**Risk assessment of *cry1C* (Event MLS 9124) by Metahelix**

The environmental safety assessment of Bt cotton hybrids included extensive studies on pollen escape out- crossing, aggressiveness and weediness, effect on non-target organisms, presence of Cry 1C protein in soil, effect of Bt cotton event MLS 9124 on soil micro-flora, confirmation of the absence of Terminator Gene, and baseline susceptibility studies.

**Studies conducted on pollen escape/out crossing**

Multi-location experiments conducted in 2005, 2006 and 2007 revealed that out-crossing occurred only upto 1.9 meters, indicating the limited pollen movement under the conditions of the field trials, sampling and analysis.

**Aggressiveness & Weediness**

To assess the weediness of Bt cotton carrying Metahelix Cry 1C gene, the rate of germination and vigor was compared by laboratory test and in soil to the non-transformed parental line. The results demonstrated that there are no substantial differences between Bt and non-Bt cotton for germination and vigor. This also indicates that there is no substantial difference between transgenic Bt and control non-Bt cotton with regard to their weediness potential.

**Studies conducted on the effect of Bt on non-target organisms**

Studies conducted during the multi-location field trials revealed that the Bt cotton hybrids do not have any toxic effects on the non-target species. The results indicated that the non-target insects and beneficial fauna levels remained normal in the trial containing transgenic cotton. Populations of beneficial fauna viz., Coccinellids, Chrysopa, Syrphids and Spiders, were comparable among the test *Bt* entries, the *Bt* check and the non-*Bt* checks indicating the safety of the Cry1C protein towards these organisms.

**Studies conducted regarding presence of Bt protein in soil**

Studies were conducted to assess the possible risk of accumulation of Bt protein in the soil, by ELISA method. Bt protein was not detected in soil samples indicating that Bt protein is rapidly degraded in the soil on which Bt cotton is grown.

The soil samples from two locations from both *Bt* and Non *Bt* fields were tested for the Cry1C protein content. All the soil samples from both the rhizosphere and the non rhizosphere zones at different depths showed very low OD value similar to that of the negative control sample. The range of detection using this kit was as low as 1 ppb to 10 ppb. The high OD value of soil sample spiked with *Bt* cotton seed powder indicates that extraction was comparable across all samples. The low OD values for the soil samples from Non *Bt* fields show that Cry1C protein was not present in the soil. Similar low values in the soil samples from *Bt* fields suggest that Cry1C protein, if present, is below the limit of detection. It was thus confirmed that the Cry1C protein will not form residues in soil and hence be considered not to pose any concern to the soil environment.

**Studies to evaluate the effect of Bt gene on soil micro-flora**

Studies were conducted to evaluate any impact of Bt protein leached by roots of Bt cotton on the soil micro-flora. The soil microbial population was comparable between the soils surrounding *Bt* cotton, MLS9124 event expressing Cry1C protein and the non *Bt* counterpart. This observation indicates that MLS *Bt* cotton, event MLS9124, does not have adverse effect on soil microflora.

**Studies to evaluate the Food/Feed Safety**

For evaluating food/feed safety, the studies conducted include: compositional analysis, allergenicity studies, toxicological study, presence of Bt gene and protein in Bt cottonseed and feeding studies on fish, laying hens, goats and buffaloes.

The composition of cottonseed and oil from Bt cotton hybrids was compared to that of non-Bt cotton hybrids and other conventional cotton varieties. The nutrients measured in the cotton seed included protein, fat, fiber, moisture, ash, amino acids, fatty acids, and the anti-nutrients gossypol. These analyses demonstrated that Bt cotton hybrids are substantially equivalent and as safe and nutritious as non-Bt hybrids and other conventional cotton varieties. Furthermore, nutritional studies in cows and buffaloes showed no difference on feed intake, milk yield and composition between Bt and non-Bt groups and safety studies confirmed the food and feed safety of Bt cotton.

The results of the mammalian acute oral toxicity studies support the specificity and the safety of the Cry1Ac protein. No significant acute effects were observed even at extremely high dose levels (4200mg/kg of body weight), when the Cry1Ac protein was administered orally to mice. Also the Cry1Ac protein expressed in the cotton plant is not expected to present a risk of dermal or inhalation toxicity. The proteins that are non-toxic by the oral route are not expected to be toxic by the dermal or pulmonary route.

Feeding experiments conducted with Bt cotton seed meal on fish, laying hens and cows indicated that Bt cotton seed meal is nutritionally equivalent, wholesome and safe as the non-Bt cottonseed meal.

Feeding studies were conducted at the Department of Toxicology, Shriram Institute For Industrial Research, Delhi. The feeding trial of transgenic and non- transgenic cottonseeds in the egg laying hens did not reveal any appreciable change. Also the 28 days feeding studies on Catfish with ‘Bt Cottonseeds and Non – Bt Cottonseeds’as a feed supplement, did not induce any observable toxic effects, when compared to its control counterparts. The 90 days repeated oral administration of ‘Bt Cottonseeds in goats did not induce any observable toxic effects as compared with the goats fed on ‘Non-Bt Cottonseeds and the control group of goats. The toxicological studies on fish and goats under Indian conditions confirmed the safety and wholesomeness of cottonseed meal derived from Bt cotton.

**Allergenicity studies**

Allergenicity studies were conducted in rabbits. Under the conditions of the study, the test substance ‘Bt Cottonseeds’ was found to be negative for Passive cutaneous anaphylaxis test (PCA), Prausnitz- Kustner (PK) Test and Enzyme Linked Immunosorbent assay (ELISA). Therefore, the test substance ‘Bt Cottonseeds’ was found to be non-allergenic and was comparable to the ‘Non-Bt Cottonseeds’. Results of the study concluded that there is no significant change in endogenous allergens of Bt cottonseed compared to non-Bt cottonseed.

Therefore, Bt cotton carrying **Metahelix- *cry1C* (Event MLS 9124)** does not present any additional safety concerns compared to conventional cotton even as a result of processing or handling of the transformed plants or fruits.

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