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Regulatory Analysis and Development, PPD
Animal and Plant Health Inspection Service (APHIS)
Station 3A- 03.8
4700 River Road Unit 118
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RE: **Comments on APHIS Docket No. APHIS-2007-0030**
Comments on APHIS's Draft Environmental Assessment for a determination of nonregulated status for insect-resistant corn derived from the MON 89034 transformation event

The Center for Food Safety appreciates the opportunity to comment on APHIS's draft environmental assessment of Monsanto's petition for determination of nonregulated status for MON 89034, corn genetically engineered for expression of two Bt insecticidal toxins, Cry1A.105 and Cry2Ab2.

The draft environmental assessment (EA) is deficient in a number of important respects. Relevant scientific evidence has not been considered. In particular, scientific studies published since the EA was drafted, and which contradict key assertions made by APHIS in the EA, have not been considered. These deficiencies, outlined below, should be redressed in the context of a full environmental impact statement prior to any decision on the regulatory status of MON 89034.

A. Insect resistance to Bt proteins in MON 89034

Agricultural scientists have long been concerned that widespread planting of transgenic insect-resistant (Bt) crops will foster rapid evolution of pest resistance to the insecticidal proteins expressed in these crops. While evolution of pest resistance to pesticides is a common phenomenon, the selection pressures exerted by Bt crops are significantly greater than those exerted by chemical insecticides, in at least three important respects: temporal, spatial, and few modes of action. First, Bt crops present susceptible insect pests with continual exposure to the insecticidal compound(s) throughout all or most of the growing season, whether the pests are present at economically damaging levels or not. Exposure is often constant over multiple

growing seasons as well, in those increasingly common situations where a Bt crop is grown year after year. This contrasts sharply with the lesser temporal exposure to chemical pesticides, which are used sporadically, as needed, to control economically damaging levels of pests. Secondly, the presence of Bt insectidal proteins in the more than 400 million acres of Bt corn and cotton grown since 1996 likely represents by far the most extensive exposure to any class of insectidal compounds in the history of agriculture. Finally, selection pressure is increased by the prevalence of a few insecticidal compounds with very few different modes of action in commercially deployed Bt crops, in comparison to the many different modes of action of chemical insectides. In short, Bt crops expose susceptible insect pests continuously, over vast expanses of land, to a very few insecticidal compounds, most of which have the same or similar mode of action. These three factors ensure enormously high selection pressure for development of pest resistance relative to chemical insecticides, "generating one of the largest selections for insect resistance ever known." (Tabashnik et al 2008).

MON 89034 is one of a growing number of commercial transgenic plants that incorporate multiple insecticidal proteins rather than just one. The "stacking" or "pyramiding" of multiple insecticides in a single plant has been presented as one means for slowing development of pest resistance to any one of these proteins. Other methods include engineering the plant for high expression levels of the insecticidal protein(s) (i.e. EPA's "high-dose" strategy), and the requirement that growers of transgenic insect-resistant (Bt) crops also plant refugia of non-Bt crops in their fields to maintain populations of susceptible insects that can then mate with those that develop resistance to "dilute" resistance genes. This strategy is most effective when the resistance mechanism is controlled by a recessive allele, and is much less effective when resistance is dominant.

APHIS states that: "There have been no documented instances of confirmed insect resistance in natural populations of target insects to Bt corn or the Cry toxins they produce..." (EA, p. 20). APHIS subsequently broadens the claim to cover all Bt crops: "Because there have been no confirmed instances of pest resistance to Bt crops currently planted, there has been no need to implement mitigation measures, and their success has not been evaluated" (EA, p. 31). However, a recently published paper that documents the first cases of pest resistance to a Bt crop (Tabashnik et al 2008) presents APHIS with a new situation. The mitigation measures that APHIS recommends as potential responses to the discovery of resistance as well as other measures (discussed further below) need to be addressed in the context of an environmental impact statement on MON 89034, now that resistance has been confirmed.

In an analysis of published monitoring data covering the years 2003 to 2006, Tabashnik et al (2008) documented field-evolved resistance of multiple populations of *Helicoverpa zea* (*H. zea*) to the Cry1Ac protein expressed in Bt cotton in Arkansas and Mississippi. Six strains of *H. zea* with resistance to more than 50 times the concentration of Cry1Ac that kills susceptible strains of the insect were found in 2003 and 2004, including four strains with resistance ratios > 100, and two strains with resistance ratios > 500. Additional strains identified in 2005 and 2006 exhibited even higher resistance levels: seven strains had resistance ratios > 100, including two strains with resistance ratios > 1,000.

While these findings of resistance involve a cotton pest, they raise serious questions about development of pest resistance with the introduction of MON 89034. Consider the following facts:

1) *H. zea* is considered one of the most economically damaging agricultural pests in the United States (University of Florida 2007).

2) *H. zea* is known to infest not only cotton, but also corn, where it is known as corn earworm. And it is highly mobile, making it possible that these pests will be exposed to both Bt cotton and Bt corn, including MON 89034.

3) *H. zea* infests an extremely broad range of crops, making development of resistance to Cry1Ac, a key insecticidal protein in many Bt microbial sprays used by organic farmers, of great concern. *H. zea* is known to infest a number of field crops, including alfalfa, clover, cotton, flax, oat, millet, rice, sorghum, soybean, sugarcane, sunflower, tobacco, vetch, and wheat, and is particularly a problem on sorghum, where it is known as "sorghum headworm." *H. zea* also attacks many vegetable crops, including artichoke, asparagus, cabbage, cantaloupe, collard, cowpea, cucumber, eggplant, lettuce, lima bean, melon, okra, pea, pepper, potato, pumpkin, snap bean, spinach, squash, sweet potato, tomatoes and watermelon. Tomatoes are particularly favored, where the pest is known as "tomato fruitworm." *H. zea* also attacks fruit and ornamental plants, including ripening avocado, grape, peaches, pear, plum, raspberry, strawberry, carnation, geranium, gladiolus, nasturtium, rose, snapdragon, and zinnia (University of Florida 2007).

4) One of the two insecticidal proteins expressed in MON 89034 is a synthetic Bt protein (Cry1A.105) based in large part on the structural features of Cry1Ac (Petition, p. 99). Hence *H. zea* strains resistant to Cry1Ac may be cross-resistant to the Cry1A.105 insecticide expressed in MON 89034. Even if this is not the case, the demonstrated propensity of *H. zea* to develop resistance to Cry1Ac suggests a higher likelihood of independent development of pest resistance to the highly similar Cry1A.105 in corn. We note also that Cry1Ab, which is the major toxin in most Bt corn (MON810 and Bt11), is highly similar to the Cry1Ac to which resistance has developed in *H. zea*, that cross-resistance to the two proteins has been documented in diamondback moths (Sayyed & Wright 2001), and may be occurring in other insect pests.

5) Besides possessing similar Cry1A proteins, MON 89034 and Bollgard II cotton possess very similar (perhaps identical) Cry2Ab2 proteins. While the structural dissimilarity of Cry1Ac and Cry2Ab2 in Bollgard II cotton and some field studies suggest lack of cross-resistance in insect pests, the EPA notes that: "Work with TBW [tobacco bollworm] and CBW [*H. zea*] resistant (to Cry1Ac) colonies indicates that ***there is some low potential for cross-resistance and that there are likely to be a range of Bt resistance mechanisms. Previously, published research indicates that there is evidence for broad cross-resistance (low levels of resistance) to Cry1A and Cry2A in laboratory-selected strains of beet armyworm (Moar et al. 1995) and TBW (Gould et al. 1992)*** (emphasis added, EPA BRAD Cry2Ab2, p. 29). This suggests the potential for pests to be cross-resistant to the highly similar pair of insecticidal compounds in MON 89034, a subject that deserves more careful scrutiny given the new findings of Tabashnik et al (2008).

6) Recent modeling research has led experts in Bt pest resistance to predict that "Cry2A resistance evolution is maximized when single-protein varieties expressing Cry1A and two-protein varieties expressing Cry1A and Cry2A were both available." (EPA BRAD Cry2Ab2, p. 30, citing unpublished work by Livingstone et al 2002). Thus, introduction of MON 89034 into a landscape dominated by Cry1Ab corn varieties like MON 810 and Bt11 may maximize the rate of Cry2A resistance evolution in insect pests. "If market forces result in a complicated mix of one- and two-gene Bt plants, the impact of the pyramided Bt plants on slowing resistance evoultion could be undermined." (Zhao et al 2005, citing Gould 2003). This is an important consideration that APHIS needs to address in the context of an EIS on MON 89034.

7) Recent experimental work with Bt insecticide-resistant diamondback moths (*Plutella xylostella*) involving exposure to various combinations of one- and two-gene Bt plants supports these modeling studies (Zhao et al 2005). Though the authors of this study conceded that Bt trait stacking may offer benefits with respect to insect resistance management, they concluded that:

> *"Because* ***the concurrent use of single- and two-gene Bt plants can offer exposed populations a ''stepping stone'' to develop resistance to both toxins****, it is important that regulatory decisions regarding the registration of plants with pyramided genes also consider the registration or de-registration of single-gene Bt plants.* ***From a resistance management perspective, pyramided Bt plants should not be deployed simultaneously with single gene plants if they share similar Bt toxins.***
>
> *Our results indicate that the introduction of pyramided plants with currently deployed single-gene plants could enhance resistance evolution to the otherwise more durable pyramided plants if they are used simultaneously in the same area.* ***Our previous data and this study suggest that it would be advantageous from a resistance management standpoint for regulatory agencies to consider de-regulating single gene plants as soon as pyramided plants are available****."(emphasis added)*

APHIS cites five potential mitigations measures that should be considered when pest resistance to an insecticidal protein is discovered (EA, p. 32). These include: 1) Informing farmers and extension agents in the affected areas of resistance; 2) Increased monitoring; 3) Implementing alternative means to control pests in the affected areas; 4) Implementing a structured refuge; and 5) Halting Bt seed sales in the affected and bordering counties until an effective local management plan has been implemented. The draft EA did not address any of these options, which must be thoroughly addressed in the context of an EIS now that pest resistance to Cry1Ac has been confirmed over a 4-year period in *H. zea*. APHIS should also address the other options suggested in the studies cited above (e.g. barring concurrent use of stacked and single-protein Bt crops that share a similar toxin) in the context of the EIS.

Organic growers have long been concerned that the massive adoption of Bt crops will speed development of pest resistance, leading to loss of efficacy of the Bt microbial pesticides upon which many rely for insect control. The Bt proteins expressed in MON 89034 are derived from Cry proteins commonly found in microbial pesticides. For instance, Cry2Ab2 is derived from *Bt kurstaki*, an active ingredient in many commercial microbial sprays, including Dipel, which contains Cry2Aa, and Cutlass, which contains Cry2Ab (Cry2Aa and Cry2Ab share 88%

sequence homology) (Petition, p. 102). *Bt kurstaki* is also known to contain Cry1Ab and Cry1Ac.

APHIS should address the potential loss in efficacy of Bt microbial pesticides that may occur as a near- or longer-term consequence of the introduction of MON 89034, in combination with other currently planted Bt crops. This should include an assessment of the value of Bt microbial pesticides to organic and other growers of various crops, and various scenarios involving differing degrees of loss of efficacy.

Another strategy to slow development of pest resistance to insecticides incorporated into Bt crops is the "high-dose" strategy. The idea behind this strategy is to generate high enough levels of the Bt toxin (25- to 50-fold higher than that required to kill susceptible pests) to kill even those rare individuals that exhibit enhanced resistance. Numerous scientific studies, however, show widely varying Bt toxin concentrations in different Bt crops, different tissues of the same Bt crop, and at different growing stages. Additional factors that may influence Bt protein expression include genetic background of the particular variety into which the Bt protein is incorporated, and environmental factors such as soil quality or abiotic stressors. Collectively, these studies raise serious questions about the viability of the high-dose strategy as a means to slow development of insect resistance. In particular, they demand fuller collection of data on Bt protein expression levels under a wider range of conditions than is presented by Monsanto in its petition for deregulation of MON 89034.

A recent published study on Cry1Ab expression in leaves of Novelis corn derived from MON810 reveals Cry1Ab levels that differ substantially from those reported by Monsanto for MON810 to the EPA and European authorities in the mid-1990s. While the mean Cry1Ab levels reported by Monsanto in four field trials in the US and Germany ranged from 8.95 to 12.15 mcg/g fresh weight, Nguyen & Jehle (2007) report mean levels ranging from 2.4-6.4 mcg/g fresh tissue, several-fold less. A study commissioned by Greenpeace found still lower levels of Cry1Ab leaf expression in MON810 hybrids grown in Germany: mean levels of 0.5 to 2.2 mcg Cry1Ab/g fresh weight, roughly an order of magnitude lower than Monsanto's figures (Greenpeace 2007). Significantly, the Greenpeace results show a substantial number of plants with leaves containing < 0.1 mcg Cry1Ab/g fresh weight, and a lesser but surprisingly high number of leaves with no detectable Cry1Ab expression at all. This two order of magnitude variation in Bt protein expression in the same tissue of Bt hybrids derived from the same transformation event has obvious implications for the viability of the high-dose strategy.

Additional studies that document the high variability in Bt protein levels that APHIS should consult include Olsen et al (2005), who found that Cry1Ac toxin levels and bioefficacy decline significantly as cotton plants mature; Wan et al (2005), who found that the toxin content in Bt cotton changed significantly over time, and that the structure, growth stage, and variety were significant sources of variability; and Abel & Adamczyk (2004), who found that Cry1A expression in corn leaves and cotton tissues varied with chlorophyll content of the tested tissue.

Bt protein expression variability in MON 89034 requires careful assessment in the context of an EIS as it has important implications for the "high-dose" strategy to slow development of insect resistance to a vital class of biopesticides.

A third strategy to slow resistance development to Bt proteins expressed in Bt crops is the planting of refugia. APHIS notes that Monsanto has requested a reduction in the size of non-Bt crop refugia required for planting of MON 89034 (EA, p. 16). We note that APHIS's brief discussion of Monsanto's request for reduced refugia is predicated on an assumption that refuges have been effective in preventing development of insect resistance to Bt. Given the confirmed development of resistance in CEW (*H. zea*), a major corn and cotton pest, to a Bt protein and the other insect resistance issues discussed above (increased speed of resistance evolution when a mosaic of two-gene and one-gene Bt crops are planted concurrently, variability of expression levels of Bt proteins potentially undermining the high-dose strategy), APHIS should assess the refuge issue (in consultation with EPA) in the context of an EIS before any decision is made on deregulation of MON 89034.

B. Non-target organism impacts

A recent study (Rosi-Marshall et al 2007) found that Cry1Ab-containing Bt corn residues as well as Bt corn pollen may pose threats to certain aquatic insects, such as caddisflies. Caddisflies are extremely important to aquatic food webs. Neither the petition nor the EA contain any reference to tests for potential adverse impacts of Cry1A.105 or Cry2Ab2 (individually or combined) on caddisflies or any other aquatic insect. The only testing cited by Monsanto in this area involved exposure of the aquatic invertebrate *Daphia magna* to pollen from MON 89034 (Petition, p. 155). In view of this study, APHIS (in consultation with EPA) should demand testing to establish whether the Bt toxins expressed in MON 89034 have adverse effects on a full range of freshwater aquatic insects, including caddisflies, and assess them in the context of an EIS. It is unacceptable to assume lack of NGO effect based on mostly theoretical speculations concerning the presumed specificity of Cry proteins to target pests (EA, p. 12). One study disputing this assumption demonstrated that the Cry1Ac protein can bind to surface proteins in the small intestine of the mouse (Vazquez et al 2000). Another factor that undermines facile assumptions about the specificity of Bt proteins is our still-evolving knowledge of the mechanism of action of Bt proteins; for instance, Broderick et al (2006) demonstrate that septicemia induced by midgut bacteria is essential to the killing action of Bt toxins, which was previously assumed to be due to starvation from Bt toxin-puncture of the midgut alone. Non-target organism impact testing should avoid the gross deficiencies uncovered in SAP (2006), and fully meet the standards for non-target organism testing set by those experts.

C. Other issues

APHIS fails to list several studies cited in the text of its EA in the bibliography, hampering public review of APHIS's assessment. These include Shelton et al (2002), Clark et al (2006), Vercesi et al (2006), Zhao et al (2005), Mehlo et al (2005), Langrock et al (2003), Lee et al (2003), Cao-Guo et al (1997), Yu et al (1997).

Respectfully submitted,

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