

US EPA ARCHIVE DOCUMENT

MEMORANDUM

TO: Mike Mendelsohn, Regulatory Action Leader
Biopesticides and Pollution Prevention Division, 7511C

FROM: Robyn Rose, Entomologist
Biopesticides and Pollution Prevention Division, 7511C

PEER REVIEW: Zigfridas Vaituzis, Ph. D., Microbiologist
Biopesticides and Pollution Prevention Division, 7511C

THROUGH: Phil Hutton, Branch Chief
Microbial Pesticides Branch
Biopesticides and Pollution Prevention Division, 7511C

SUBJECT: Review of ecological non-target lady beetle effects for Monsanto's EUP request to allow testing and further development of their *Bacillus thuringiensis* Cry3Bb1 Field Corn (EPA Reg. No. 524-LRA; Barcode No. D262045; Case No. 06622; Submission No. S572997).

CLASSIFICATION: Acceptable for Experimental Use Permit; Supplemental to testing pollen feeding by lady beetles for a full commercial registration.

BPPD CONCLUSIONS:

This study adequately demonstrates that lady beetles feeding on corn plant tissue or prey that have fed on corn plant tissue containing Cry3Bb1 will not be adversely affected. However, lady beetles routinely feed on corn pollen. Direct exposure to Cry3Bb1 is therefore possible if it is expressed in the pollen. This is a concern since Cry3Bb1 is active against coleopterans. In order to obtain a full commercial registration, Monsanto should conduct a test demonstrating the effect of lady beetles feeding on corn pollen containing Cry3Bb1.

BACKGROUND:

According to 40 CFR §158.590, effects on nontarget insect predators and parasites should be evaluated when registering a new pesticide. The OPPTS 885.4340 guideline titled "Nontarget insect testing" recommends performing tests on three species of insects including parasitic dipterans, predaceous hemipterans, predaceous coleopterans, predaceous mites, predaceous neuropterans, and parasitic hymenopterans. BPPD routinely requests studies on the effects of Bt proteins on lady beetles (coleopteran), green lacewings (neuropteran), and a parasitic Hymenoptera. As part of this requirement, Monsanto sponsored a test on the effects of the Cry3Bb Bt protein against *Hippodamia convergens*, a representative lady beetle.

DATA EVALUATION REPORT

REVIEWED BY: Robyn Rose, Entomologist
Biopesticides and Pollution Prevention Division
SECONDARY REVIEWER: Zigfridas Vaituzis, Ph.D., Microbiologist
Biopesticides and Pollution Prevention Division
STUDY TYPE: Non-target effects of Bt Protein 11231 on lady beetles
MRID NO.: 44903-14
STUDY NO.: WL-98-299
SPONSOR: Monsanto Company, 700 Chesterfield Parkway North, St Louis,
MO 63198
PERFORMING LAB: Monsanto Company, 700 Chesterfield Parkway North, St Louis,
MO 63198 and Wildlife International Ltd., 8598 Commerce Dr.,
Easton, MD 21601
TEST MATERIAL: *Bacillus thuringiensis* Protein 11231
AUTHOR: Susan J. Palmer and Henry O. Krueger, Ph.D
STUDIES DATED: August 19, 1999

Study Summary

Title: *Bacillus thuringiensis* Protein 11231: A Dietary Toxicity Study With the Ladybird Beetle (*Hippodamia convergens*)

Authors: Susan J. Palmer and Henry O. Krueger, Ph.D (Wildlife International Ltd.).

Objective: To evaluate the toxicity of *Bacillus thuringiensis* Cry3Bb1 (analogous to Protein 11231) in diet to lady beetles (*Hippodamia convergens*)

Methods: Diet containing *Bacillus thuringiensis* (Bt) and honey was fed to *H. convergens* at rates one and 20 times the maximum protein concentration found in corn leaf tissue. Diets contained 400 and 8,000 µg Cry3Bb1/mL of diet as well as a control group containing a honey/water mixture and two substance reference groups containing 1,000 or 10,000 µg potassium arsenate/mL diet. Four hundred and 8,000 µg Cry3Bb1/mL of diet are approximately equivalent to 1X and 20X the maximum Bt protein concentration in plant tissue. Mortality and other signs of toxicity were recorded twice within four hours after test initiation and daily thereafter until 20% mortality was reached in the control group. Results from groups fed diet containing Cry3Bb1 were compared to control groups.

Results: Results from this study showed that the no-observed-effect-concentration NOEC for Cry3Bb1 when incorporated in diet and fed to *H. convergens* is 8,000 µg Bt protein/mL diet. The study was terminated ten days after initiation because mortality in the control group reached 24%. There was 33% *H. convergens* mortality when fed 400 µg Cry3Bb1/mL diet and 35% *H. convergens* mortality when fed 8,000 µg Cry3Bb1/mL diet. These results demonstrate no significant differences in mortality rate between lady beetles fed 400 and 8,000 µg Cry3Bb1/mL of diet. At the 400 µg Cry3Bb1/mL diet dose, there was a small number of beetles that became immobile but this was determined not to be related to the treatment. Mortality for the 1,000 and 10,000 µg potassium arsenate/mL diet groups were 55% and 95% respectively at day 10. This

demonstrates that toxicity can be measured by mixing a test substance with diet.

Monsanto Conclusions: Lady beetles do not feed on corn plant tissue. They do, however, prey on pest insects that may feed on corn tissue and contain Cry3Bb1 in their gut, thus exposing lady beetles to the Bt protein. According to Monsanto, there is approximately 390 µg Cry3Bb1/g fresh weight corn tissue. Lady beetle exposure is expected to be significantly lower than this since the corn tissue would be metabolized, eliminated, or otherwise degraded within the prey species. Since the NOEC was found to be 8,000 µg Cry3Bb1/mL diet which is 20 times higher than maximum expected exposure levels, risk of Cry3Bb1 to lady beetles is expected to be minimal.