

specific insecticidal properties of VIP3A to certain *lepidopteran* insects in cotton as well as its lack of effects on nontarget organisms such as mammals, birds, fish, and beneficial insects.

2. *Magnitude of residue.* A determination of the magnitude of residue at harvest is not required for residues exempt from tolerances. However, the petitioner has provided data on the quantity of VIP3A protein measured in various plant parts including seeds of VIP3A cotton, as measured by enzyme linked immunosorbent assay (ELISA). Additionally, the petitioner has provided data on the quantity or presence of VIP3A protein in processed cottonseed products.

3. *A statement of why an analytical method for detecting and measuring the levels of the pesticide residue are not needed.* An analytical method is not required because this petition requests an exemption from tolerances. However, the petitioner has submitted an analytical method for detection of the VIP3A protein in cottonseed by ELISA analysis.

C. Mammalian Toxicological Profile

The VIP3A(a) gene expressed in event COT102 cotton is very similar (ca. 99% homology) to VIP3A or VIP3A-like genes that appear to occur commonly in *Bt* strains from a variety of sources. In addition, it has been determined that the VIP3A protein demonstrates insect specific toxicity and must be ingested to be active. Once in the insect gut, the VIP3A protein binds to specific receptors (different from those bound by Cry1A proteins), inserts into the membrane and forms ion-specific pores. These events disrupt the digestive processes and cause death of the insect. The lack of mammalian toxicity has been confirmed in numerous safety studies conducted in laboratory animals, which are traditional experimental surrogates for humans. These studies, summarized herein, demonstrate the lack of toxicity of the VIP3A protein following high-dose acute oral exposures to mice, rapid degradation of VIP3A upon exposure to simulated gastric fluid, and the lack of amino acid sequence similarity of the VIP3A protein to proteins known to be mammalian toxins or human allergens. It can be concluded from these studies that the VIP3A protein will be non-toxic to humans.

When proteins are toxic, they are known to act via acute mechanisms and at very low doses (Ref. 1). Therefore, when a protein demonstrates no acute oral toxicity in high-dose testing using a standard laboratory mammalian test

species, this supports the determination that the protein will be non-toxic to humans and other mammals, and will not present a hazard under any realistic exposure scenario, including long-term exposures.

Studies conducted to assess the mammalian safety of VIP3A protein have demonstrated no toxicity. Four acute oral toxicity studies in mice have been completed. Three of the VIP3A test substances used were produced via microbial expression systems and one prepared by extracting protein from leaves of VIP3A event Pacha-derived corn plants. The four test substances contained VIP3A protein that differed from the VIP3A protein expressed in event COT102 by zero to two amino acids. At maximum dosage the microbially expressed test substance was administered at a level of 5,000 milligrams/kilogram (mg/kg) with an estimated acute lethal dose (LD)₅₀ by gavage determined to be >3,675 mg VIP3A/kg mg/kg/bwt/wt. Because toxicity was not observed at this dose, it can be concluded that the LD₅₀ for pure VIP3A protein is >3,675 mg/kg body weight. The VIP3A protein in both the microbial and plant derived test substance was determined to be substantially equivalent to VIP3A produced in event COT102 derived cotton plants, as measured by biological activity, protein size, immunoreactivity, mass spectral analysis of amino acid sequence, and apparent lack of post-translational modifications.

The amino acid sequence of VIP3A is not homologous to that of any known or putative allergens described in public data bases. The VIP3A protein is not derived from a known source of allergens and does not display characteristics commonly associated with allergens, including glycosylation or stability to heat and food processing. Additionally, VIP3A is susceptible to gastric digestion by pepsin and did not provoke an allergic response in an experimental atopic dog model of human food allergy.

VIP3A protein appears to be present in multiple commercial formulations of *Bacillus thuringiensis* microbial insecticides at concentrations estimated to be ca. 0.4 32 parts per million (ppm). This conclusion is based on the presence of proteins of the appropriate molecular weight and immunoreactivity (by SDS-PAGE and western blot), and quantitation by ELISA. Therefore, it is conceivable that small quantities of VIP3A protein are present in the food supply because VIP3A or a very similar protein, based on size and immunoreactivity appears to be present in currently registered insecticide

products used on food crops, including fresh market produce. These commercial *Bacillus thuringiensis* products are all exempt from food and feed tolerances.

D. Aggregate Exposure

1. *Dietary exposure—i. Food.* Food products derived from cotton (refined cottonseed oil and cellulose linters fiber) are highly processed and are essentially devoid of any proteins. Moreover, no VIP3A protein was detected in refined cottonseed oil or cotton fiber produced from event COT102-derived VIP3A cotton plants. Therefore, no human dietary exposure to VIP3A protein is expected to occur via VIP3A cotton. Even if dietary exposure to VIP3A protein were to occur, data derived from bioinformatic analyses as well as direct *in vitro* and *in vivo* testing collectively indicate that the VIP3A protein is unlikely to have allergenic potential. The amino acid sequence of VIP3A is not homologous to that of any known or putative allergens described in public data bases. The VIP3A protein is not derived from a known source of allergens and does not display characteristics commonly associated with allergens, including glycosylation or stability to heat and food processing. Additionally, VIP3A is susceptible to gastric digestion by pepsin and did not provoke an allergic response in an experimental atopic dog model of human food allergy.

ii. *Drinking water.* No exposure to VIP3A and the genetic material necessary for its production in cotton via drinking water is expected. The proteins are incorporated into the plant and will not be available. However, if exposure were to occur by this route, no risk would be expected because the VIP3A protein is not toxic to mammals.

2. *Non-dietary exposure.* Non-dietary exposure is not anticipated, due to the proposed use pattern of the product. Exposure via dermal or inhalation routes is unlikely because the plant-incorporated protectant is contained within plant cells. However, if exposure were to occur by non-dietary routes, no risk would be expected because the VIP3A protein is not toxic to mammals.

E. Cumulative Exposure

Because there is no indication of mammalian toxicity to the VIP3A protein, it is reasonable to conclude that there are no cumulative effects for this plant-incorporated protectant.

F. Safety Determination

1. *U.S. population.* The lack of mammalian toxicity at high levels of exposure to the VIP3A protein