Decisions taken in the 126th meeting of the Genetic Engineering Appraisal Committee (GEAC) held on 04.01.2016

The 126th meeting of the GEAC was held on 4th January 2016 in the Ministry of Environment, Forest and Climate Change under the chairmanship of Shri Hem Pande, Special Secretary, MoEFCC and Chairman, GEAC.

The deliberations and decisions taken in the GEAC meeting in respect of Agenda item 3 are as follows:

Agenda item No. 3: Consideration for permission for Environmental release of transgenic mustard hybrid DMH-11 and parental lines containing events bn 3.6 and modbs 2.99 developing using barnase, barstar and bar genes by M/S CGMCP, University of Delhi:

3.1 Based on the decisions in the 125th GEAC meeting held on 11th December, 2015, Prof. Deepak Pental, CGMCP, University of Delhi made a detailed presentation before the Committee. He further mentioned that the BRL-I and BRL II studies were carried out in a technically robust manner and also followed all the prescribed guidelines. Members have interacted with Dr. Pental both on technical and procedural issues.

3.2 The Committee deliberated on the history of safe use of the *barnase, barstar* and *bar* genes. *Barnase-barstar* based system has been used for hybrid seed production in rapeseed (transgenic Canola, a crop closely related to mustard) in Canada (1996) followed by USA (1999) and Australia (2003). The Committee noted, inter alia, the following:

i. Transgenic rapeseed oil and meal is being consumed around the world – oil for human consumption and meal for feeding cattle and poultry. The Committee noted that in the acute oral toxicity studies, Bar, Barnase and Barstar were tested at dose level of 1000, 1000 and 1700 mg/kg, respectively.
ii. The administered dose of BAR protein is 6382 X of the estimated human daily dietary exposure, taking into account the average daily intake of green vegetables (100 grams /day) and Maximum expression level of Bar protein in leaves (94 micrograms/gram weight).
iii. The weediness potential of the DMH11 hybrid.
iv. Cross pollination between transgenic brassica occur though at low frequency and is even low when the distance is more. Even if outcrossing occurs, the progeny of such crosses may not have any survival advantages unless sprayed with specific herbicide. Therefore, the chances of increase in the frequency plants receiving transgene would be low in subsequent generations in the absence of any selection pressure.
v. In rare cases if barnase gene is transferred, the resultant plant would be male sterile and unless pollinated with pollen from wild type plant, and therefore the chances of producing progeny and their persistence in the environment is low.
vi. The dry matter yield appears non-linear with yield contributing components and yield, irrespective of the location.
vii. Several inconsistent data observation/presentation often leading to different interpretation.
viii. Minor variations in the microbial count in in the BRLI and BRL II trails. However, members also noted that the absence of any detectable level of Barnase, Barstar and Bar protein in the soil samples.

3.3 The Committee therefore sought further clarifications from the Applicant which include the following:

i. Various genetic components such as promoters, polyA signals, spacer regions, etc. are to be indicated in the binary construct for clarity and the interpretation of the Southern data.
ii. The information related to the presence of potential ORFs in the spacer element derived from two different truncated genes from two different sources used in *Barnase* Gene construct was not given in the dossier. However, the applicant clarified the absence of any potential ORFs in his presentation. The same information need to be submitted.
iii. Clarification on whether the T_DNA insertion leads to disruption of any endogenous ORF at the integration site of T-DNA needs to be submitted.
iv. The committee advised for continuous monitoring and further investigation for fitness and the transfer of transgenic trait from DMH11 to their progenies and feral population will be essential for implementing management strategies to minimize persistence and dissemination from release site
v. Minor variations observed in the microbial counts may not be correlated with the exudates from the transgenic plants.
vi. LSD at 5% has not been reported for oil content, dry matter yield and other measured parameters leading to statistical non-validation. Similarly, the unit of measurement may be provided.
vii. The evidence that the barnase protein purified from E. coli is as active as other preparations of barnase protein be provided. The validity of the acute toxicity study hinges on this assumption.
viii. The sub-chronic toxicity studies, the non-transgenic hybrid assessment be submitted.
ix. The kidney weight of female animals in barnase group are significantly higher? What might the higher kidney weight imply?
x. What is the status of the original patents on the barbase-barstar system of fertility control?
xi. Is the tapetum specific promoter covered by patents?
xii. Was the site of T-DNA integration in Bn and modBs lines mapped and information on the sites of integration known? Does the integration alter the existing genes? Has the group developed event-specific markers for the two events? What are the molecular 'signatures' to be used for the 'bn36' and 'modBs' events?

3.4 Keeping the detailed deliberations in view, the GEAC constituted a sub-Committee under the Chairmanship of Dr. K. Veluthambi, co-Chair of the GEAC with the following Members to further deliberate on issues raised during the deliberation in the Committee including issues mentioned at para 3.3 and other issues that may be cropped up during deliberation in the sub-Committee and submit the report to the Committee within 15 days for further consideration:

i.	Dr. K. Veluthambi	Chairman
ii.	Dr. S R Rao, Adviser, DBT,	Member
iii.	Dr. S.K. Apte	Member
iv.	Dr. Ramesh V Sonti	Member
v.	Dr. B. Sesikeran,	Member
vi.	Dr. C R Babu	Member
vii.	Dr. K V Prabhu, Joint Director (Research), IARI	Member
viii.	Member Secretary, GEAC	To facilitate the sub-Committee

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