

exposure to pesticide residues. First, EPA determines the toxicity of pesticides. Second, EPA examines exposure to the pesticide through food, drinking water, and through other exposures that occur as a result of pesticide use in residential settings.

III. Toxicological Profile

Consistent with section 408(b)(2)(D) of the FFDCA, EPA has reviewed the available scientific data and other relevant information in support of this action and considered its validity, completeness, and reliability and the relationship of this information to human risk. EPA has also considered available information concerning the variability of the sensitivities of major identifiable subgroups of consumers, including infants and children.

Data were submitted, reviewed, and determined acceptable for product characterization of Cry1F expressed in cotton (construct pAGM281). Adequate product characterization data also were submitted to demonstrate that the Cry1F protein expressed in or on cotton and Cry1F protein expressed in or on corn were the same protein (Ref. 2). The registrant requested that the data submitted for corn (construct PHI 8999) be used to support the acute oral toxicity, *in vitro* digestibility, and heat stability studies for Cry1F protein expressed in or on cotton based on the substantial similarity to Cry1F protein expressed in corn which is already exempt from the requirement of a tolerance (40 CFR 180.1217). EPA reviewed the product characterization data for both Cry1F expressed in or on cotton and corn and determined that the Cry1F proteins are the same. Therefore, EPA has concluded that the data which supported the tolerance exemption for Cry1F and its genetic material necessary for its production in corn can also support Cry1F and its genetic material necessary for its production in or on cotton.

Adequate data also was submitted to demonstrate that the Cry1F test material derived from microbial cultures was biochemically and, functionally similar to the protein produced by the plant-incorporated protectant expressed in cotton (Ref. 2). Production of microbially-produced protein was chosen in order to obtain sufficient material for testing and because a diet of only cotton seed would not provide an adequate diet for test animals. The FIFRA Scientific Advisory Panel has supported this approach. See Mammalian Toxicity Assessment Guidelines for Protein Plant Pesticides (SAP Report No. 2000-03B, September 28, 2000) at <http://www.epa.gov/scipoly/>

[sap/2000/June/finbtmamtox.pdf](http://www.epa.gov/scipoly/sap/2000/June/finbtmamtox.pdf). Given that the Cry1F protein produced in corn and cotton have been determined to be the same protein, EPA has determined that the acute oral toxicity (MRID numbers 446911-01 and 450201-18), heat stability (MRID numbers 452748-01 and 449717-01), and *in vitro* digestibility (MRID number 447149-03) studies which support 40 CFR 180.1217 also support this exemption from the requirement of a tolerance. Although Cry1F expression level data were required for an environmental fate and effects assessment, residue chemistry data were not required for a human health effects assessment of the subject plant-incorporated protectant ingredients because of the lack of mammalian toxicity.

Data were submitted and reviewed which demonstrate the lack of mammalian toxicity at high levels of exposure to the pure Cry1F protein (Ref. 3). These data adequately demonstrate the safety of the Cry1F protein at levels well above maximum possible exposure levels that are reasonably anticipated in the cotton crops (Ref. 2). This is similar to the Agency position regarding toxicity and the requirement of residue data for the microbial *Bacillus thuringiensis* products from which this plant-incorporated protectant was derived. See 40 CFR 158.740(b)(2)(i). For microbial products, further toxicity testing and residue data are triggered by significant acute effects in studies such as the mouse oral toxicity study, to verify the observed effects and clarify the source of these effects (Tiers II and III). Refer to the *Bacillus thuringiensis* Plant-Incorporated Protectants Reassessment Biopesticide Regulatory Action Document (BRAD) dated October 15, 2001 (Ref. 3).

The acute oral toxicity data (MRID numbers 446911-01 and 450201-18) submitted support the prediction that the Cry1F protein is non-toxic to humans. Male and female mice (5 of each) were dosed with 15% (w/v) of the test substance, which consisted of *Bacillus thuringiensis* var. *aizawai* Cry1F protein at a net concentration of 11.4%. Two doses were administered approximately an hour apart to achieve the dose totaling 33.7 milliliter/kilogram (mL/kg) body weight. Outward clinical signs and body weights were observed and recorded throughout the 14-day study. Gross necropsies performed at the end of the study indicated no findings of toxicity. No mortality or clinical signs were noted during the study. A lethal dose (LD)₅₀ was estimated at greater than 5,050 mg/kg body weight of this microbially produced test material. The actual dose

administered contained 576 mg Cry1F protein/kg body weight. At this dose, no LD₅₀ was demonstrated as no toxicity was observed. Cry1F cotton seeds contain 0.0017 to 0.0034 mg of Cry1F/gram of cotton tissue which is a much lower level than the highest no observable effect level.

When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels (Ref. 1). Therefore, since no effects were shown to be caused by the plant-incorporated protectant, even at relatively high dose levels, the Cry1F protein is not considered toxic. Further, amino acid sequence comparisons showed no similarity between Cry1F protein to known toxic proteins available in public protein data bases.

Since Cry1F is a protein, allergenic sensitivities were considered. Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid, and proteases may be glycosylated and present at high concentrations in the food (Ref. 3). Data were submitted and reviewed, and these data demonstrate that the Cry1F protein is rapidly degraded by gastric fluid *in vitro* and is non-glycosylated. In a solution of Cry1F:pepsin at a molar ratio of 1:100, complete degradation of Cry1F to amino acids and small peptides occurred in 5 minutes. A heat lability study demonstrated the loss of bioactivity of Cry1F protein to neonate tobacco budworm larvae after 30 minutes at 75 °C. Studies submitted to EPA using laboratory animals have not indicated any potential for allergic reactions to *Bacillus thuringiensis* or its components, including the delta-endotoxin of the crystal protein. Additionally, a comparison of amino acid sequences of known allergens uncovered no evidence of any homology with Cry1F, even at the level of eight contiguous amino acids residues. The potential for the Cry1F protein to be a food allergen is minimal (Ref. 2).

Regarding toxicity to the immune system, the acute oral toxicity data submitted support the prediction that the Cry1F proteins are non-toxic to humans. When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels (Ref. 1). Therefore, since no effects were shown to be caused by the plant-incorporated protectant, even at relatively high dose levels, the Cry1F protein is not considered toxic.

IV. Aggregate Exposures

In examining aggregate exposure, section 408 of the FFDCA directs EPA to consider available information