

Because it would be difficult, or impossible, to extract sufficient biologically active protein from plants to perform safety tests, Cry2Ab2 protein from bacteria was produced. Product analysis data show that the microbially expressed and purified Cry2Ab2 delta-endotoxin is sufficiently similar to that expressed in the plant to be used for safety assessment purposes. Plant- and microbially produced Cry2Ab2 delta-endotoxins were shown by these studies to have similar molecular weights and immunoreactivity (SDS-PAGE and Western blots), to lack detectable post-translational modification (glycosylation), to have identical amino acid sequences in the N-terminal region and to have similar results in bioassays against *Heliothus virescens* and *Heliocoverpa zea*. The combined results of the above studies indicate a high probability that these two sources produce proteins that are essentially identical by available protein analytical assays.

2. *Magnitude of residue at the time of harvest and method used to determine the residue.* The Cry2Ab2 protein is derived from *Bacillus thuringiensis* class of Cry2A proteins which are designated to have a greater than 95% sequence identity. Data characterizing the Cry2Ab2 protein used in cotton have been submitted to EPA, because it would be difficult, or impossible, to extract sufficient biologically active protein from plants to perform safety tests, Cry2Ab2 protein from bacteria was produced. Product analysis data show that the microbially expressed and purified Cry2Ab2 delta-endotoxin is sufficiently similar to that expressed in the plant to be used for safety assessment purposes. Plant- and microbially produced Cry2Ab2 delta-endotoxins were shown by these studies to have similar molecular weights and immunoreactivity (SDS-PAGE and Western blots), to lack detectable post-translational modification (glycosylation), to have identical amino acid sequences in the N-terminal region and to have similar results in bioassays against *Heliothus virescens* and *Heliocoverpa zea*. The combined results of the above studies indicate a high probability that these two sources produce proteins that are essentially identical by available protein analytical assays.

3. *A statement of why an analytical method for detecting and measuring the levels of the pesticide residue are not needed.* A validated extraction and qualitative analytical method (ELISA) for the detection of Cry2Ab2 protein has been submitted to the Agency.

C. Mammalian Toxicological Profile

The data submitted regarding potential health effects of Cry2Ab2 include information on the characterization of the expressed protein in cotton. The acute oral toxicity data submitted support the determination that the Cry2Ab2 protein is non-toxic to humans. When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels.

The acute oral toxicity data submitted support the prediction that the Cry2Ab2 protein would be non-toxic to humans. Male and female mice (10 of each) were dosed with 67,359 and 1,450 milligrams/kilogram body weight (mg/kg bwt) of Cry2Ab2 protein. Outward clinical signs were observed and body weights recorded throughout the 14-day study. Gross necropsies performed at the end of the study indicated no findings of toxicity attributed to exposure to the test substance. No mortality or clinical signs attributed to the test substance were noted during the study. When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels (Sjogblad, Roy D., et al. "Toxicological Considerations for Protein Components of Biological Pesticide Products," Regulatory Toxicology and Pharmacology 15, 3-9 (1992)). Therefore, because no effects were shown to be caused by the Cry2Ab2 proteins, even at relatively high dose levels, the Cry2Ab2 protein is not considered to be toxic. Furthermore, amino acid sequence comparisons showed no similarity between Cry2Ab2 proteins and known toxic proteins available in public protein data bases.

Data were submitted that demonstrate that the Cry2Ab2 delta-toxin is rapidly degraded by gastric fluid *in vitro*. In a solution of simulated gastric fluid (U.S. Pharmacopeia), complete degradation of detectable Cry2Ab2 protein occurred within 15 seconds. Incubation in simulated intestinal fluid resulted in a 50 kDa protein digestion product. A comparison of amino acid sequences of known allergens uncovered no evidence of any homology with Cry2Ab2.

Collectively, the submitted data on Cry2Ab2 protein, as well as the history of safe use with other plant-expressed and microbially produced *Bacillus thuringiensis* products, establishes the safety of the Cry2Ab2 protein.

The genetic material necessary for the production of the Cry2Ab2 protein is nucleic acid (DNA) which is common to all forms of plant and animal life and there is no instance where these nucleic acids have been associated with toxic

effects related to their consumption as a component of food.

D. Aggregate Exposure

1. *Dietary exposure—i. Food.* Monsanto has considered available information on the aggregate exposure levels of consumers (and major identifiable subgroups of consumers) to the protein residue and to other related substances. These considerations include dietary exposure under the existing tolerance exemption and all other tolerances or exemptions in effect for the plant-incorporated protectant residue, and exposure from non-occupational sources. Oral exposure at very low levels may occur, but lack of mammalian toxicity and digestibility has been demonstrated.

ii. *Drinking water.* Movement of the Cry2Ab2 protein to drinking water is highly unlikely given that Cry proteins are known to rapidly degrade in the soil. Oral exposure at very low levels may occur but lack of mammalian toxicity and the digestibility of this protein have been demonstrated.

2. *Non-dietary exposure.* Exposure to Cry2Ab2 proteins via dermal exposure or inhalation is unlikely given that these proteins are contained in the plant and are not exuded and are not volatile. Therefore, worker and bystander exposure resulting from plant pesticides will be negligible and would be unlikely to add measurably to any worker or bystander exposure resulting from microbial or other *Bacillus thuringiensis* formulations.

E. Cumulative Exposure

Available information on the cumulative effects of such residues and other substances that have a common mechanism of toxicity have been submitted. Because there is no indication of mammalian toxicity from this plant-incorporated protectant, there are no cumulative effects.

F. Safety Determination

1. *U.S. population.* There is reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to the Cry2Ab2 protein and the genetic material necessary for its production. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. This conclusion is based on the low toxicity of the protein, lack of allergenicity, digestibility, and low dietary exposure.

2. *Infants and children.* Nondietary exposure to infants and children is not anticipated due to the patterns of use for