EXECUTIVE SUMARY

Monsanto Company has developed biotechnology-derived, insect-protected soybean MON 87701 that produces the Cry1Ac insecticidal crystal (δ-endotoxin) protein[1] derived from *Bacillus thuringiensis* (Bt) subsp. *kurstaki*. Cry1Ac provides protection from feeding damage caused by targeted lepidopteran pests and will reduce or replace current insecticide applications in tropical and subtropical production regions, where these insects cause significant plant damage and yield loss. The *cry1Ac* gene was transferred into the genome of soybean cells using *Agrobacterium tumefaciens*-mediated transformation. The data and information presented in this application demonstrate that the foods derived from MON 87701 are as safe and nutritious as those derived from commercially-available conventional soybean.

Soybean is the most commonly grown oilseed in the world. In 2007/08, approximately 218.8 million metric tons (MMT) of harvested soybean seed were produced, representing 56% of the world's oilseed production. The impact and severity of insect pest infestations vary greatly across global soybean production regions, primarily due to the different climate and weather conditions, the distribution and environmental tolerance of insect species, and agricultural practices. MON 87701 will be initially commercialized in South America for the control of targeted lepidopteran pests, including the velvetbean caterpillar (*Anticarsia gemmatalis*), soybean looper (*Pseudoplusia includes*), soybean axil borer (*Epinotia. aporema*), and sunflower looper (*Rachiplusia nu*). The Cry1Ac protein produced in MON 87701 is expressed at relatively high levels in leaf tissue throughout the entire growing season and provides efficacious control of these target pests.

MON 87701 was produced by *Agrobacterium*-mediated transformation of soybean with PV-GMIR9, which is a binary vector containing two transfer DNAs (2T-DNAs). The first T-DNA, designated as T-DNA I, contains the *cry1Ac* gene cassette. The second T-DNA, designated as T-DNA II, contains the *cp4 epsps* gene cassette. During transformation, both T-DNAs were inserted into the soybean genome. The *cp4 epsps* gene was used as the selectable marker to select transformed cells and plants. After the transformed cells and subsequent plants were identified, the selectable marker gene was no longer needed. Therefore, a traditional breeding process was deployed to isolate plants that contain only the *cry1Ac* expression cassette (T-DNA I), thereby, producing marker-free MON 87701 plants.

The genetic modification in MON 87701 has been comprehensively characterized. These studies confirm that MON 87701 contains a single insert with the intended sequence, the insert is maintained stably over multiple generations, and the insertion will not result in unintended gene products with similarity to known allergens or toxins. The strategy used to characterize the genetic modification included: 1) Southern blot analyses to assay the entire soybean genome for the presence of DNA derived from the transformation plasmid to confirm that one copy of T-DNA I (*cry1Ac* expression cassette) is present at a single locus in the genome, that sequences from T-DNA II (*cp4 epsps* expression cassette) are absent, and that the insert was stably inherited; 2) DNA sequence analysis to confirm that the sequence of the inserted DNA matched the T-DNA I sequence of the transformation vector; and, 3) segregation analysis to confirm that the inserted DNA is inherited according to Mendelian laws of genetics. Additionally, open reading frame (ORF) bioinformatic analyses of the junction site between the insert and soybean genomic DNA confirm that no relevant

[1] Hereafter referred to as Cry1Ac or Cry1Ac protein.

similarities exist between any putative polypeptides and known toxins or allergens. The results confirm that MON 87701 contains a single copy of T-DNA I inserted at a single locus of the genome, that the backbone sequences are absent in the insert, and that the integrated DNA is stably inherited and segregates according to the Mendelian laws of genetics.

A detailed characterization and safety evaluation of the newly expressed Cry1Ac protein confirm that it is safe for human and animal consumption. The assessment included: 1) physicochemical and functional characterization; 2) an examination of similarity to known allergens, toxins and other biologically active proteins known to have adverse effects on mammals; 3) an evaluation of digestibility in simulated gastrointestinal fluids; 4) documentation of a history of safe consumption and of structural and functional homologues that lack adverse affects on human or animal health; 5) an investigation of potential mammalian toxicity by an oral gavage assay; and 6) estimation of expression levels for a dietary exposure assessment. All data confirm that Cry1Ac is safe for human and animal consumption.

Because the expression of Cry1Ac in MON 87701 seed is low, it was necessary to produce the quantities needed for Cry1Ac studies in a high-expressing host organism, *E. coli;* tests confirmed that the MON 87701-produced Cry1Ac was physicochemically and functionally equivalent to the *E. coli*-produced Cry1Ac. The MON 87701-produced Cry1Ac contains four additional amino acids at the N-terminus derived from a chloroplast targeting sequence (CTP). Therefore, the Cry1Ac produced by *E. coli* was designed to match the exact amino acid sequence of its counterpart expressed in MON 87701. The Cry1Ac produced in MON 87701 shares an amino acid identity of >99% with the naturally occurring Cry1Ac from Bt subsp. *kurstaki* and shares 100% identity with the Cry1Ac produced in Bollgard® and Bollgard II® cotton, except for the four additional amino acids at the N-terminus.

The safety of the MON 87701-produced Cry1Ac protein was confirmed by both well-established scientific methods and a significant history of safe use. Bioinformatics assessments showed that Cry1Ac does not share amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins that have adverse effects on mammals. Digestive fate experiments demonstrated that the full-length Cry1Ac is rapidly digested in simulated gastric fluid (SGF), a characteristic shared among many proteins with a history of safe consumption. A small, transiently stable Cry1Ac fragment from the SGF digestion was very quickly (within 30 sec) degraded during short exposure to simulated intestinal fluid (SIF). Taken together, these data indicate that it is highly unlikely that the Cry1Ac and its fragment will reach absorptive cells of the intestinal mucosa, resulting in low to no allergenic risk.

The safety of Cry1Ac is further confirmed by a history of safe use, low toxicity potential, and no anticipated risk to humans and animals from its presence in the diet. Cry1Ac is a member of the family of Bt Cry proteins that have been used in agriculture as microbial pesticides for over 50 years with no evidence of adverse effects to human or animal health. Since 1996, a number of insect-resistant biotechnology crops expressing Bt Cry proteins have been commercialized. These include Bollgard and Bollgard II cotton that express a Cry1Ac that shares 100% amino acid identity to the MON 87701-produced Cry1Ac (except for the four N-terminal amino acids), and YieldGard® Corn Borer corn that expresses Cry1Ab with an amino acid identity of ~90% with the MON 87701-produced Cry1Ac. Mouse acute oral toxicity tests demonstrate that the Cry1Ac protein is not acutely toxic and does not cause any

® Bollgard, Bollgard II and YieldGard are registered trademarks of Monsanto Technology LLC.

adverse effect, even at the highest dose levels tested (1290 and 1460 mg/kg body weight for females and males, respectively). A dietary safety assessment based on the expected expression levels of Cry1Ac in the seed of MON 87701, acute toxicity data, and soybean product dietary patterns shows that the margin of exposure (MOE) for the overall U.S. population is $\geq 2.93 \times 10^6$. A dietary assessment for animals shows that the expected consumption of Cry1Ac as part of the daily protein intake in feed rations is 0.0498% for the dairy cow and less than 0.0012% for both the broiler and pig. Taken together, these data indicate that food and feed derived from MON 87701 containing the Cry1Ac protein are safe for consumption as the food and feed derived from conventional soybean.

MON 87701 is as safe and nutritious as conventional soybean based on a comprehensive compositional and nutritional assessment. The assessment compared the composition of the seed and forage of MON 87701 to a conventional control and commercially available soybean varieties, harvested in 2007 from five field sites located in major U.S. soybean growing regions. Compositional analyses included important nutrients (protein, fat, carbohydrates, fiber, ash, moisture, amino acids, fatty acids, and a vitamin), and anti-nutrients, consistent with OECD guidelines. In each assessment, MON 87701 was compared to an appropriate conventional control, which had a genetic background similar to MON 87701 but did not possess the introduced trait. In addition, the same analytes were assessed in 20 conventional soybean varieties to establish a 99% tolerance interval for each of the analytes for the population of commercial conventional soybean varieties grown concurrently at the same sites. The results show that MON 87701 is nutritionally and compositionally equivalent to, and as safe and nutritious as, conventional soybean.

In a further assessment, the composition data for MON 87701 and the conventional soybean control were statistically compared in a combined-site analysis, followed by individual-site analyses. The combined-site analysis for harvested seed and forage samples showed no significant difference ($p>0.05$) between MON 87701 and the conventional control, for 40 of 55 comparisons. For the majority of analytes where differences were noted ($p<0.05$), the magnitude of differences were generally low (most <5%), the differences were not observed consistently across all sites (individual-site analyses), and the mean values for MON 87701 were within the 99% tolerance interval. Vitamin E levels for MON 87701 were significantly higher than the control in the combined-site analysis (7.69 vs. 6.24 mg/100g DW) and in four of five individual-site analyses, but were within the calculated 99% tolerance interval. It is concluded that the statistical differences represent the natural variability for these soybean analytes and they were not regarded as biologically meaningful. Harvested seed and forage analyte values were comparable to values published in the scientific literature and reported in the International Life Sciences Institute-Crop Composition Database (ILSI-CCD). This further supports the conclusion that harvested seed and forage from MON 87701 are compositionally equivalent to those of conventional soybean.

The data and information presented in this application demonstrate that the foods derived from MON 87701 are as safe and nutritious as those derived from commercially-available conventional soybean. The conclusion of food safety of MON 87701 was confirmed based on well established lines of evidence:

1. A detailed molecular characterization of the inserted DNA, where the results confirm the insertion of a single functional *cry1Ac* expression cassette at a single locus within the soybean genome;

2. An extensive set of biochemical evaluations that demonstrate the equivalence of the full-length Cry1Ac protein produced in MON 87701 to the *E. coli*-produced Cry1Ac protein used for safety evaluation;
3. An assessment of the toxic and allergenic potential of Cry1Ac based on a history of safe use, extensive information collected and safety evaluations performed, demonstrates that Cry1Ac is unlikely to be a toxin or allergen; and,
4. The compositional and nutritional assessment confirmed that the seed and forage from MON 87701 are compositionally and nutritionally equivalent to, and as safe as, those of conventional soybean.

All data strongly support the conclusion that food derived from MON 87701 will be as safe and nutritious as food derived from conventional soybean, and that the consumption of MON 87701 soybean would be in compliance with the Australian New Zealand Food Standards Code.