

May 3, 2001

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Information Resources and Services Division (7502C)  
Office of Pesticide Programs  
Environmental Protection Agency  
1200 Pennsylvania Avenue, NW  
Washington, D.C. 20460

**COMMENTS SUBMITTED TO DOCKET NUMBER OPP-30509:  
MONSANTO'S ONE-YEAR SEED-PRODUCTION REGISTRATION  
APPLICATION FOR CRY3Bb TRANSGENIC CORN MODIFIED TO  
CONTROL CORN ROOTWORMS**

These comments, prepared by Dr. Charles Benbrook, are submitted on behalf of the Union of Concerned Scientists<sup>1</sup> to the Environmental Protection Agency (EPA) in response to a March 19, 2001, *Federal Register* notice announcing receipt of an application from Monsanto for a one-year, contained, 22,875 acre pre-commercial seed-increase registration of corn genetically engineered to express the *Bacillus thuringiensis* Cry3Bb proteins for control of corn rootworms. A seed-production registration is a prelude to a request for full commercialization. The EPA docket, 30509, established for this seed-production registration application contains Monsanto's June 20, 2000, request for full registration and an amendment of a previous request for an exemption from the requirement of a tolerance for this plant pesticide protein (PP 7F4888: EPA File Symbol 524-LEI).

The comments that follow are based primarily on the June 2000 submission and apply both to Monsanto's one-year seed-production registration request (docket OPP-30509) as well as the company's request for full registration.

This application seeks registration of Cry3Bb protein for "all corn lines and varieties," including field corn, sweet corn and popcorn. The later two crops are grown for direct human consumption. Because of the much higher expression levels of this protein in edible grain tissues relative to earlier *Bt*-corn events, the EPA must proceed more cautiously and thoroughly in its scientific assessment of the human allergenicity and food safety of this technology, given that people will be exposed to much greater quantities of Cry proteins than arising from any previously reviewed/approved *Bt* corn technology.

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<sup>1</sup> The Union of Concerned Scientists is a nonprofit, public-interest group advocating the transition to a sustainable food system.

In these comments, we first highlight in Part A new risk issues and uncertainties evident in or raised by the data in Monsanto's multi-volume June 2000 submission. Part B assesses the potential benefits and sustainability of Cry 3Bb transformed corn. We challenge Monsanto's claims regarding reductions in pesticide use, highlight promising alternative pesticide-based and IPM alternatives, and address resistance issues. Part C discusses in more detail ecological and human food safety risks.

***UCS urges EPA to turn down Monsanto's request for a one-year seed-production registration and not act on any applications for full registration of Cry3Bb corn until Monsanto has submitted the data needed to address the ecological and human health risks and the resistance-management concerns posed by the new product.***

## **A. Monsanto's Submission Raises Major New Concerns**

This new submission contains more data and discussion of the MON 863 event, the transgene used to develop it, and its impacts on hybrid corn lines than the seriously flawed initial application from Monsanto submitted August 19, 1999 (MRID # 44904300-19). Some of the major deficiencies in the August 1999, submission were highlighted in March 20, 2000, comments submitted to EPA by UCS (comments available at [http://www.ucsusa.org/food/epa\\_mar00.html](http://www.ucsusa.org/food/epa_mar00.html)) and other public interest organizations (accessible at <http://www.biotech-info.net/rootworm.html#EPA>).

The August 1999 application sought approval for any Cry 3Bb transformation event, in effect a blank check to introduce the Cry 3Bb gene into corn in any way that emerges as possible. The 1999 application presented data on several transformation events but did not specify the event that would be commercialized. The submission presented arguments "bridging" health and safety data covering previously approved Cry 1Ab and Cry 1Ac *Bt*-corn events to all and any Cry 3Bb corn event. The submission also explained that the approval of Cry 1Ab and Cry 1Ac events had been based largely on the long history of past, safe use of foliar *Bt* insecticides.

It is important to stress that, while this submission is an improvement over the 1999 application, Monsanto still has not submitted sufficient scientific information in the June 2000 submission to support a full assessment of the health and environmental impacts of MON 863 corn. The majority of the submission focuses on –

- The molecular characterization, stability, and expression levels of the MON 863 event.
- The equivalency of the MON 863 event to other Cry 3Bb events tested previously, which were the subject of the August 1999 registration application – i.e., the case for incorporating data from past submissions/events in determining the safety of MON 863 corn.

- The environmental fate of the proteins expressed by MON 863 proteins in soil.
- A recommended resistance management plan.

The submission includes no new data on allergenicity or human health effects and only sparse new data on impacts on a small set of nontarget organisms.

Given that the U.S. corn industry has been struggling with StarLink issues for nearly a year with no end in sight, and that MON 863 corn expresses levels of Cry 3Bb proteins in grain orders of magnitude higher than most earlier *Bt*-transgenic corn events (see Table 1), it is surprising that Monsanto has asked EPA to approve this registration largely on the basis of equivalency and incorporating data from earlier approved Cry 1Ab and Cry 1Ac varieties, which in turn were approved largely on the basis of highly questionable equivalency and data from foliar *Bt* insecticides in use since the 1960s.

### **1. Expression Levels Are Higher than Other *Bt*-Corn Events**

The application refers to the expression levels of the Cry 3Bb protein as “moderate to low.” (MRID # 451845-01, page 5). But from the perspective of likely environmental impacts and potential food safety risks, the expression levels are very high.

Table 1 presents an overview of corn plant tissue expression levels in MON 863 Cry 3Bb corn in contrast to previously approved *Bt* corn events. One volume in the Monsanto submission reports Cry 3Bb and NPTII (antibiotic-resistance marker gene) expression levels in various tissues of transformed corn plants (MRID # 451568-02). With the exception of whole plant levels in Cry 9c (StarLink) corn, Cry 3Bb expression levels are higher in every category for every other event previously reviewed and registered. (In Table 1, some expression levels in leaf tissues, roots, and whole plants for the Cry 1 and Cry 9 events may represent levels at a different growth stages, and hence somewhat skew the comparisons to Cry 3Bb levels.)

In leaf tissue, so critical in terms of nontarget impacts, the Cry 3Bb level is 10 to 40 times higher than other currently registered *Bt* events. In roots, Cry 3Bb levels are over 500 times higher than Syngenta’s Event 176 and 328 times higher than the Cry 1Ac event. The level is twice as high as in MON 810 corn and 1.6 times the level in StarLink.

In grain, the level is at least 50 times higher than all other events, except for StarLink (3.8 fold). Cry 3Bb protein levels in corn grain kernels are 233 times higher than the MON 810 Cry 1Ab event, the most common in *Bt* corn planted by U.S. farmers in recent years.

These data show clearly that Cry 3Bb expression levels are extraordinarily high relative to previously approved *Bt*-corn events. In addition, relatively high levels exist in

plant leaf tissue, pollen and grain, where they are not needed and can do harm to nontarget organisms. High levels in these tissues are useless because the corn rootworm is only susceptible to the Cry 3Bb endotoxin in its larval stage, as it feeds in the soil on plant roots. Adults are essentially not susceptible and hence any feeding later in the season will have no appreciable impact on pest levels.

The Cry3Bb expression level is characterized by Monsanto as “low to moderate” relative to the ability of Cry3Bb endotoxins, at the levels expressed in plant roots, to control corn rootworm larvae, the principal target pest at its most vulnerable stage. It is not that the Cry3Bb expression level is “low,” but rather it takes a lot of Cry3Bb proteins to effectively manage corn rootworm larvae.

## **2. No Data Are Presented on Root Exudates**

Nowhere in the submission does Monsanto present information on the levels of Cry 3Bb endotoxin exuded through root systems and into the soil. The lack of such information is curious, given that root exudates would presumably enhance efficacy and surely have been measured by Monsanto.

EPA should insist that data on root exudates is submitted, along with properly conducted soil assays to determine the persistence of Cry 3 Bb proteins in the soil. Information on the total flow of Cry 3Bb and NPTII proteins into the rhizosphere via roots and root exudates, and the environmental fate of these proteins, are essential to determine the possible impact on soil microbial communities.

## **3. MON 863 Incorporates an Antibiotic-Resistance Marker**

The MON 863 event was created using gene gun technology with a 4,691 base pair ZMIR13L construct. This construct incorporates the NPTII neomycin phosphotransferase II marker gene directly adjacent to the cauliflower mosaic virus 35S promoter. Many questions have been raised about the wisdom of using antibiotic-resistance marker genes in the development of GMO crop cultivars. There is a global consensus that use of such selectable marker genes may be risky, are no longer necessary, and hence should be phased out.

While concerns have been raised before regarding human dietary exposure to NPTII antibiotic marker gene proteins in GMO foods, data in the public arena suggest that the levels of NPTII expressed in edible portions of previously approved *Bt*-corn plants are very low. The Monsanto submission reports higher expression levels of NPTII proteins than earlier *Bt*-corn events.

## **4. Missing DNA Warrants Further Study**

In the study entitled “Molecular Analysis of Corn Event MON 863” (MRID #

451568-01), Cavato et al. describe the techniques used to confirm that just one copy of the transgene cassette was expressed and that expression is stable in subsequent generations of plants bred from the MON 863 line.

The ZMIR13L vector used in engineering MON 863 corn contains two major sections. The first contains the Cauliflower mosaic 35S promoter and the NPTII selectable marker gene, flanked by the NOS 3' regulatory nopaline synthase gene of *Agrobacterium tumefaciens* T-DNA. The purpose of this regulatory gene is to end transcription and direct polyadenylation. Molecular analysis of the MON 863 event suggests that just one copy of this sequence has been expressed and that expression is stable.

The second section within the ZMIR13L plasmid contains the Cry3Bb1.11098 gene. It is flanked to the left by –

- An activating sequence (4-AS1);
- “wt CAB,” a 5' untranslated leader of the wheat chlorophyll a/b protein; and
- An intron from the rice actin gene.

To the right, the Cry3Bb1.11098 gene is flanked by “tahsp17,” which is described in the submission as a “3' nontranslated region on the coding sequence for wheat heat shock protein 17.3 which ends transcription and directs polyadenylation.” This second regulatory sequence is coupled to a *HindIII* restriction endonuclease “that cuts DNA at specific locations,” as well as a *MluI* restriction endonuclease, also incorporated to assist in the proper placement and truncation of the transgene within the corn genome.

The Cavato et al. report states that –

“Genomic flanking sequence indicates that while the *HindIII* site at the 3' end of PV-ZMIR13L is missing, the entire tahsp 17 3' polyadenylation sequence is present in event MON 863.”

The “missing” DNA is approximately 10 base pairs and includes the *HindIII* site and *MluI* half site. The authors stress that despite the missing DNA, the Cry3Bb gene and its regulatory sequence are properly and stably expressed in the MON 863 event. The authors do not explain, however, what may have happened to the 10-base pair segment of DNA containing the two restriction endonucleases. Presumably, Monsanto explored whether this missing DNA lodged somewhere else in the transformed corn lines. We would expect that the company has developed probes capable of detecting these restriction endonucleases. The lack of further explanation of what they found in their search for the missing DNA raises important questions.

Possible implications have been suggested in research by Nielsen and colleagues.

Their work focused on the impacts of transgenic sugar beets on bacterial gene flow in the soil. The transgene used to develop the modified sugar beets included several of the same promoter and marker genes as the Cry 3Bb transgene. They found in their work that the “presence in transgenic plants of various prokaryotic markers and vector sequences may facilitate additive insertion of foreign genetic material into bacterial hosts after homology-based heteroduplex formation” (Nielsen et al., 2000).

The restriction enzymes missing in Cry 3Bb transformed corn lines could trigger unexpected and possibly detrimental pleiotropic effects, depending on whether and where they are lodged in the genome of various corn hybrids including the MON 863 transgene. Monsanto must develop a mechanism to reliably determine the fate of this missing DNA and make it widely and freely available. Lacking such a probe and ongoing scrutiny of all the hybrids into which the MON 863 event is placed, the U.S. corn industry will be vulnerable to a loss in consumer confidence and export sales if, a few years down the road, it is discovered that this missing DNA had, in certain varieties under certain circumstances, been expressed in a way leading to detectable changes in corn grain or forage composition or product performance.

### **5. High Toxin Levels May Jeopardize Margins of Safety for Soil Organisms**

Monsanto reports that the maximum environmental concentration expected in the soil is 13.3 mg/kg, based on the assumption that corn plants are tilled into the top six inches of soil when leaf tissue expression levels are peaking. Yet exposure levels are likely to be much higher for organisms that move through the soil and seek out the nutrient enriched zone within a few centimeters of roots. Moreover, plant residues and stalks worked into the soil will create an uneven distribution of Cry 3Bb in the soil profile; organisms that happen to be feeding in the vicinity of decomposing plant tissues will be exposed to relatively higher levels.

Cry 3Bb endotoxins are lethal to earthworms at 57 mg/kg in the soil, resulting in just a 4-fold difference between the known high-level concentrations in soil. Such a narrow margin of safety is not acceptable in any other regulatory context. While under “normal” conditions, a 4-fold margin of safety may spare most earthworms in fields planted to corn varieties expressing the Cry 3Bb endotoxin, many factors can enhance the vulnerability of earthworms – or other nontarget organisms – to the effects of any toxin.

Earthworms moving through “hot spots” could be exposed to lethal levels. Exposure to other insecticides or herbicides or drought stress also can make earthworms more vulnerable than normal. Tillage systems, irrigation, or soil conditions might alter their movement and feeding habits, increasing or decreasing their exposures levels.

## **6. Efficacy Data Are Skewed; Key Question Is Raised**

The submission includes a summary of efficacy trials carried out in seven locations (Volume 1). Plants were mechanically infested at the V2 growth stage with 1,200 to 1,600 eggs per square foot. Before the plants were dug up to inspect roots for damage, ELISA kits were used in the field to identify any plants not expressing the Cry 3Bb endotoxin. According to the submission, “negative plants were removed” (Volume 1, page 56).

Removing plants not expressing the Cry 3Bb endotoxin in an efficacy trial of MON 863 engineered corn would seem to undermine the basic purpose of the study. At a minimum, Monsanto should have reported the number of plants not expressing the Cry 3Bb protein, as well as the percent of plants not expressing the endotoxin. If the percent of plants not expressing the Cry 3Bb endotoxin is in fact above the normal rate at which corn hybrids fail to express a specified trait, Monsanto should have offered some explanation of why, since non-expression will have significant implications for product performance and in the design of resistance management plans.

## **7. Data on Risks of Stacked *Bt* and Roundup Ready Genes Are Lacking**

The resistance management plan document (MRID 451845-01) states that the Cry3Bb protein “...will be released both as a stand-alone product and in combination with the Cry 1Ab gene for lepidopteran control because of the dual needs of many farmers. “

Monsanto’s intentions are unclear regarding future combinations of the Cry 3Bb gene with the Cry 1Ab, Cry 1Ac (DeKalb-Monsanto event), or other Cry proteins for European corn borer (ECB) control. Dual-*Bt*-transgenic hybrid varieties could be produced from parental corn lines, one containing the Cry 3Bb gene, the second MON 810 Cry 1Ab genes. This would be a logical way to proceed, since it would simplify the backcrossing process.

Alternatively, a single event could be engineered to express both Cry 3Bb and Cry 1Ab or Cry 1Ac endotoxins. When crossed into commercial hybrids both endotoxins would presumably be expressed. A method to engineer two *Bt* endotoxins into cotton plants has been the subject of a Monsanto-Aventis patent dispute, which was recently settled as part of a broader set of IPR agreements between the two companies (Agrow, 2001). It is possible that Monsanto will use this technology, or adapt it in creating stacked gene *Bt*-corn varieties expressing Cry 3Bb and Cry 1Ab or Cry 1Ac endotoxins.

These alternative strategies would have profoundly different regulatory consequences. The former approach, crossing two parental lines, each with a single Cry gene, might not be subject to regulatory review, since no new genetic transformation event would be used. At this time, EPA plays at most a very minimal role in the review

of plant varieties made through the crossbreeding of two parental lines, one or both of which are genetically engineered.

The second alternative – a single event expressing two Cry proteins -- would probably require the development and review of a complete new data package. Monsanto would have to generate new data on expression levels, environmental fate in the soil, nontarget impacts, and resistance management. The fact that the former strategy would receive little if any regulatory scrutiny while the later would trigger a thorough evaluation is an artifact of regulatory laws and policy significantly out of step with the practical applications of the tools of biotechnology as applied to plant varietal improvement.

Regardless of how dual-*Bt* corn hybrids are developed, the stacking of two *Bt* genes in the same corn lines will raise many critical risk assessment science questions relative to impacts on expression levels, gene stability, food safety, efficacy, resistance management and environmental impacts. Clearly, EPA needs to clarify the additional data and regulatory review processes that will be followed if and when such varieties are developed, regardless of how they are developed.

In addition, we fully expect that Monsanto also intends to incorporate the Cry 3Bb gene in corn varieties engineered to be Roundup Ready via modification of the EPSPS gene, a critical regulatory protein within the shikimate pathway of all plants. The aromatic amino acids are synthesized within this pathway and play multiple, critical roles in plant growth and development and plant defense responses to pest attack and abiotic stress.

Recent evidence has emerged that in some Roundup Ready plants, changes in the levels of expression of phenylalanine (Padgett et al., 1995), or other aromatic amino acids or their secondary products (Sidhu et al., 2000), may be impairing plant defense mechanisms and retarding or weakening the systemic acquired resistance (SAR) response of soybean and corn plants (see Benbrook, 2001).

In corn varieties with stacked *Bt* and Roundup Ready genes, it is probable that different combinations of pest and abiotic stresses will trigger a wide array of unusual protein expression patterns. Some of these combinations will surely impact product performance and the efficacy of resistance management plans, while others may pose allergenicity or other food safety risks.

For this reason, EPA must make it clear in response to this and other applications that additional data must be submitted on each proposed stacked gene product sufficient to settle uncertainty regarding the unique risks posed when a given variety is genetically engineered in two or more ways, especially when the modifications target different physiological processes and pathways that are known to interact, as the case with *Bt* and Roundup Ready corn varieties.

## **B. Potential Benefits and Sustainability of Cry3Bb Corn**

A critical change has occurred in corn insect population dynamics as a result of a behavioral adaptation of the Western corn rootworm (WCR). Rotating corn with soybeans has been recommended for decades and widely practiced, and is one of the great success stories in IPM. On continuous corn acres in the 1990s, farmers apply corn rootworm insecticides on 90 percent or more of acres planted, while less than 15 percent of rotated corn has been treated.

The reliability of the corn-soybean rotation in limiting WCR pressure set the stage for trouble. Both farmers and scientists were lulled into thinking that no other steps were needed in managing this pest. Evolution is a powerful and patient force. In the mid-1980s University of Illinois corn IPM specialist Dr. Michael Gray documented the first signs of trouble -- isolated but economically significant Western corn rootworm damage in first-year corn following soybeans.

Over the next 10 years the pattern of WCR infestation was uneven and sporadic. Some seasons, like the wet crop year in 1998, did not favor WCRs and populations never reached damaging levels. But in recent years it has become clear that the Western corn rootworm has developed *behavioral resistance* to a management-based control strategy, in this case crop rotation. This change in rootworm behavior has led to an increase in insecticide use since 1996 in parts of the Midwest. It has also heightened grower interest in Integrated Pest Management (IPM) strategies, including area-wide population suppression strategies being developed by a multi-state USDA-funded team, as well as non-engineered varieties bred to resist corn rootworm feeding damage.

### **Corn Rootworms Pose Tough Management Challenges**

Only a few insects are resistant to more insecticides than the corn rootworm (CRW). Populations are resistant to chemicals in all major classes of insecticides. The Monsanto document setting forth the Cry 3Bb resistance management plan provides a brief summary of the historical evolution of resistance.

Organochlorine (DDT) use started in the late 1940s and resistance was first documented in Nebraska in the late 1950s. Aldrin and dieldrin use began in 1952 and heptachlor use in 1954. Resistant populations to these cyclodiene insecticides emerged about 10 years later and were 100 to 1,000-fold less sensitive than susceptible populations.

Organophosphate and carbamate insecticides were introduced in the 1960s and pockets of resistance began to emerge in the 1970s. Synthetic pyrethroids were introduced in the 1980s and provided farmers another option to rotate families of chemistry, a common practice that has slowed the evolution of resistance.

The challenge in managing resistance to Cry3Bb corn varieties will be significant. According to Dr. Gray –

“I suggest that the potential for resistance development by corn rootworms is much more acute than for European corn borer, *Ostrinia nubilalis* (Hubner)... Even with [resistance management] strategies in place, in my opinion, resistance will develop eventually.” (Gray, 2000).

According to Gray, “Corn rootworms have shown repeatedly that they are superbly capable of adapting to a variety of insecticides and even to a cultural practice. Any notion that they will not develop resistance to transgenic insecticidal cultivars at some point is foolhardy” (Gray, 2000).

Corn rootworm adults are highly mobile. During its adult stage, the Southern corn rootworm is also known as the spotted cucumber beetle, a common vegetable pest that can thrive on a wider variety of crops than Northern and Western corn rootworms. The Monsanto application cites data showing that the Western corn rootworm can survive on 21 species and the Northern corn rootworm on 14 species.

## **1. Impacts on Insecticide Use**

Citing USDA pesticide use data, the June 2000 Monsanto application states that 32 percent of corn acres were treated in 1998 with an insecticide registered for corn rootworm control. While narrowly true, this statement is misleading since some insecticides registered for both ECB and CRW control are applied largely or fully for ECB control, or to manage other insects.

Tables 2 and 3 provide an overview of insecticide use on corn, also drawing on official USDA pesticide use survey results going back to 1971. Table 2 reports data on the percent of corn acres treated. Table 3 presents pounds applied by active ingredient. The tables disaggregate insecticide use into those products applied predominantly for ECB control, those applied predominantly for corn rootworm control, and a set of 11 active ingredients applied over the years for control of both the ECB and the CRW, and on some farms, control of other insects.

Several entomologists were consulted in developing Tables 2 and 3. There is little disagreement over the designation of insecticides applied predominantly for ECB control or predominantly for corn rootworm control. There is uncertainty, however, in how to assign a portion of the acres treated with insecticides applied for “ECB and Other [Insect pest] Control” to the ECB versus the CRW. In the tables that follow, it is assumed that one-quarter of the acres treated with “ECB and Other Control” insecticides were applied to manage the ECB and one-half was applied targeting the corn rootworm. We also assume that the remaining one-quarter of “ECB and Other Control” acres treated

and pounds applied were sprayed to manage other insect pests.

While the percent of national corn acres treated for control of the corn rootworm went up from 1995 to 1998, reflecting the emergence of CRWs over wintering in soybean fields, the portion of acres treated went down in 1999 to about 23 percent – well less than the 32 percent implied by Monsanto. There is, moreover, a clear shift away from OP insecticides and toward synthetic pyrethroids, especially the effective, relatively new product tefluthrin, now the market leader.

Table 3 reports pounds applied from 1971 through 1999 and shows an almost 2 million pound decrease in corn insecticides applied for corn rootworm control from 1998 to 1999. The drop in terbufos and fonofos pounds applied accounts for much of the drop. Unfortunately, use of the highly toxic carbamate insecticide carbofuran has increased, although only about 1 percent of acres were treated.

Corn accounts for the majority of insecticide use in the Midwest. An estimated 75 percent of corn insecticide use in 1999 was triggered by the need to manage the corn rootworm (Table 3). Reducing reliance on highly toxic, broad-spectrum corn insecticides such as terbufos, carbofuran, and chlorpyrifos would contribute significantly to regional efforts to enhance water quality and lessen bird and fish kills.

### **Pesticide Alternatives**

There are over 25 pesticides registered as corn insecticides and about 15 are used on significant acreage. Use is declining of the two most frequently applied OPs -- terbufos and chlorpyrifos. Both were applied to about 5 percent of national corn acres in 1999 at a rate just over one pound of active ingredient per acre.

Together these two insecticides accounted for just over 75 percent of total insecticide use on corn in 1999. Both are highly toxic, broad-spectrum products, which pose a range of risks to soil organisms, birds and fish, and the farmers and applicators handling the material. When applied to cornfields before or at planting, these products rarely show up as residues in the food supply. Chlorpyrifos is found in a high percentage of the nation's grain supply, including corn and soybeans, as a result of use during storage (see the annual results of the USDA's "Pesticide Data Program" residue testing).

The most promising insecticide alternative to OPs and carbofuran is the relatively new synthetic pyrethroid, tefluthrin (Force). In 1999 this product emerged as the clear market leader, with 7 percent of acres treated, 2 percent more than the next most widely used insecticides. The average rate of application of tefluthrin is just one-tenth of a pound per acre. In addition, tefluthrin is far less toxic than the OPs and carbofuran, especially to birds. For this reason the widespread adoption of tefluthrin at the expense of OPs and carbamates represents a significant decline in the overall impacts of corn insecticide use.

Rhone Poulenc recently received full registration for the reduced risk insecticide fipronil, which was applied to about 1 percent of national acres in both 1998 and 1999. Fipronil is also applied at a relatively low rate – one-tenth of a pound per acre. In addition there are several other new active ingredients in the product development pipelines of pesticide manufacturers. Most will be applied at low rates and will meet EPA’s reduced risk criteria.

### **IPM Alternatives Abound**

The behavioral change in Western corn rootworms has undermined the confidence of some farmers in the efficacy of crop rotations. Still, rotations remain the backbone of corn IPM systems. According to Illinois entomologist Michael Gray, additional control tactics and attention to IPM-system details are needed to augment rotation-based IPM systems (Gray, 2000).

Less than 10 percent of the acres planted to corn following soybeans or another crop require an insecticide treatment. The extra expense of Cry 3Bb corn would also not be justified on these acres. Gray writes that, “Economic infestation levels of corn rootworm larvae do not occur in most cornfields. This knowledge supports the use of established scouting techniques for adult corn rootworms in late summer and the use of transgenic hybrids for corn rootworms the subsequent spring in only those fields that exceeded economic thresholds.” (Gray, 2000).

In addition, area-wide CRW population suppression systems are under development and show promise. New approaches show promise in targeting insects at multiple stages in their lifecycle, when control interventions can be carried out economically and safely. Area-wide management strategies, coupled with new ways to design field-edge habitats supportive of natural enemies, may prove to be highly cost-effective and adequate to restore the former efficacy of the corn-soybean rotation in management of corn rootworms (Landis, et al., 2000).

### **2. Resistance Management Relies on an Unacceptable Low-to-Moderate Toxin Dose Against the Corn Rootworm**

The June 2000 submission includes a 245-page volume entitled “Insect Resistance Management for a Transgenic Corn Rootworm Control Product.” Monsanto envisions a Cry 3Bb resistance management plan (RMP) that “transparently overlays the plan presently used for lepidopteran-control products” (MRID 451845-01, page 4). The need for compatible RMPs is driven by Monsanto’s future technology development and marketing plans –

“[Cry 3Bb corn] will be released both as a stand-alone product and in combination with the Cry 1Ab gene for lepidopteran control because of the dual needs of many farmers.” (MRID 451845-01, page 4).

The Cry 3Bb RMP would include 20 percent refuges planted to non-Cry 3Bb corn. Growers would have the option to both plant insecticide-treated seed and spray insecticides for corn rootworms or cutworms.

It is highly significant—and flies in the face of unambiguous recommendations from EPA Scientific Advisory Panels for high dose/refuge strategies—that the RMP document states that – “The transgenic event to be commercialized expresses Cry 3Bb at low to moderate levels” and that the Cry3Bb protein has “little or no impact on adult CRW (corn rootworms).” EPA should reject Monsanto’s resistance-management plan, based as it is on an unacceptable low-to-moderate toxin dose.

Some argue that there is no cause for alarm if resistance to Cry 3Bb endotoxins were to emerge in CRW populations because there are no products registered for soil application that contain the comparable endotoxin from *Bt tenebrionis* (*Btt*). This reasoning is faulty on two grounds.

First, it is too short term. Some companies have worked on and others will no doubt commercialize biopesticides containing an endotoxin much like Cry 3Bb for beetle control. Investment in a soil applied *B.t* product will be greatly accelerated if the Food Quality Protection Act leads to the phase out of some of the more toxic OP and carbamate insecticides. Another factor could dramatically change the market – the spread of resistance to synthetic pyrethroid insecticides, a very real concern.

Second, it has been established that once resistance emerges in an insect population to one *Bt* endotoxin, the selection pressure and time required for resistance to emerge to others will fall dramatically. For this reason, there remains great pressure on the biotechnology industry, and the EPA and USDA, to assure that effective resistance management plans (RMP) are developed and adopted in step with commercial introduction of any and all *Bt*-corn events.

Recently published research in *Nature Biotechnology* sheds light on some key design features of *Bt*-transgenic crop resistance management plans (RMPs). The important paper by Shelton and colleagues shows that refuges need to be separate from crop fields and that the spraying of refuges undermines their effectiveness (Shelton et al., 2000). This observation raises unique RMP challenges in the case of corn rootworms.

Mating occurs during the adult stage of the rootworm lifecycle soon after females emerge during the summer months. Few if any farmers spray cornfields in the summer and fall for corn rootworm adults, yet it is during this period when refuges must serve

their purpose by assuring that resistant beetles mate with susceptible adults.

Cornfields are sometimes sprayed during these periods for European corn borer. Applications are made typically with broad-spectrum insecticides, some of which can markedly depress adult rootworm populations. Accordingly, the EPA and registrant will have to take into account the need to avoid the spraying of refuge acres with insecticides targeting the ECB which might also undermine the effectiveness of the CRW RMP, and vice versa.

## **C. Ecological and Food Safety Risks**

The June 2000 submission contains little new data on Cry 3Bb food safety and environmental risks. In most cases the submission refers to data submitted with the August 1999 application (that covered other events) or data supporting the earlier approvals of Cry 1Ab or Cry 1Ac corn events. In addition, the lack of appreciable risk from years of use of foliar *Bt* insecticides is noted as evidence that Cry 3Bb corn will also be safe.

EPA should reject the application until there are substantial data to support product safety. Otherwise, the agency will expose the American public and the corn industry to major risks if it bases safety judgments on such a cursory treatment of environmental and food safety risks

### **1. Data on Impacts on Nontarget Organisms Are Inadequate**

The need for a much more thorough assessment of nontarget impacts is among the lessons learned from the EPA's review and approval of *Bt*-corn varieties engineered to control the European corn borer.

Most pesticides, including traditional *Bt* insecticides, have been tested in standard laboratory assays involving a half-dozen to as many as ten indicator species. Such assays are reasonably useful as screening tools to determine whether there is much risk of adverse impacts on populations of beneficial or nontarget organisms. But there are profound differences in Cry3Bb exposure pathways and levels and qualitatively different studies will be needed to more fully assess impacts on nontargets. A British research team led by T.H. Schuler has published an excellent review of the impacts on *Bt*-transgenic crops on arthropod natural enemies, which points out that –

“Feeding by herbivores induces changes in the emission of plant volatiles (termed herbivore-induced synomones) making the plant more attractive to parasitoids. Most current transgenes target the digestive system of insects and could therefore affect this induction process” (Schuler et al., 1999).

Monsanto's registration application states that the Cry3Bb proteins are active

against species in “several families of Coleoptera,” some of which are likely to play beneficial roles as predators or part of food webs. Yet, Monsanto has submitted a bioassay on only one Coleoptera species (ladybird beetles) in a family that is generally known not to be susceptible to Cry3Bb toxins. Much more comprehensive nontarget organisms testing is needed and must include both acute and sub-acute effects, as well tritrophic level studies.

### **Indirect Impacts Must Be Assessed**

The adverse impacts of Cry3Bb on nontarget organism will be largely indirect, the result of changes in species composition and food webs. Work by Angelika Hilbeck and colleagues at the Swiss Research Station for Agroecology and Agriculture has shown that *Bt*-transgenic corn can have direct and indirect impacts on both nontarget organisms and beneficial insects.

Two studies published in 1998 showed that the Cry1 toxins expressed in *Bt* corn plant tissue can have an adverse impact on the development and populations of *Chrysoperla carnea* (green lacewings), a common generalist predator (Hilbeck et al., 1998a; Hilbeck et al., 1998b). Mortality as high as two-thirds was observed among green lacewing larvae reared on insects that had fed on corn plants expressing *Bt* toxins. Similar mortality was found when activated *Bt* toxins (the truncated form of *Bt* expressed in corn plant tissues) were fed to green lacewing larvae in an artificial diet (Hilbeck et al., 1998a).

In both studies, Hilbeck and colleagues used Egyptian cotton leafworms. These insects fed on the *Bt* corn, or diets containing the activated *Bt* toxins, and suffered only minor, non-lethal effects. But when the lacewings fed on the cotton leafworms, they suffered both impaired development and mortality. In addition, green lacewings feeding on *Bt*-fed prey progressed through developmental stages more slowly than the controls fed on prey not exposed to *Bt* toxins.

One of the study’s intriguing findings is that passage of *Bt* toxins and protoxins through the gut of certain insects, in this case Egyptian cotton leafworms, appears to potentiate the effects of the *Bt* toxins when another insect (i.e., green lacewings) preys upon the cotton leafworms (Hilbeck et al., 1999). This and other findings led the team to conclude “tritrophic level studies are necessary to assess the long-term compatibility of insecticidal plants with common natural enemies” (Hilbeck et al, 1999) and to make a critical point:

“The ubiquitous and temporally extended availability of *B. thuringiensis* proteins in the field in addition to its modified form of release, makes it necessary to verify and monitor the compatibility of this new pest management strategy with natural enemies. The long-term agroecological safety of the combined use of transgenic

crop plants and *B. thuringiensis* insecticides cannot simply be deduced from the past record of safe *B. thuringiensis* insecticide use when *B. thuringiensis* compounds were available in the field only during short periods.” (Hilbeck et al., 1999).

## **2. Impacts on Soil Health and Productivity Must Be Assessed**

Healthy soil is a prerequisite for profitable farming on a sustained basis. Soil quality is generally defined as the capacity of a soil to take in, store and purify water, to hold and recycle nutrients, to support a diverse and robust biotic community, and to suppress pathogens and other pests.

Soil quality can and has been degraded through erosion, excessive tillage, compaction, use of broad-spectrum insecticides and soil fumigants, depletion of nutrients, and the build up of salt and other minerals. Research over 50 years has documented the significant and largely irreversible impacts of soil loss and degradation on average attainable corn yields (NRC, 1993). Plus, recent work by soil ecologists is beginning to explain the many ways that soil microbial communities can impact plant growth and development, and perhaps even more important, contribute to the microbial biocontrol of soil borne pathogens and microarthropods.

Kerry has highlighted the impact of plant root exudates on nematode populations “by influencing both the dynamics of the nematode host and structure and dynamics of the community of antagonists and parasites in the rhizosphere” (Kerry, 2000). He stresses that little is known about how root exudates impact soil microbial communities, as well as microbial biocontrol processes. Introduction of Cry 3Bb corn hybrids will add another layer of complexity for soil ecologists.

A key 1998 study in *Nature* by van der Heijden and colleagues showed that the diversity of mycorrhizal fungi plays a key role in determining the productivity of soil ecosystems (van der Heijden et al., 1999). An overview by Read in the same issue speculates that greater fungal biodiversity expands the range of mechanisms through which microbial interactions can help plants deal with various sources of stress and competition (Read, 1999).

### **Persistence of Cry3Bb Toxins in the Soil Must Be Measured**

A disadvantage of foliar *Bt* products has always been a constraint on their commercial development – foliar *Bt* sprays break down quickly when exposed to sunlight, and become inactive within 48 hours to a few days. Engineering *Bt* into plant tissues helps solve this problem. Varieties like Cry 3Bb corn that express *Bt* in roots, and presumably as well through root exudates, will assure even slower breakdown of *Bt* proteins.

Important work by Stotzky and colleagues reported in *Nature* shows that *Bt* proteins are exuded from the roots of *Bt* corn and can bind with clay soil particles or humic acid and remain active for over 120 days in the soil (Saxena et al., 1999). This work needs to be repeated with Cry3Bb corn varieties to document the extent to which they exude Cry3Bb protein toxin and whether it too binds to soil particles.

Stotzky's team has shown that *Bt* proteins can bind to clay particles and humic acids and become very stable in the soil (Saxena et al., 1999). In some farming systems and on certain soils, bound *Bt* may move off fields with eroding soil and enter streams, ponds and lakes, and aquatic ecosystems. Understanding the environmental fate and movement of such bound Cry 3Bb endotoxins will require careful field research.

If approved, Monsanto's Cry3Bb corn could be adopted widely within a few years on millions of acres (Gray, 2000). It will deliver into the root zone concentrations of Cry3Bb toxins that are orders of magnitude above what occurs naturally, possibly triggering serious effects on soils, soil food webs, and soil-borne-pest dynamics.

### **3. Data Needed to Assess Human Health Risks Are Inadequate**

*Bt* foliar insecticides have been used safely for more than two decades. *Bt*'s safety record played a major role in convincing the EPA that only a cursory assessment of human and mammalian health risks from dietary ingestion of truncated *Bt* endotoxins in plant tissues was required prior to approval of today's Cry1 and Cry2 *Bt.k*-transgenic plant varieties.

Monsanto's current application for Cry3Bb transgenic corn rests heavily upon traditional toxicity studies that were originally submitted to the agency by Ecogen to support registration of Raven, a foliar *Bt* product containing Cry3Bb1, the endotoxin from *Bt tenebrionis* (*Btt*). *Btt* studies on file are routine toxicological feeding experiments and were not designed to detect mammalian health hazards. In addition, the Ecogen studies were carried out with *Btt* derived from bacteria via fermentation, which produces a larger molecule and is different from the truncated form of protein expressed in Cry3Bb transgenic corn plants.

In the summary of the registration application, Monsanto states that the safety of corn containing Cry3Bb proteins to mammals rests on:

- Multiple studies showing very high NOELS ("No Observable Effect Levels") in acute feeding studies with a variety of Cry proteins in a number of species.
- Similarity of Cry3Bb proteins to other Cry proteins and *Bt* insecticides, especially Raven, which contains a mixture of two Cry3 proteins and Cry1Ac.
- A 40-year history of safe use of *Bt* insecticides and Cry proteins.

There are indeed very wide margins of safety in terms of acute poisoning when mammals ingest Cry proteins. Acute toxicity should still be investigated through dermal, inhalation and dietary routes of exposure, given the high expression levels in MON 863 transformed corn. Allergenicity should also be explored much more thoroughly. The submission refers to a review of a database of known allergens, which produced no evidence of a match.

There is little known about the degree of similarity or differences between Cry3Bb proteins and other Cry proteins in terms of the health effects of greatest concern, which would include allergenicity and digestive system impacts.

The third point is also not reassuring. The American population has not been extensively exposed to any Cry proteins over 40 years. Exposure has been almost non-existent to Cry3 proteins. No foliar *Bt* insecticide containing *Bt tenebrionis* has achieved significant market share in any crop. In addition, there is very little if any dietary exposure to residues left after foliar *Bt* applications. Because *Bt* insecticides break down quickly when exposed to sunlight (within 48 hours in most cases), residues are long-gone by the time the produce reaches consumers.

The only other possible route of exposure since 1997 would be transgenic potatoes containing Cry3A proteins (New Leaf potatoes), but they have never accounted for more than a few percent of national potato acreage.

### **Antibiotic-Resistance Marker Should Be Removed**

Nielsen and colleagues point out in a recent article in *Applied and Environmental Microbiology* that –

“Of the 15 different resistance genes incorporated into plants, several encode resistance to clinically used antibiotics....Recently, uptake of transgenic plant-harbored DNA fragments by bacteria based on restoration of a partially deleted (10- or 317-bp internal deletion) bacterial kanamycin (KM) resistance gene (*nptII*) after recombination with transgenic plant-inserted homologues was demonstrated” (Nielsen et al., 2000).

Recent evidence documenting how freely bacteria can exchange genes has rekindled concerns over the emergence of new strains of antibiotic resistant bacteria as a result of use of antibiotic selectable marker genes. The Cry 3Bb gene cassette contains the 0.79 Kb *nptII* gene from a transposon isolated from *E. coli*. It confers resistance to kanamycin and neomycin<sup>2</sup>.

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<sup>2</sup> Kanamycin and neomycin are antibiotics used to control disease-causing bacteria (Berkow, 1992). FDA has approved a food additive petition from Calgene, Inc., (now owned by Monsanto) for use of the *nptII* gene in genetically engineered crops.

When the first *Bt*-transgenic plant applications were reviewed, there was little evidence that antibiotic marker genes might trigger resistance problems. Now the transfer of antibiotic marker genes in plant cells to soil bacteria has been demonstrated (Gebhard and Smalla, 1998). In a key article in the journal *Applied and Environmental Microbiology*, Gebhard and Smalla hypothesize that:

“...the introduction of bacterial genes into the plant genome leads to a higher probability of gene transfer from plants to bacteria due to the presence of homologous sequences. However, until now, there has been a lack of clear experimental evidence that successful gene transfer from plants to bacteria can occur at all.” (Gebhard and Smalla, 1998).

The scientists studied gene flow from transgenic sugar beets to *Acinetobacter* species (common soil bacteria) and found, for the first time, “transformation of naturally competent bacteria by transgenic plant DNA, even with plant homogenates...” They have also shown that *Acinetobacter* bacteria that have taken up an antibiotic-resistance gene from a transgenic plant can persist up to two years in soil (Gebhard and Smalla, 1999).

Also contrary to conventional wisdom, genes that confer antibiotic resistance have been shown to move readily between people and from bacteria in the gut of farm animals to people (Tschape, 1994). A team in Denmark has shown that indigenous soil bacteria can serve as a sink for plasmid-borne antibiotic resistance traits from *E. coli* entering agricultural soils from animal manure or other wastes (Sorensen et al., 1999).

Until recently it was also thought that foreign DNA, such as the gene constructs in Cry 3Bb corn, would pass through the mammalian digestive without being activated and without consequence. The transfer of foreign DNA in food into the blood and organ systems in mice has now been demonstrated (Schubbert et al., 1997). In addition some of the transgenic DNA was found to covalently bind to mouse DNA, in effect becoming a part of the mouse genome. The authors modestly summed up this article by stating -- “The medical and evolutionary implications of these observations may be considerable” (Schubbert et al., 1997).

## **Conclusions and Recommendations**

In light of the substantial risks documented convincingly in the Monsanto submission, as well as many critical data gaps and unresolved technical questions, we urge EPA to deny this registration application for Cry3Bb transgenic corn. The supporting documents do not come even close to providing adequate health, safety, efficacy, and ecological impacts data to support a judgment that the benefits of this technology exceed the risks posed by it.

Moreover, we believe that as more information is compiled on Cry 3Bb corn and as more balanced, independent assessments are completed, the magnitude of the projected benefits will shrink and the scope and severity of possible risks will rise. In particular, Monsanto's plan to "stack" Cry 3Bb and Cry 1Ab endotoxins in the same corn varieties, perhaps in conjunction with the Roundup Ready trait, will raise new and complex risk assessment science and regulatory policy issues.

We appreciate the opportunity to submit these comments and await the agency's decision on the pending registration application.

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