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DATA EVALUATION REPORT

STUDY TYPE: Determination of potential for sublethal effects on *Chrysoperla carnea* (green lacewing) fed on Bt corn consuming prey.

CITATION: Angelika Hilbeck. September 1996. INVESTIGATIONS ON SIDE-EFFECTS OF TRANSGENIC BT-CORN ON BENEFICIAL INSECTS. Report for the Swiss National Science Foundation. (SSP Biotechnologie, Gesuch Nr. 5002-042598).

Published as: Hilbeck, A., M. Baumgartner, P.M. Fried, and F. Bigler.1998. Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae), Environ. Entomol.,27(2):480-487

DP BARCODE: D236803 **CASE**: 044773

REG./FILE#: 66736-1 **CHEMICAL/BIOL#**: 006430 BT CORN

COMPANY/SPONSOR: A research report submitted by:
Novartis Seeds, Inc.
Seeds Biotechnology Research Unit
P.O.Box 12257
Research Triangle Park, North Carolina 27709-2257

Novartis Seeds, Inc. submitted to BPPD a copy of the above mentioned research report to be reviewed for any potential impact on Event 176-derived transgenic B.t. corn ecological effects determination.

TEST MATERIAL: Event 176-derived "Bt corn" (EPA Reg. No. 66736-1) and non-transgenic corn provided by Novartis Seeds Ltd.

REVIEW CONCLUSION: The data are inconclusive and not useful for risk assessment of Event 176-derived Bt corn

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ADEQUACY OF STUDY: Not a valid study

RECOMMENDATIONS: In order to have beneficial insect risk assessment value this study should be conducted as a field test (in the presence of environmental influences). Before a field test the study should be repeated using pure Bt toxin with a healthy natural diet in all larval development stages.

STUDY OBJECTIVES: (1) To determine the effects of consumption of prey fed on Bt corn on the survival and development of the green lacewing, and (2) to determine whether the observed effects were due to exposure to Bt toxin in the prey, or if the observed effects were due to the indirect influence of consumption of sick prey fed a suboptimal diet.

MATERIALS & METHODS:

Plants: Two corn hybrids provided by Novartis Seeds (CIBA) were used in the experiments. One produced a Bt var. *kurstaki* HD-1 CryIA(b) delta endotoxin. The second was an identical hybrid not containing the CryIA(b) delta endotoxin gene. The plants were cultivated in greenhouses for 2 to 4 months up to a height of 50-80 cm (5-8 leaf stage).

Insects: The predatory green lacewing larvae (*Chrysoperla carnea*) were maintained in a laboratory colony on pea aphids (*A. pisum*). The adults were kept on a mixture of yeast, honey and water.

No-choice feeding experiments: The predatory green lacewing larvae (*Chrysoperla carnea*) were presented with the European corn borer (ECB, *Ostrinia nubilalis)* and the Egyptian cotton leafworm (ECL, *Spodoptera littoralis)* larvae that had been fed Bt hybrid corn plants. The control larvae were fed non-hybrid Bt corn plants. These two insects (ECB and ECL) were chosen because one is susceptible to the Bt toxin (ECB), and one is resistant to the Bt toxin effects (ECL).

The experiment was replicated four times. Fifty (50) lacewing larvae were used for each treatment resulting in 200 lacewing larvae per replicate (800 total larvae for 4 replicates).

During the first two instars of development ECB and ECL larvae were the only food source presented to the lacewing larvae. During the third instar this prey-only diet was supplemented with *Ephestia kuehniella* eggs.

Sampling: Stage specific mortality and development time was recorded daily.

Test Conditions: Temperature 25° C for 10 h during photophase and 20° C for the remaining 14 hours, relative humidity 70%, 18:6 light/darkness hr.

REPORTED RESULTS: Mortality was significantly higher for lacewing larvae reared on Bt-

exposed prey (62%) as compared to lacewing larvae reared on prey that had been fed on control plants (39%). Development time of lacewing larvae was prolonged by 2.5 days (31.6 days total) when Bt-exposed ECB larvae were consumed as prey. The development time was comparable when lacewings were reared on (Bt versus non-Bt) ECL larvae.

STATISTICAL METHODS: For the statistical analyses a regular ANOVA was carried out using a model that tested for significant run and treatment main effects and, where appropriate, also for interaction effects. Analyses were performed using the GLM procedure of the SAS statistical package (SAS Institute, 1988).

STUDY AUTHOR'S CONCLUSIONS: In reference to the increased mortality, the author stated that "This finding suggests that the reduced fitness of chrysopid larvae raised on Bt prey is caused by Bt-related factors." In reference to the slight increase in development time when fed Bt-ECB, the author states that this was "possibly due to a combined effect of Bt exposure through the ECB larvae and nutritional deficiency caused by sick prey of poor nutritional value."

QUALITY ASSURANCE MEASURES:

There is no QA statement indicating whether the study was conducted according to GLP.

DISCUSSION/REVIEWER'S COMMENTS:

The study author's conclusions are prefaced with the terms "suggests" and "possibly due to." These statements indicate less than unambiguous results derived from a sound data set. The data are weak for the following reasons.

The high lacewing **negative control mortality (39%) is a sign of a less than optimal (unhealthy) test system.** No data are presented to show the amount of prey consumed by each test group to make an independent assessment of unpalatable and sick prey effects like starvation effects due to food avoidance. In addition, the lack of quantitation of consumed Bt makes it impossible to determine correlation between exposure to Bt and the observed responses. Also, the lacewing larvae were not given a choice between Bt exposed and unexposed prey species throughout the test period. As a result, **the submitted study does not account for the effects of a suboptimal/starvation diet** (consisting of sick and dying larvae) which may have been unpalatable to the lacewings and therefore of limited nutritional value.

Therefore **there is no** unequivocal **support for the conclusion that the Bt toxin was directly responsible for the observed differences in lacewing mortalities.** The author tried to address this problem by including ECL larvae (which were thought to be resistant to Bt toxin) as a "healthy" control unaffected by, but containing Bt toxin. However, a recently published study shows that the ECL is also susceptible to the CryIA(b) protein in a 5-day feeding study [Muller-Cohn, *et al.* Journal of Economic Entomology. Aug 1996. v. 89 (4) p. 791-797]. Therefore the authors "may effect" conclusions are partially based on faulty assumptions in the

test system.

Likewise **there is no clear support for the conclusion that the Bt toxin was directly responsible for the observed differences in lacewing development times** in the laboratory study. The ECB larvae were the only food source available to the green lacewings during their early development. The experimental design did not permit a distinction between a direct effect due to the Bt toxin versus an indirect effect of consuming a suboptimal diet consisting only of sick or dying prey. The prey may have been septicemic (and therefore indirectly toxic), of limited nutritional value, or otherwise unpalatable to the lacewing. The lacewings did not have a choice of diet throughout their development, which they have in a field setting.

Environmental influences were also not taken into account when speculating that Bt corn may pose a risk to beneficial insects. In a field setting it is highly improbable that chrysopid larvae will mature exclusively on a diet of ECB larvae. **In nature the lacewing does not rely upon a single food source for development.** The lacewing larvae have a choice of other insects or eggs to feed on. **Furthermore, in Bt corn fields the ECB will not be available to the lacewings, since the ECB will be practically eliminated by the Bt toxin in corn plants. In addition, the surviving ECB larvae would normally be within the corn plant most of their larval life and not available for consumption by chrysopids. Thus it is highly unlikely that in the field the lacewings, or other beneficial insects, will ingest the amounts of Bt that the larvae were forced to consume in the laboratory study** (i.e. there is a very low Bt toxin exposure to predators in the field). Therefore, actual field studies are necessary to ascribe practical significance to these controlled laboratory feeding experiments in order to assess the ecological relevance of the reported single species laboratory findings.

In general the reported laboratory findings are inconclusive and cannot be used to assess the effects on beneficial insects in a field use setting. **The author, A. Hilbeck, agrees with this by stating that "No conclusions can be drawn at this point as to how results from our laboratory trials might translate in the field. Further experimentation under field conditions is necessary."**

In summary, any conclusion of detrimental effects on the lacewing populations can be confirmed only by a field study. All available in-house and published data do not show significant detrimental effects due to Bt delta endotoxin on the lacewing (e.g., no effect on lacewing larvae fed 16.7 ppm pure CryIA(b) protein for seven days). Therefore, based on the available laboratory data in the Agency's files and the low field exposure of lacewing larvae to Bt containing ECB, the risk to lacewing populations due to the toxicity of Bt corn should be minimal to nonexistent. The submitted study does not supply data that would be useful in changing the Agency's risk assessment at the present time. In addition, the alternative to the use of Bt corn (i.e. the use of conventional chemical pesticides) would pose a much greater hazard by practically eliminating the lacewing and other beneficial insect populations from the corn fields.

Therefore, the submitted data do not provide information that would alter the Agency's risk assessment of the effects to green lacewing populations or to beneficial insect communities from Event 176 derived Bt corn.

Adequacy of the Study:

1. Validation Category: Invalid

2. Rationale: This study was not performed according to EPA Guidelines, the negative control mortality is unacceptably high and there are major flaws in the study design.

[This study was not required, since the risk assessment based on Tier I maximum hazard dose nontarget beneficial insect testing data on file with the Agency showed no adverse effects to the green lacewing larvae.]