

needed to obtain sufficient quantities of the protein for use in safety testing.

2. *Magnitude of residue at the time of harvest and method used to determine the residue.* Cry3Bb1 residue data should not be required for a human health effects assessment because of the demonstrated lack of mammalian toxicity.

3. *A statement of why an analytical method for detecting and measuring the levels of the pesticide residue are not needed.* A validated method for extraction and direct enzyme linked immunosorbent assay (ELISA) analysis of Cry3Bb1 in corn grain has been submitted to the Agency.

C. Mammalian Toxicological Profile

Cry proteins have been used safely and effectively as pest control agents in microbial *Bacillus thuringiensis* formulations for more than 40 years. The numerous toxicology studies conducted with these microbial products show no significant adverse effects, and demonstrate that the products are practically nontoxic to mammals. An exemption from the requirement of a tolerance has been in place for these products since at least 1971 (40 CFR 180.1011).

Data have been submitted demonstrating the lack of mammalian toxicity at high levels of exposure to purified Cry3Bb1 proteins. These data demonstrate the safety of the proteins at levels well above maximum possible exposure levels that are reasonably anticipated in crops. This conclusion is consistent with the Agency position regarding toxicity and residue data requirements for the microbial *Bacillus thuringiensis* products from which this plant-incorporated protectant was derived (40 CFR 158.740(b)(2)(i)). For microbial products, further toxicity testing and residue data are only 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 (*i.e.*, Tier II and Tier III testing).

Acute oral toxicity studies have been submitted for three Cry3Bb1 variants. These variants of the wild type Cry3Bb1 protein contain a small number of amino acid substitutions (four to seven) to enhance insecticidal activity against corn rootworm larvae. The acute oral toxicity data submitted support a prediction that Cry3Bb1 protein will be nontoxic to humans. Male and female mice (10 per sex per dose level) were dosed with 36, 396, or 3,780 milligrams/kilogram bodyweight (mg/kg bwt) of Cry3Bb1 protein for one variant. Mice were dosed with 39, 419, or 2,980 mg/kg bwt for a second Cry3Bb1 variant.

Mice were dosed with 400, 1,100, or 3,200 mg/kg bwt of Cry3Bb1 for a third Cry3Bb1 variant. In one study, two animals in the high dose group died within a day of dosing. These animals both had signs of trauma probably due to dose administration (*i.e.*, lung perforation or severe discoloration of lung, stomach, brain, and small intestinal tissue). No clinical signs were observed in the surviving animals and body weight gains were normal throughout the 14-day study for the remaining animals. Gross necropsies performed at the end of the study indicated no findings of toxicity attributed to exposure to the test substance in any of the three studies. No other mortality or clinical signs attributed to the test substance were noted in any of the studies.

When proteins are toxic, they are known to act via acute mechanisms and at very low levels (Sjoblod, R. *et al.* "Toxicological Considerations for Protein Components of Biological Pesticide Products." *Regulatory Toxicol. Pharmacol.* 15:3–9, 1992). Since no acute effects were shown to be caused by Cry3Bb1 proteins, even at relatively high dose levels, they are not considered toxic. Furthermore, amino acid sequence comparisons between the Cry3Bb1 variants and known toxic proteins available in public databases showed no similarities.

Since Cry3Bb1 variants are proteins, the potential for allergic sensitivities was evaluated. Current scientific knowledge suggests that common food allergens are present at high concentrations in food, are resistant to pepsin digestion, may be resistant to acid or heat, and can be glycosylated. Data have been submitted demonstrating that the Cry3Bb1 proteins are rapidly degraded by gastric fluid *in vitro*. In a solution of simulated gastric fluid (pH 1.2 U.S. Pharmacopeia), complete degradation of detectable Cry3Bb1 protein occurred within 30 seconds. Insect bioassay data indicate that the protein loses insecticidal activity within 2 minutes of incubation in simulated gastric fluid. Incubation in simulated intestinal fluid resulted in a ~59 kDa protein digestion product. Cry3Bb1 protein produced in corn is not glycosylated and it is not detectable in grain following baking at elevated temperatures.

An analysis of amino acid sequences of known allergens uncovered no evidence of sequence homology with Cry3Bb1, even at the level of eight contiguous amino acid residues. The potential for Cry3Bb1 proteins to be food allergens is minimal. Regarding toxicity to the immune system, the acute

oral toxicity data submitted support the prediction that Cry3Bb1 proteins will be nontoxic to humans.

The genetic material encoding the Cry3Bb1 proteins and the regulatory regions controlling expression of the nucleotide sequence encoding Cry3Bb1 proteins are nucleic acids deoxyribonucleic acid/ribonucleic acid ((DNA) and (RNA)). DNA and RNA occur in all forms of plant and animal life and there is no documented instance of nucleic acids being associated with toxic effects when consumed as a component of food. Data characterizing the genetic material necessary for the production of Cry3Bb1 in corn has been provided to the Agency. No mammalian toxicity is anticipated from dietary exposure to the genetic material necessary for production of the subject plant-incorporated protectant.

D. Aggregate Exposure

Data have been submitted with which to evaluate aggregate exposure levels for consumers to residues of the plant-incorporated protectant.

1. *Dietary exposure.* Cry3Bb1 is a plant-incorporated protectant in corn, thus dietary exposure is deemed to be the most relevant route for assessing human risk.

i. *Food.* Oral exposure, at very low levels, may occur from ingestion of processed corn products. However, a lack of mammalian toxicity and the rapid digestibility of the plant-incorporated protectant have been demonstrated.

ii. *Drinking water.* Oral exposure from ingestion of drinking water is unlikely because the protein is present at low levels within the plant and submitted studies demonstrate that Cry3Bb1 is rapidly degraded in soil.

2. *Non-dietary exposure.* Exposure via skin or inhalation is not likely since the plant-incorporated protectant is contained within plant cells, which essentially eliminates these exposure routes or reduces them to negligible. The use sites for Cry3Bb1 proteins are all agricultural for control of insects. Therefore, exposure via residential or lawn use to infants and children is not expected.

E. Cumulative Exposure

Since there is no indication of mammalian toxicity to the plant-incorporated protectant there will be no opportunity for cumulative toxic effects.

F. Safety Determination

1. *U.S. population.* Sufficient data have been submitted for the purpose of assessing health risk of Cry3Bb1