Safety Assessment of Bollgard® Cotton Event 531

Executive Summary

Bollgard cotton, developed by Monsanto and field tested since 1992, produces an insect control protein (Cry1Ac) derived from the naturally occurring soil bacterium, *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*). Production of the Cry1Ac protein in the cotton plant provides effective season-long protection against key Lepidopteran insect pests, including tobacco budworm, pink bollworm and cotton bollworm (Wilson *et al.*, 1994; Betz *et al.*, 2000). Microbial formulations of *Bacillus thuringiensis* that contain the Cry1Ac insecticidal protein have been registered in numerous countries worldwide, and have been safely used for control of Lepidopteran insect pests for more than 40 years (Luthy *et al.*, 1982, Baum *et al.*, 1999). The Cry1Ac protein produced in Bollgard cotton is nearly identical in structure and activity to the Cry1Ac protein found in nature and in commercial *B.t.k.* microbial formulations. *Bacillus thuringiensis* and *B.t.k.* microbial formulations have been shown to be specific to the target insect pests and do not have deleterious effects to non-target organisms such as beneficial insects, birds, fish, and mammals, including humans (U.S. EPA, 1988).

The primary benefits of Bollgard cotton are reduced insecticide use, improved control of target insect pests, improved yield, reduced production costs, improved profitability, reduced farming risk, and improved opportunity to grow cotton, resulting in improved economics for the cotton growers (Edge *et al*., 2001; Carpenter and Gianessi, 2001; Betz *et al.*, 2000; Economic Research Service/USDA, 2000; Falck-Zepeda *et al.*, 1998; Falck-Zepeda *et al.*, 2000; Fernandez-Cornejo and McBride, 2000; Gianessi and Carpenter, 1999; Klotz-Ingram *et al.*, 1999; Traxler and Falck-Zepeda, 1999; Xia *et al.*, 1999). Planting of Bollgard cotton since 1996 in the US has resulted in a reduction in insecticide use of 2.7 million pounds of insecticidal active ingredients and a reduction in 15 million insecticide applications (Carpenter and Gianessi, 2001). US cotton growers planting Bollgard cotton showed a 260 million pound increase in cotton production per year which resulted in an estimated $99 million increase in net income in 1999 (Carpenter and Gianessi, 2001). There also are a number of secondary benefits associated with the reduction in insecticide use, which include enhanced populations of beneficial insect and wildlife populations, reduced potential runoff of insecticides, and improved safety for farm workers by reducing potential exposure.

The genetically improved Bollgard cotton product was produced using *Agrobacterium tumefaciens*-mediated transfer of the *cry1Ac* gene into the genome of a conventional cotton variety, Coker 312, using a binary plasmid vector. The *nptII* gene, which encodes a selectable marker enzyme, neomycin phosphotransferase II (NPTII), was also present on the plasmid to facilitate selection of insect-protected plants. The NPTII protein served no other purpose and has no pesticidal properties. The plasmid also contained the antibiotic resistance *aad* gene, which encodes the bacterial selectable marker enzyme 3"(9)-O-aminoglycoside adenyltransferase (AAD). This gene confers resistance to the antibiotics spectinomycin and streptomycin, and facilitated the selection of bacteria containing the plasmid in the initial steps of transforming the cotton tissue. The *aad* gene is under the control of a bacterial promoter and the encoded protein is not detected in Bollgard cotton plant tissue.

In assessing the nutritional and compositional equivalence of Bollgard cotton to conventional cotton varieties, more than 2,500 separate analyses were performed on 67 components of the cottonseed and oil. These analyses included protein, fat, moisture, calories, minerals, amino acid, cyclopropenoid fatty acid and gossypol levels. The results of these analyses clearly demonstrate that, other than the production of the Cry1Ac and NPTII proteins, Bollgard cotton is compositionally equivalent to and is as safe as conventional cotton varieties currently available (Berberich *et al.*, 1996).

The following summary provides information on the methods used to develop Bollgard cotton event 531 and a summary of the food, feed and environmental safety studies that support the safety of Bollgard cotton. In addition to the molecular characterization, the following safety studies were conducted: safety of the produced proteins, food/feed composition, and environmental safety. On the basis of this evaluation, Bollgard cotton and its processed fractions were found to be substantially equivalent to conventionally bred

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cotton, taking into consideration the natural variation seen among cotton varieties, with the exception of the expression of the Cry1Ac and NPTII proteins. The Cry1Ac and NPTII proteins were shown to be safe for human and animal consumption and to the environment.