

EUP-I(8), 29964-EUP-1, 29964-EUP-3, 29964-EUP-U(4), and 29964-EUP-L(5)

Section 408(c)(2)(A)(i) of the FFDCA allows EPA to establish an exemption from the requirement for a tolerance (the legal limit for a pesticide chemical residue in or on a food) only if EPA determines that the exemption is "safe." Section 408(c)(2)(A)(ii) of the FFDCA defines "safe" to mean that "there is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information." This includes exposure through drinking water and in residential settings, but does not include occupational exposure. Section 408(b)(2)(C) of the FFDCA requires EPA to give special consideration to exposure of infants and children to the pesticide chemical residue in establishing a tolerance and to "ensure that there is a reasonable certainty that no harm will result to infants and children from aggregate exposure to the pesticide chemical residue. . . ." Additionally, section 408(b)(2)(D) of the FFDCA requires that the Agency consider "available information concerning the cumulative effects of a particular pesticide's residues and other substances that have a common mechanism of toxicity."

EPA performs a number of analyses to determine the risks from aggregate 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 have been submitted demonstrating the lack of mammalian toxicity at high levels of exposure to pure Cry34Ab1/Cry35Ab1 proteins. These data demonstrate the safety of the products at levels well above maximum possible exposure levels that are reasonably anticipated in the crops. This is similar to the Agency position regarding toxicity and the requirement of residue data for microbial pesticides.

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).

The acute oral toxicity data submitted support the prediction that the Cry34Ab1 and Cry35Ab1 proteins would be non-toxic to humans. The test substance was administered to five female and five male mice (5,000 milligrams/kilogram (mg/kg) body weight) in a 1:4.6 mixture of the two proteins, 14 kDa and 44 kDa. A single dose gavage (25 milliliter/kilogram (mL/kg)) delivered as a 20% mixture in 0.5% methycellulose. All animals survived the 2-week study. One female mouse exhibited protruding or bulging eyes on days 6 and 7, but this resolved thereafter. This observation was not attributed to the treatment as it was an isolated observation (i.e., no other animals exhibited this). No other clinical signs were noted for any animals during the study. An initial weight loss was observed in two mice at test days 1 and 2, but both gained weight for the remainder of the study. All other animals gained weight throughout the study. No gross treatment related observations were recorded during the study as represented by gross pathologic observations. An acute oral LD₅₀ was calculated for this study based upon a dosage of a 1:4.6 ratio mixture of Cry34Ab1 (54% pure) and Cry35Ab1 (37% pure) proteins at greater than 5,000 mg/kg, and greater than 2,000 mg/kg for an equimolar mixture (1:3) of the pure proteins.

When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels (Ref. 3). Therefore, since no acute effects were shown to be caused by the plant-incorporated protectants, even at relatively high dose levels, the Cry34Ab1 and Cry35Ab1 proteins are not considered toxic.

Since Cry34Ab1 and Cry35Ab1 are proteins, 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. Data have been submitted that demonstrate that the Cry34Ab1 and Cry35Ab1 proteins are rapidly degraded by gastric fluid *in vitro* and are non-glycosylated. Two *in vitro* digestibility studies were conducted to determine the lability of the Cry34Ab1 and Cry35Ab1 proteins in

an acid environment containing pepsin. In the first *in vitro* digestibility study, 1 microgram (µg) of the 14 kDa protein (Cry34Ab1) were loaded and was visible on the SDS-PAGE gel up to the 15 minute sample point and on the Western blot, which has greater sensitivity, up to the 20 minute time point. Two micrograms of the 44 kDa protein (Cry35Ab1) was loaded on the SDS gel. A single band was observed on the 44 kDa SDS-PAGE at approximately 15 to 16 kDa. Western blot bands were observed at approximately 42 kDa and 14 kDa. These bands were only observed at the one minute time point, but not afterwards. It was concluded that both proteins are susceptible to degradation in the simulated gastric environment, but that the Cry35Ab1 was more rapidly degraded. In the second *in vitro* digestibility study, the digestibility of Cry34Ab1 was further investigated and enzyme kinetics were used in evaluating the data. In this study, 0.36 µg of the protein was loaded in the SDS gel. The protein appears to have approached full degradation by 7.5 minutes. Volumes remaining at the 10 and 15 minute time points were excluded from the calculations since they were below background levels. Using this first order decay model, the DT50 and DT90 for this protein in the simulated gastric fluid GF were estimated to be 1.9 and 6.2 minutes, respectively. The Cry34Ab1 protein is rapidly degraded in the simulated gastric fluid using this assay and detection methodology. The conditions of the assay are biologically appropriate in temperature, pH, and chemical makeup of the digestive solution. The first order decay rate kinetics accurately portray the digestion of Cry34Ab1.

Submitted studies regarding heat stability of the Cry34Ab1 and Cry35Ab1 proteins demonstrate that these proteins are inactivated at ≤90 °C and ≤60 °C, respectively. A comparison of amino acid sequences of known allergens uncovered no evidence of any homology with Cry34Ab1 or Cry35Ab1, even at the level of 8 contiguous amino acids residues. The potential for the Cry34Ab1 and Cry35Ab1 proteins to be food allergens is minimal.

Regarding toxicity to the immune system, the acute oral toxicity data submitted support the prediction that the Cry34Ab1 and Cry35Ab1 proteins would be non-toxic to humans. When proteins are toxic, they are known to act via acute mechanisms and at very low dose levels (Ref. 3). Therefore, since no effects were shown to be caused by the plant-incorporated protectants, even at relatively high dose levels, the