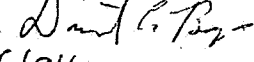


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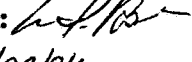
DATA EVALUATION REPORT

1. Chemical: Transgenic potato plants which express *Bacillus thuringiensis* subsp. *tenebrionis* (Btt) protein.
2. Test Material: Russet Burbank CPB (Colorado Potato Beetle) Resistant Potato tuber Powder, Lines Bt 06, Bt 16, Bt 18, Bt 23 and a control (parental) line of Russet Burbank Potato.
3. Study/Action Type: An Avian Dietary Study, Species: Northern Bobwhite Quail (*Colinus virginianus*) (71-2).
4. Study Identification: A Dietary Toxicity Study With Russet Burbank Potatoes in the Northern Bobwhite. By Susan M. Campbell, Joann B. Beavers and Mark Jabers. Prepared By Wildlife International LTD, April, 1993. Project No. 139-357. Submitted By Monsanto Agricultural Company. Saint Louis, Missouri. EPA Acc. No. 429322-15.

5. Reviewed By: David C. Bays, PhD.
Microbiologist
EFED/EEB

Signature: 
Date: 6/16/94

Robert I. Rose, PhD.
Entomologist
EFED/EEB

Signature: 
Date: 06/23/94

6. Conclusions:

The study is scientifically sound and no treatment mortality, differences in food consumption or behavior was observed between the birds fed 50,000 ppm (5% w/w) lyophilized CPB resistant potato powder (from three different potato lines) in the diet and birds fed the same level of Russet Burbank control (parental line) potatoes or basal diet. The no-observed effects level was considered 50,000 ppm. This indicates that the transgenic potato plants are practically nontoxic to birds. The study will be considered core and fulfills EPA Guideline requirements for an avian dietary test.

7. Recommendations: N/A

8. Materials and Methods:

A. Test Organisms: Healthy day old northern bobwhite quail, phenotypically indistinguishable from wild birds, were obtained from Fritts' Quail Farm, Phillipsburg, New Jersey and acclimated until they were 10 days old. The quail were distributed into 3 treatment groups and 2 control groups of 10 birds each (3 replicates), without regard for the sex of the bird. Water and feed, a game bird ration formulated by Wildlife International Ltd, were provided ad libitum during the acclimation and testing periods.

B. Dosage Form: The four test substances (each a different experimental potato line), all tan powders (lyophilized potato tissue), were directly mixed into the test diet for the birds (5% of the total weight). The suspension was prepared daily and then frozen. Fresh, frozen test diet at the nominal dietary test concentration of 50,000 ppm was given to the birds daily. The dietary concentration was not adjusted for the purity of the test substance, so the test concentration is reported as parts per million of the test substance received. The freeze drying process did not adversely affect the stability of the Btt protein based on a study (Volume 3: Rogan, J.G., Anderson, J.S., McCreary, J.A. and Lavrik, P.B. "Determination of the Expression Levels of Btt and NPTII Proteins in Potato Tissues Derived from Field Grown Plants" (1993), Study Number 92-01-37-02, an unpublished study conducted by Monsanto Company) submitted by the registrant.

C. Referenced Protocol: The dietary concentrations used in the study were established using known toxicity data. The control consisted of the bird diet with enough carrier added to equal the largest amount used in the treatments. The birds were given the treated and control feed for 5 days and then given untreated feed for 3 days.

All birds were acclimated and tested in brooding pens (72x90x23 cm high) assigned by random draw and housed indoors. Average ambient room temperature for the study was 23.2 ± 0.3 C with an average relative humidity of 28 ± 5 % and with an average brooding compartment temperature of 38 ± 2 C. The photoperiod (monitored by a time clock) was 16 hours of light per day during acclimation and throughout the study. The light was provided by Chroma 50 fluorescent lights (5000 Kelvin) which closely approximated noon-day sunlight (4870 Kelvin). The birds received approximately 130 lux of illumination. Housing and husbandry practices were based upon the "Guide for the Care and Use of Laboratory Animals", NIH Publication No. 85-23, 1985.

All birds were observed daily during acclimation and any exhibiting abnormal behavior or physical injury were not used. After test initiation and continuing until termination, all birds were observed at least twice daily with all mortality, signs of toxicity or abnormal behavior being recorded. Body weights of the test birds were recorded individually prior to dosing, on Day 5 and on Day 8. Average estimated feed consumption was measured for days 0-5 during the exposure period, and 6-8 during the post-exposure observation period.

D. Statistical Analysis: The test data in this study did not facilitate the use of the C. E. Stephan computer program for

calculating an LC_{50} value. Therefore, the LC_{50} value was estimated using visual inspection of the mortality data.

12. Reported Results:

| <u>Dosage</u> | <u>ppm</u> | <u>Number Dead/Number Exposed</u> <u>(At 30 Days After Dosing)</u> |
|---------------|------------|---|
| Phase I | | |
| Negative | 0 | 0/10 |
| Control | 0 | 0/10 |
| | 0 | 0/10 |
| Bt-Control | 50,000 | 1/10 |
| | 50,000 | 0/10 |
| | 50,000 | 0/10 |
| Bt 06 | 50,000 | 0/10 |
| | 50,000 | 0/10 |
| | 50,000 | 0/10 |
| Bt 16 | 50,000 | 0/10 |
| | 50,000 | 0/10 |
| | 50,000 | 0/10 |
| Phase II | | |
| Negative | 0 | 2/10 |
| Control | 0 | 0/10 |
| | 0 | 0/10 |
| Bt-Control | 50,000 | 0/10 |
| | 50,000 | 0/10 |
| | 50,000 | 3/10 |
| Bt-23 | 50,000 | 1/10 |
| | 50,000 | 0/10 |
| | 50,000 | 0/10 |
| Bt-18 | 50,000 | 0/10 |
| | 50,000 | 0/10 |
| | 50,000 | 0/10 |

$LC_{50} > 50,000$ ppm

No mortalities occurred in the basal diet control groups. No differences in mortality occurred between the control and

treated groups. There were no apparent effects on body weight or feed consumption between the control and the treated groups.

13. Study Author's Conclusions/Quality Assurance Measures:

LC₅₀ > 50,000 ppm

"This study was conducted as to conform with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR 160, 17 August 1989; OECD, ISBN 92-84-12367-9, Paris 1982; and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984 with the following exceptions: Characterization of the test substance was the responsibility of the Sponsor and Practical analytical methods are not available to determine the concentration, uniformity and stability of the test substance incorporated in the avian diet, and therefore was not performed." Signed by study director, Susan M. Campbell and Joan B. Beavers.

14. Reviewer's Discussion and Interpretation of the Study:

- A. Test Procedures: The procedures used follow those recommended by EPA in the 1989 Pesticide Testing Guidelines for Microbial and Biochemical Pest Control Agents, Subdivision M.
- B. Statistical Analysis: None was needed since there were no mortalities.
- C. Discussion/Results: An LC₅₀ > 50,000 ppm indicates that Bt expressed in the potato lines tested in this study are practically non-toxic to birds.
- D. Adequacy of the Study:
1. Validation Category: Core
 2. Rationale: Adequately fulfills guideline requirements

15. Completion of the One-liner: