

6-24-94

SUBJECT: Section 3 Registration Submission for
Bacillus thuringiensis subsp. *tenebrionis* (CryIII/A)
Insect Control Protein Produced in Potato.

TO: Phil Hutton (PM 18)
Insecticide-Rodenticide Branch
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DP BARCODE: 195923

REG./FILE #: 000524-UTU

BACKGROUND: On September 3, 1993, Monsanto Company submitted to OPP a Section 3 registration document which included eight nontarget organism tests. The tests were as follows:

1. Stability of *Bt* in sucrose and honey solutions (429322-08)
2. Dietary effects on honey bee larvae (429322-09)
3. Dietary effects on honey bee adults (429322-10)
4. Dietary toxicity on parasitic Hymenoptera (429322-11)
5. Dietary toxicity on Ladybird Beetles (429322-12)
6. Dietary toxicity on Green Lacewing larvae (429322-13)
7. Dietary toxicity on Northern Bobwhite with *Bt* lines 10, 12, & 17 (429322-14)
8. Dietary toxicity on Northern Bobwhite with *Bt* lines 06, 16, 18 & 23 (429322-15)

This assessment will determine the adequacy of the data submitted by Monsanto and will recommend any additional requirements which may be necessary for completing a risk assessment.

This review is only in response to the ecological effects data requirements presented by the registrant. Review of other portions of the document will be left to the appropriate disciplines (ie. Fate, Health Effects, Registration).

RECOMMENDATIONS:

Avian data requirements: The registrant has conducted two dietary avian toxicity studies using the bobwhite quail and seven different *Btt* lines (Guideline # 71-2). The studies were both scientifically sound and no treatment mortality, differences in food consumption or behavior was observed between the dosed (50,000 ppm from potato tubers) and control birds. These studies adequately address the avian toxicity concerns for *Btt* expressed in potato. No additional avian studies should be needed in order to make a risk assessment.

Aquatic data requirements: The registrant did not submit any aquatic studies for this product. Since the *Btt* insect control protein is contained within the potato tissue, exposure to aquatic organisms is considered to be unlikely. Therefore, aquatic testing will not be necessary.

***Btt* protein comparisons:** To ensure that the truncated CryIIIA protein expressed in the potato plants will not have an altered host-range of susceptible insects relative to the native full-length protein, comparative insect host-range studies have been submitted by the registrant. These studies were reviewed by the Health Effects Division (HED) of OPP (March 8, 1994 memorandum). The data consisted of SDS-PAGE comigration, Western blot analysis, staining for carbohydrate residues, N-terminal amino acid sequence analysis, and biological equivalence. The results demonstrated that the *Btt* protein expressed by the potato plant was equivalent to the native protein with respect to the parameters tested.

Determining equivalence is important because the registrant used native full-length *Btt* in the honeybee, mammalian and nontarget insect testing. These results indicate that the native *Btt* is very similar to the *Btt* endotoxin produced in the potato plants. This proven similarity will allow the registrant to use the native *Btt* instead of actual plant tissue or an extract from the plant itself.

Non-target and beneficial insects: The Agency recommended that the registrant use a beneficial insect that would be exposed to the *Btt* protein produced in the potato plants. Instead, the registrant submitted the three standard nontarget insect studies (parasitic wasp, ladybird beetle and green lacewing). These studies will be adequate for making a risk assessment, but it would have been more appropriate to submit a study using insects actually exposed to the product. The results of these studies indicated that potato *Btt* was practically nontoxic to parasitic hymenoptera (*Nasonia vitripennis*), green lacewing (*Chrysopa carnea*) and lady bird beetle (*Hippodamia convergens*).

An additional field study on the comparative impacts of foliar-applied microbial *Btt*, transgenic potato plants, and conventional insecticides on non-target arthropods was submitted by the registrant. Beneficial arthropods (ie. lady beetles, damsel bugs, flower flies, soldier beetles, big-eyed bugs, spiders, minute pirate bugs, green lacewings, brown lacewings, stink bugs, and ground beetles) were significantly more abundant in plots containing genetically modified potato plants and foliar-applied microbial *Btt* than in those treated with conventional chemical insecticides. Aphid control was achieved in the plots containing transgenic potatoes solely through predation by natural enemies, while aphid populations rose to high levels in plots where beneficial arthropods were eliminated and no chemical aphid control was applied.

The registrant also submitted a study which tested the sensitivity of selected insect species to the *Btt* protein produced in the potato plants. The tested species were as follows: 3 coleopterans-Colorado potato beetle, boll weevil and southern corn rootworm; 4 lepidopterans-European corn borer, tobacco hornworm, corn earworm and tobacco budworm; 1 dipteran-yellowfever mosquito; 1 orthopteran-German cockroach; and 1 hemipteran-green peach aphid. The results demonstrated that no species other than the Colorado potato beetle displayed significant mortality. There was a slight reduction in the amount of honeydew produced by the Green peach aphid which was an indication of reduced feeding.

These studies indicate that *Btt* produced in potato plants should not adversely affect the nontarget insects studied in these tests. Since *Btt* is specific to coleopterans it is not surprising that the non-coleopteran insects were not affected by this toxin. However, nontarget coleopteran insects that feed on these potato plants will, in all likelihood, be adversely affected by the *Btt* protein. Since any coleopteran insect that feeds on these plants would be considered a plant pest, this should not present a risk to nontarget, non-pest insects.

Nontarget soil organism testing: The registrant did not submit any testing on soil organisms. Because of literature reports describing adverse effects on soil invertebrates from conventional *Bt* products and the great potential for exposure from *Bt* protein in the plant debris left in the field after harvest that soil organisms will feed upon, these studies will need to be submitted by the registrant. The preferred organisms to be tested would be the earthworm and a soil invertebrate such as *Collembola* (springtails). Test protocols using *Collembola* and earthworms have been developed and are available from a number of sources.

Honeybee toxicity study: In light of the production of *Bt* endotoxin protein in pollen and its subsequent exposure to honeybees, the registrant was required to submit a larval honeybee study. The registrant also submitted an adult honeybee study (154-24) which was not required for registration. The adult and larval honeybees were dosed with *Btt* in a sucrose and honey solution. The

registrant wanted to ensure that the *Btt* endotoxin was stable in this type of solution. Testing indicated that there was no significant loss of *Btt* protein bioactivity in honey or sucrose solutions when maintained for up to 7 days at approximately 28C.

The adult honeybee study was found to be invalid due to excessive mortality in the controls. Since this study was not required, it will not have to be repeated. The larval honeybee study was scientifically sound and demonstrated that *Btt* in potato is practically non-toxic to honeybee larvae. However, the study was not validated using a positive control (ie. B-exotoxin). The registrant will need to validate the study to ensure that the negative results are an indication of no effect of the toxin and not an invalid test. Therefore, this study will need to be validated and the results submitted by the registrant. The registrant will need to repeat the *Btt* test using a positive control. Also, it would improve the test if 1-2 day larvae were used instead of 3 day larvae, because the younger larvae would feed more actively on the test substance and would get a larger exposure.

Endangered species considerations: At this time, the Agency has not determined if a may effect has been triggered for *Btt* protein expressed in potato plants. A decision should be forthcoming and the registrant will be advised of that decision as soon as it has been made.

Mammalian Toxicity: HED has reviewed a mammalian toxicity study using the 68 kD and 55 kD *Btt* proteins (March 10, 1994 memorandum). These proteins were found to be nontoxic by oral gavage when mice were dosed with up to 5220 mg/kg body weight (Tox Category IV). Therefore, the *Btt* proteins should not present a risk to nontarget mammalian species.

CONCLUSIONS: *Btt* protein produced in transgenic potato plants should not cause adverse effects to avian species, wild mammals, and non-target and beneficial insects. The aquatic testing was waived based on a lack of exposure because the *Btt* protein is contained in the potato tissue. The results of the honeybee larval test indicated that the protein is not toxic to honeybee larvae, but the test needs to be validated using a positive control in order to be certain. A determination on whether there are endangered species risk concerns has not been made at this time. This issue will need to be resolved before a risk assessment can be made.

The registrant will need to complete the following testing requirements in order for a risk assessment to be completed on this product:

1. Soil Organism Testing (*Collembola* and earthworm)
2. Repeat the Larval Honeybee Study using a positive control (also, use 1-2 day old instead of 3 day old larvae). 4.
3. An evaluation of the potential risks to Endangered Species has been initiated and a decision (on may effect trigger) will be deferred until these issues have been resolved.