

equivalence against *Leptinotarsa decemlineata*. These data are adequate to support the equivalence of the microbially produced and plant-produced protein for use in the toxicology studies.

3. *Characterization of the major tryptic fragment from Colorado potato beetle active bacillus thuringiensis subsp. tenebrionis*. The purity and activity of a 55kD protein released with tryptic digestion of the B.t.t. delta endotoxin purified from *E. coli* was shown to have a similar size, immunoreactivity, and amino acid sequence to the 55kD fragment found in potato tubers. The 55kD protein had somewhat higher bioactivity than the 68kD full-length delta endotoxin from B.t.t. These data support the contention that both the 55kD and 68kD forms of the CryIII(A) delta-endotoxin found in the plant were similar to those occurring in B.t.t.

4. *Characterization of Colorado potato beetle active bacillus thuringiensis subsp. tenebrionis protein produced in escherichia coli*. The method of preparing by fermentation the delta endotoxin from B.t.t. in *E. coli* was presented. The protein was characterized for purity and stability after purification. These data indicate that normal fermentation techniques were used to produce the plant equivalent, microbial CryIII(A) delta-endotoxin.

5. *Compositional comparison of Colorado potato beetle (CPB) active bacillus thuringiensis subsp. tenebrionis proteins produced in CPB-resistant potato plants and commercial microbial products*. The CryIII(A) delta-endotoxin as expressed in potato tissue or an *E. coli* alternative gives a similar immunoreactivity and electrophoretic mobility to registered microbial products producing the same delta-endotoxin.

Toxicology Assessment

Toxicity

The delta-endotoxin proteins of *B. thuringiensis* have been intensively studied, and no indications of mammalian toxicity have been reported. Furthermore, approximately 176 different *B. thuringiensis* products have been registered since 1961, and the Agency has not received any reports of dietary toxicity attributable to their use. This is especially significant because FIFRA section 6(a)(2) requires registrants to report any adverse effects to EPA. Therefore, EPA does not expect any mammalian toxicity from this protein in plants based on the use history of *B. thuringiensis* products.

The data submitted by Monsanto support the prediction that this protein would be nontoxic to humans. Adequate information was submitted to show that the test material derived from microbial cultures was essentially identical to the protein as produced by the potatoes.

Production of a plant equivalent, microbial CryIII(A) delta-endotoxin, was chosen to obtain sufficient material for mammalian testing. In addition, the *in vitro* digestibility studies indicate the protein would rapidly be degraded following ingestion.

The genetic material necessary for the production of the *Bacillus thuringiensis* CryIII(A) delta endotoxin are the nucleic acids (DNA and RNA) which comprise the CryIII(A) gene and its controlling sequences. DNA and RNA are common to all forms of life, including plants, and the Agency knows of no instance where these nucleic acids have been associated with toxic effects related to the consumption of food. These ubiquitous nucleic acids as they appear in the subject active ingredient have been adequately characterized by the applicant. Therefore, no mammalian toxicity is anticipated from dietary exposure to the genetic material necessary for the production of the *Bacillus thuringiensis* CryIII(A) delta endotoxin in potatoes.

Allergenicity

Despite decades of widespread use of *Bacillus thuringiensis* as a pesticide (it has been registered since 1961), there have been no confirmed reports of immediate or delayed allergic reactions from exposure. Such incidents, should they occur, are required to be reported under FIFRA section 6(a)(2) and as a data requirement for registration of microbial pesticides (40 CFR 158.740 and Subdivision M of the FIFRA testing guidelines, NTIS # PB89-211676).

Studies done in laboratory animals or as reported in the literature also have not indicated any potential for allergic reactions to *B. thuringiensis* or its components, including the delta-endotoxin in the crystal protein. Recent *in vitro* studies also confirm that the delta endotoxin would be readily digestible *in vivo*.

Current scientific knowledge suggests that common food allergens tend to be resistant to degradation by heat, acid, and proteases, are glycosylated, and are present at high concentrations in the food. The delta endotoxins are not present at high concentrations, are not resistant to degradation by heat, acid and proteases, and are apparently not glycosylated when produced in plants. The company has submitted data to indicate that the CryIII(A) delta endotoxin

is rapidly degraded by gastric fluid *in vitro*, is not present as a major component of food, and is apparently nonglycosylated when produced in plants.

Submitted Data

1. *Acute oral toxicity of B.t.t. protein*. The B.t.t. proteins were determined to be stable and the dosing concentrations were determined to be 74.9 mg/mL, 14.62 mg/mL, and 7.4 mg/mL. B.t.t. protein was not toxic by oral gavage when mice were dosed with up to 5220 mg/kg body weight. These results placed this protein in Tox Category IV.

2. *In-vitro digestibility of B.t.t. protein*. The 68 kD and 55kD B.t.t. proteins degraded within 30 seconds in simulated gastric fluid when analyzed by western blot and were not active against Colorado potato beetles after degradation. The 68kD B.t.t. protein degraded to 55kD within 2 hours of incubation in simulated intestinal fluid. The 55 kD form remained unchanged after 14 hours of incubation and retained its bioactivity and western blot results. These results indicate that, following ingestion by humans, the B.t.t. proteins will be degraded like other proteins to amino acids and peptides similar to those occurring in a normal human diet.

Scientific Advisory Panel Subpanel on Plant Pesticides

A Subpanel of the FIFRA Scientific Advisory Panel (SAP) met on March 1, 1995, to discuss the Agency's Preliminary Scientific Review for this use and concluded that "The Monsanto B. t. potato presents little potential for human dietary toxicity. At a dose of one million-fold greater than that contained in a potato (a 150-gram potato contains about 300 micrograms B.t. protein, 70 kg person = 4.5 micrograms/kg), no toxicity was observed. Moreover, several studies of B.t. potatoes are indistinguishable from strains of wild-type potatoes in nutritive content (total protein, total sugars, vitamin C, minerals, etc.). Furthermore, the B.t. toxin is rapidly digested by pepsin and is inactivated by heat encountered in cooking."

Conclusions

In summary, based upon the submitted studies and other available information, the Agency does not foresee any human health hazards from the use of the *Bacillus thuringiensis* CryIII(A) delta-endotoxin and the genetic material necessary for its production.

Based upon submitted data and a review of its use, EPA has found that when used in accordance with good