From: Hicks, Lebelle
Sent: Monday, December 03, 2007 1:34 PM
To: John Jemison (E-mail); 'Andrei. Alyokhin (E-mail)'; 'Eric Sideman'; 'James Dill'; 'Lauchlin Titus'; 'Tom Cote (E-mail 2)'
Cc: Jennings, Henry; Fish, Gary; Schlein, Paul B; Hicks, Lebelle
Subject: FW: Monsanto caddisfly technical review posted to monsanto.com

FYI

-----Original Message-----
From: MORIN, KIMBERLY A [AG/1000] [mailto:kimberly.a.morin@monsanto.com]
Sent: Monday, December 03, 2007 12:28 PM
To: Hicks, Lebelle
Cc: LAUCHLIN TITUS
Subject: Monsanto caddisfly technical review posted to monsanto.com

Hi Lebelle,

A Monsanto technical review of the caddisfly study was posted to our website if you are interested. Go to http://www.monsanto.com/products/techandsafety/safetysummaries.asp. The intro is listed under "Supplemental Information on MON 810."

Kimberly Morin
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MONSANTO

Technical & Safety Info

Supplemental Information for MON 810

On October 8, 2007 the results of a study published in the Proceedings of the National Academies of Science (PNAS) suggested the potential for Bt corn to have effects on aquatic ecosystems in corn producing regions.

Scientific and regulatory authorities have acknowledged the potential risks associated with genetic modification of all kinds, including traditional cross-breeding, biotechnology, chemical mutagenesis and seed radiation, and have established a safety assessment framework for biotech crops designed to identify any potential food, feed and environmental safety risks prior to commercial use. The insect-protected Bt corn event MON 810 was rigorously assessed for safety by authorities prior to commercial introduction in 1997, and has been extensively studied by independent scientific experts before and after commercial approval.

Monsanto takes the benefits, stewardship and safety of all of our products very seriously. We are confident in the safety and benefits of Bt corn for humans, animals and the environment. While this study did not establish any field-level effects of Bt proteins on aquatic insects, it has raised questions that our scientific experts have carefully considered. These experts have thoroughly reviewed the publication and we have elected to make this review public to improve understanding of the study and its findings.

Click here for complete technical review (pdf 97KB)

Technical Review
Monsanto Company

Toxins in transgenic crop byproducts may affect headwater stream ecosystems

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PNAS 104: 16204-16208, October 9, 2007

Background:

On October 9, researchers from several Midwestern universities reported findings that suggest potential harm to aquatic ecosystems in areas where pollen and other plant parts from *Bt* corn may wash into streams near cornfields. In a press release the researchers stated, *"The agency [EPA] did not evaluate the impacts of Bt corn on organisms that live in streams…"* consequently, their team undertook a comprehensive evaluation of organisms that live in streams draining agricultural lands. The authors stated, *"Overall, our study points to the potential for unintended and unexpected consequences from the widespread planting of genetically engineered crops."*

The authors reported that consumption of *Bacillus thuringiensis* (*Bt)* crystal protein (Cry1Ab) expressed in plant parts such as corn pollen, stalks and cobs increased mortality and reduced growth in caddisflies. These effects were observed in a laboratory setting. Caddisflies are in the insect order Trichoptera and are found in stream ecosystems. There are two feeding guilds of trichopterans: the scrapping (or algae-filtering) pollen feeder (*Helicopsyche borealis*) and the detritivorous leaf-shredder (*Lepidostoma liba*). They are related to lepidopteran insects such as European corn borers, which are the targeted pests of Cry1Ab protein expressed in *Bt* corn.

Reviewer's Findings:

- **The researchers reported no effects of *Bt* corn pollen on growth or mortality of caddisflies under natural conditions in a previous study**

Researchers that were co-authors of the PNAS paper presented relevant findings in June 2007 at the North American Benthological Society's annual meeting.

From the abstract: http://www.benthos.org/database/allnabstracts.cfm/db/Columbia2007abstracts/id/370)

"*To address this hypothesis, we developed flow-through chambers to measure individual growth rates in situ. [...]* ***The presence of Bt corn pollen did not influence growth or***

mortality. Results of this particular study do not indicate that Bt corn pollen poses a threat to filtering caddisflies. *However, caddisflies in our study were exposed to relatively low concentrations of pollen and further research is needed to assess potential adverse effects of Bt corn pollen on other stream-dwelling invertebrates."*

Given the importance of these results, it is surprising that this information was not included in the PNAS paper.

- **The artificially high levels of exposure used in the laboratory bioassay are not indicative of risk under natural conditions**

The effects on caddisflies from a laboratory experiment reported in the PNAS paper were the result of exposure levels estimated to be 2-3 times the maximum level of corn pollen observed in field conditions. Consequently, the "potential for unintended and unexpected consequences" to aquatic ecosystems claimed by the authors are not supported by their data.

- **The absence of mortality effects on the scraping caddisfly when fed non-*Bt* and *Bt* corn pollen at field concentrations suggests minimal risk under natural conditions**

It is puzzling why the researchers emphasize in the PNAS paper the potential for toxicity from *Bt* corn pollen since the laboratory pollen feeding study shows no significant differences in mortality between *Bt* and non-*Bt* corn pollen at exposure levels encountered in the field**.** In fact, this result is expected since pollen from the currently available *Bt* corn hybrids is virtually devoid of *Bt* toxins as compared to vegetative tissues. For example, MON 810 has a content of about 10 ng *Bt* protein per milligram of tissue, but its pollen has less than 0.09 ng/mg *Bt* protein (Mendelsohn et al., 2003). The perception that corn pollen has toxic levels of *Bt* protein likely is based on the controversial report by Losey et al. (1999). It was later determined that *Bt* pollen from the hybrid used by Losey et al. (1999) did *not* harm monarch butterflies. Their results were an artifact of the way that the pollen was harvested, which resulted in anther wall fragments being fed to the caterpillars (Hellmich et al., 2001), and it was the anther wall fragments, not the pollen, that were toxic to the caterpillars. Given the potential for contamination of pollen with other *Bt* corn tissues and no information on the method of pollen collection used by the researchers, it is not possible to determine if the reported effects of pollen were confounded with anther contamination.

- **The researchers reported that macroinvertebrate communities studied in two natural streams were not affected by *Bt* corn detritus under natural conditions**

From the abstract: (http://nabs.confex.com/nabs/2007/techprogram/P1698.HTM)

"Laboratory experiments showed that Lepidostoma, trichopterans that are related to the lepidopteran targets of Bt toxins, grew slower when fed Bt corn (instantaneous growth = 0.022 d^{-1}) compared to traditional corn (0.049 d^{-1}) (p=0.049). However, trichopteran biomass and total shredder biomass was similar in Bt and traditional corn litterbags, and there was no difference in decay rates of Bt and traditional corn litter. Macroinvertebrate communities were similar between the two stream types, and trichopterans were poorly represented in all streams (1% of total biomass). ***Results demonstrate that Bt corn detritus can slow growth of shredding caddisflies, but in situ it did not have significant***

adverse effects on invertebrates in these highly degraded streams.*"*

The findings reported above demonstrate that macroinvertebrate communities and caddisflies were unaffected by decaying plant residues from *Bt* corn. These data and findings were not included in PNAS paper. They provide direct evidence that *Bt* corn residues entering streams near cornfields do not pose a significant risk to caddisflies under natural conditions. Importantly, these findings do not support the researcher's statement in the PNAS paper, which concluded "*Bt* crop byproducts could represent an additional stressor to these systems, which has implications for stream restoration and riparian management in agricultural landscapes."

- **The researchers reported that *Bt* protein leaches out of corn leaves in water, thus reducing the actual exposure of caddisflies to *Bt* toxin**

From the abstract: (http://www.benthos.org/database/allnabstracts.cfm/db/Columbia2007abstracts/id/477)

*"**Within 1hr, 61% of the Bt δ-endotoxin leached from the leaves and then remained constant for the next 7hrs.**"*

The loss of *Bt* protein from *Bt* corn debris in streams is extremely relevant when considering the potential for effects on caddisflies. This information was not included in the PNAS paper. The loss of *Bt* protein from the system reinforces that it is essential to quantify the level of potential toxicants or nutritionally important factors in any study where the cause of the effect is unknown. Unfortunately, this information is not included in the PNAS paper.

- **The *Bt* protein content of the *Bt* corn residues fed to caddisflies was not given in the PNAS paper**

Quantification of the toxicant under evaluation is fundamental to any study examining the effects on living organisms. The researchers do not provide an analysis of the *Bt* protein content and assume that any differences between *Bt* and non-*Bt* corn results are due to the presence of the *Bt* protein. Unfortunately, without a quantitative analysis, it is not possible to differentiate potential effects from *Bt* protein toxicity and potential effects from differences in the nutritional and/or anti-nutritional value of the *Bt* corn residues.

- **The researchers reported evidence that *Bt* corn and non-*Bt* corn residues may not be equivalent for supporting optimal growth and development of caddisflies**

From the abstract: (http://www.benthos.org/database/allnabstracts.cfm/db/Columbia2007abstracts/id/477)

"While microbial respiration was higher on sediments in Bt streams compared to non-Bt streams before harvest (0.42mgO_2/gAFDM/h vs. 0.34mgO_2/gAFDM/h; p=0.02), respiration was lower in Bt streams after harvest (0.11mgO_2/gAFDM/h vs. 0.20mgO_2/gAFDM/h; p<0.001). These results suggest that leached Bt δ-endotoxin has the potential to reduce microbial respiration in streams."

The researchers found differences in microbial respiration on sediments from streams draining *Bt* corn field compared to streams draining non-*Bt* corn fields. Given that *Bt* corn hybrids are generally more intact and healthy at harvest than comparable non-*Bt* hybrids that have been damaged by insect pests, there is a reasonable likelihood that differences would exist in the nutritional and/or anti-nutritional value of the residues.

Instead of analyzing the composition of the corn residues to understand potential differences, the researchers conclude that the differences in microbial respiration are due to leaching of *Bt* toxin from *Bt* corn. This conclusion is not supported by the data provided in the PNAS paper.

- **There is no direct comparison of *Bt* corn hybrids to corresponding non-*Bt* isolines and no information is provided on the nutritional and/or anti-nutritional value of the detritus fed caddisflies in the PNAS study**

From the PNAS paper:

"However, Bt corn detritus has 33–97% higher lignin concentrations than respective non-Bt isolines (35), meaning that the presence or absence of Bt in a given isoline is confounded with nutritional quality. Therefore, we matched Bt and non-Bt detritus with similar lignin content and C/N to standardize nutritional value of the detritus."

Interestingly, the researchers acknowledge that nutritional quality is an important consideration but do not provide an informative analysis.

- **Use of an isogenic control and/or near isogenic comparators would allow the effects of the expressed *Bt* protein to be evaluated separately from the many other factors that can vary among corn varieties and affect insect growth**

The researchers chose to match samples of *Bt* and non-*Bt* corn detritus using similar values for lignin content and C/N for the laboratory growth and mortality experiments using caddisflies. While this approach attempts to make the *Bt* and non-*Bt* treatments comparable except for the presence of *Bt* protein, the approach fails to assure that other nutrients and anti-nutrients, including secondary metabolites and chemical treatments are also comparable. Differences in secondary metabolites, anti-nutrients, or chemical pest management treatments between the *Bt* and non-*Bt* corn hybrids could easily explain the observed differences in growth rate of caddisflies. In order to determine potential effects of *Bt* corn varieties on insects, a well-designed experiment requires both isogenic and near isogenic comparators, and control of chemical treatments to draw meaningful conclusions of potential effects.

In addition, the claim by the authors that *Bt* corn has higher lignin content than non-*Bt* corn is not valid. The report cited by the researchers used potted corn plants (Saxena and Stotzky, 2001), however the effects observed were not reproduced when studied in the field (Jung and Scheaffer, 2004). A multi-environment field study of six commercial Bt/non-*Bt* near-isogenic hybrid pairs did not detect consistent differences in lignin concentration of lower stem internodes or whole plants. Jung and Schaeffer (2004) concluded: *"Contrary to some earlier reports, presence of the cry1Ab transgene did not alter lignin concentration or other forage quality traits of maize stover in commercial maize hybrids."* Also, if the results from the pot studies were confirmed and *Bt* corn hybrids manifested "up to 97% difference in lignin content," such a change in composition and nutritional value would affect the feed performance of *Bt* corn compared to non-*Bt* corn and likely affect crop yield and plant degradation. Numerous studies have established that *Bt* corn is nutritionally equivalent to comparable non-*Bt* corn (CAST, 2006) and no disparities in yield or degradation have been documented in commercialized transgenic corn.

- **As a rule, any extrapolation from laboratory results to field results requires caution, particularly when key factors were not monitored, measured, or controlled appropriately.**

Although laboratory results are a useful starting point, before making statements or drawing conclusions it is essential to validate laboratory findings in the relevant ecological setting. More importantly, when studying ecological systems, it is critical that all potentially relevant factors be characterized and appropriately controlled. Unfortunately, in the PNAS paper the researchers have not provided important information needed to draw meaningful conclusions from their study. In addition to the concerns above, the following information also would help to assess the relevance of this work.

- **The researchers should have reported the specific commercial corn hybrids used for pollen and test tissue so that the *Bt* protein(s) present could have been identified**.

This paper mentions only Cry1Ab, yet there are many different types of *Bt* endotoxins (Crickmore et al., 1998), and different *Bt* toxins have been introduced into different commercial *Bt* corn hybrids (see registry at www.agbios.com). Importantly, the various commercialized *Bt* corn proteins differ in their specificity, and some target lepidopteran pests while others target coleopteran pests. While *Bt* proteins are usually specific to select orders of insects, they tend to have the highest activity against just one or a few families within an order (OECD, 2007). Knowing the specific *Bt* proteins involved would help to assess the likelihood that the effects on trichopterans are related to the presence of one or more *Bt* proteins.

- **The researchers should have reported the amount of corn leaves used in the laboratory experiment and established a dose-response for the alleged toxicant.**

The Materials and Methods states, *"Leaves were added to aquaria as needed."* This information does not allow the study to be reproduced and suggests that a dose-effect relationship was not established. The lack of a dose-response makes it difficult to draw meaningful conclusions from the study that would be relevant to natural conditions.

Conclusion

Appropriate studies on non-target insects can help ensure new products of biotechnology provide maximum benefit with a minimum of unintended detrimental effects on ecosystems. However, this study, while innovative and ambitious, has too many design and methods problems, which make it impossible to draw any useful conclusions from the data as presented. Furthermore, the omission of results previously presented by the authors in the same year at the North American Benthological Society is troublesome. Inclusion of these findings likely would have changed the experimental design and interpretation of the results, and potentially may have impacted the overall conclusions of the PNAS paper.

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