee opined, though the JE-CV live virus vaccines offers considerable promise in terms of efficacy and cost, the applicant may be advised to submit the following information:

i. Seroconversion rate of JE-CV vaccine in comparison with the available brain mouse vaccine.

ii. Complete Phase-II clinical trial and safety data which includes information on adverse reactions, if any.
iii. Whether the Phase-II trials have been conducted without involving measles vaccine as directed by the GEAC in its letter dated 28.1.2008?
iv. Number of patients and age group to be tested in Phase-III clinical trials.

5.2.4 In view of the above stated facts, decision on the proposal was deferred.

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