March 20, 2000

Public Information and Records Integrity Division
Information Resources and Services Division (7502C)
Office of Pesticide Programs
Environmental Protection Agency
401 M Street S.W.
Washington, D.C. 20460

COMMENTS SUBMITTED TO DOCKET NUMBER OPP-30487a: REGISTRATION APPLICATION FOR CRY3Bb TRANSGENIC CORN MODIFIED TO CONTROL THE CORN ROOTWORM

These comments are submitted on behalf of Environmental Defense[1], the Institute for Agriculture and Trade Policy[2], the Science and Environmental Health Network[3], the Center for Food Safety, and the Consumer Policy Institute/Consumers Union[4]. Our focus is the application from Monsanto Company for full registration of corn genetically engineered to express the *Bacillus thuringiensis* Cry3Bb proteins for control of corn rootworms. Monsanto's application covers not just field corn, but also sweet corn and pop corn, crops clearly intended for direct human consumption.

We urge the Environmental Protection Agency (EPA) to not register Cry3Bb transgenic corn. Monsanto's application does not come close to providing the health, safety, efficacy, and other information necessary to carry out basic risk and benefit assessments. Lacking such information, the agency has no basis to determine that the benefits of Cry3Bb corn will outweigh associated risks.

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[1] Environmental Defense is a national nonprofit organization that does research and advocacy on a range of environmental issues. Headquartered in New York City, Environmental Defense has eight offices in the United States and more than 300,000 members. For more information, see <http:www.environmentaldefense.org>.

[2] The Institute for Agriculture and Trade Policy is based in St. Paul, Minnesota. Its mission is to create environmentally and economically sustainable rural communities and regions through sound agriculture and trade policy. See <http://www.iatp.org> for further information.

[3] The Science and Environmental Health Network is dedicated to the development of sound environmental and risk assessment policies, and is based in Windsor, North Dakota. See <http://www.sehn.org> for further information.

[4] Consumers Union is a nonprofit membership organization chartered in 1936 under the laws of the State of New York to provide consumers with information, education and counsel about goods, services, health, and personal finances and to initiate and cooperate with individual and group efforts to maintain and enhance the quality of life for consumers. Consumers Union's income is solely derived from the sale of *Consumer Reports*, its other publications and from noncommercial contributions, grants and fees. In addition to reports on Consumers Union's own product testing, *Consumer Reports* regularly carries articles on health, product safety, marketplace economics and legislative, judicial and regulatory actions that affect consumer welfare. Consumers Union's publications carry no advertising and receive no commercial support.

Background

Corn rootworms are beetles (Order Coleoptera) that start their life cycle in the soil. Emerging larvae feed on the roots of young corn plants. The heavier the feeding, the more plants that are weakened and subject to lodging (falling over prior to harvest). Between reductions in corn yields and the cost of insecticides applied, corn rootworms account for an estimated $1 billion in economic impacts annually (Volume 1, Administrative Materials).

Problems with corn rootworms are much more serious in fields planted to corn year after year. Indeed, for forty years, farmers have used crop rotation to keep corn rootworm populations below damage thresholds. Until recently, crop rotation has almost always been effective in avoiding damage. In parts of the Cornbelt, some species of corn rootworms appear to have changed their behavior and are now successfully over-wintering in soybean fields, where they emerge the next spring and can attack freshly planted corn (Gray, 2000). This change in rootworm behavior has led to an increase in insecticide use since 1996 and has heightened grower interest in novel Integrated Pest Management (IPM) strategies, as well as varieties bred to resist corn rootworm feeding damage.

About one-third of corn acres were treated with an insecticide in 1998 according to U.S. Department of Agriculture pesticide use data. Corn rootworms were the target of insecticide applications on about 16 million acres of corn in 1998, about 22 percent of the 71.4 million acres surveyed by USDA (see Appendix Table 1 which reports the percent of acres treated with corn insecticides by target pest since 1971).

The other major corn insect pest – the European corn borer (ECB) (a moth, Order Lepidoptera) – is episodic. Corn borer damage is typically not serious. Yields might be reduced 10 percent to 20 percent in one or two years in 10. While clearly a problem in some years, the costs of controlling ECB with insecticides are significant and the results are spotty, since the insect tunnels inside the corn plant or hides under leaf tissue.

Corn accounts for the vast majority of insecticide use in the Midwest. About 77 percent of corn insecticide use in 1998 was triggered by the need to manage the corn rootworm (see Appendix Table 2 for details). Reducing reliance on highly toxic, broad-spectrum corn insecticides would contribute significantly to regional efforts to promote biodiversity and protect avian and fish species. For this reason, extensive efforts are underway across the Cornbelt to develop and implement biologically based corn IPM systems. University of Illinois corn IPM specialist Dr. Mike Gray has done a recent, excellent overview of corn rootworm IPM challenges and control options (Gray, 2000).

Much of the public debate over the proposed Cry3Bb technology, and ultimately EPA's registration decisions on this and other transgenic corn technologies, will focus on the relative risks and benefits of alternative corn rootworm management strategies. We return to these critical, broader issues in Part D. of these comments.

Status of Application and Regulatory Review

In June 1999, Monsanto applied for the first Experimental Use Permit (EUP) on Cry3Bb transgenic corn. The EUP application was published in the Federal Register on December 8, 1999 and covered three transformation vectors. The EUP sought approval to plant about 1,600 acres each of three Cry3Bb transformed corn varieties in 27 states and Puerto Rico. If approved, the EUP will sanction in crop year 2000 the first field-scale experiments with this technology. A maximum of about 4,800 acres could be planted under the terms of the EUP.

Two weeks later on December 22, 1999, the EPA published a second Federal Register notice announcing receipt of an August 19, 1999 application from Monsanto for this technology – this time seeking full registration under Section 3 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).

The timing was unusual. Typically, EUP-sanctioned field trials are carried out over two or three years prior to a company seeking a full registration of a new transgenic crop variety or a new pesticide active ingredient. The purpose of EUPs is to collect detailed data on environmental impacts and performance under field conditions in order to satisfy registration requirements more convincingly.

Flaws in the EUP Application

Some of the major deficiencies in the EUP application were described in comments submitted to the agency January 7, 1999. The comments were prepared by the Institute for Agriculture and Trade Policy, the Science and Environmental Health Network, and the Consumer Policy Institute/Consumers Union and can be accessed on the Internet at <http://www.biotech-info.net/Corn_EUP.pdf>.

Environmental Defense concurs with the need for solid answers to the questions raised in the January 7th comments. Several new questions and concerns are highlighted in these comments in light of information in the registration application and supporting documents.

Our comments highlight the appalling deficiencies in the data supporting the application for registration as well as worrisome implications of new science relevant to the potential risks posed by Cry3Bb transgenic corn. Comments follow in five parts –

- Part A., major conclusions and recommendations.
- Part B., environmental and ecological concerns including resistance, soil health, and impacts on non-target organisms.
- Mammalian health risks and unresolved issues are addressed in Part C., with emphasis on horizontal gene flow and the fate of Cry3Bb proteins and gene constructs in mammalian digestive systems.
- Part D. describes the need to utilize Cry3Bb transgenic corn varieties within an Integrated Pest Management system.

- ❖ A recommended set of criteria and decision-rules and a framework for decision-making are the focus of Part E.

A. Conclusions and Recommendations

Monsanto's application is premature and incomplete and should be withdrawn or denied. Most of the data and justification for approval rests upon the assertion that Cry3Bb transgenic corn is substantially equivalent to Cry1 and Cry2 transgenic corn varieties, to Cry3A1 potatoes, and to standard formulations of *Bt* liquid insecticides that contain Cry3 proteins.

Monsanto asserts that these technologies have all been reviewed by the EPA and judged safe and that therefor the current application should also be approved. We urge EPA to reject this logic for two major reasons. First, because there are clear and substantial differences between Cry3Bb corn and other previously approved *Bt*-based technologies, which among other things are not directed at below-ground insects. Second, there are several critical unresolved safety, health and performance questions associated with all *Bt*-transgenic crops. Rather than compound possible problems by approving another *Bt*-transgenic corn technology, the EPA must now carry out a thorough and deliberate appraisal of Cry3Bb risks and benefits drawing on insights gained and lessons learned from recent research on Cry1 and Cry 2 *Bt*-corn varieties.

Moreover, it should go without saying that the EPA should never approve technology intended for use annually on millions of acres without any field scale data on ecological and environmental impacts. Educated guesses and extrapolation from data developed to support the registration of other *Bt* insecticides and *Bt*-transgenic crops are no substitute for well-designed, properly conducted field studies.

The Application Lacks Critical Information and is Excessively Open-ended

We are disturbed that the registration application seeks open-ended approval for "the class of Cry3Bb proteins without identifying specific transformation events" for all types of corn (Letter dated August 19, 1999 from Monsanto to EPA, accompanying the registration application). The letter goes on to say –

> "A non-event specific registration is consistent with conversations we have had with Agency staff over the last number of months…Monsanto expects that such a non-event specific registration would allow the commercialization of all events containing the *cry3Bb* gene whose protein levels fall within ranges listed on the Confidential Statement of Formula."

This request is akin to a blank check. To concur with this request, EPA would have to agree with the registrant that there is no difference between Cry3Bb proteins and transformation events in terms of any relevant environmental or human health risk – a ridiculous contention repeatedly contradicted in the data submitted by Monsanto in this very registration application.

In the same August 19, 1999 letter Monsanto announces its intention to amend a pending tolerance petition to add Cry2Ab toxins, suggesting that in the future an application will be forthcoming for corn expressing Cry2Ab. The open-ended nature of the registration Monsanto is seeking suggests that the company has not yet decided on the transformation event and Cry proteins that it will incorporate in transgenic corn lines for rootworm control. Accordingly, it is hard to evaluate the adequacy of supporting data and to assess the magnitude of potential health, ecological and environmental risks. Likewise, it is almost impossible to assess efficacy and the reliability of resistance management plans.

Data Supporting Assertions of Safety is Inadequate

The table on the next page summarizes the 19 new studies submitted by Monsanto in support for approval of an unspecified number of transformation events containing various Cry3Bb proteins.

The table shows clearly that a modest volume of new data has been submitted in support of registration. Ecological impacts were tested in just one acute feeding trial in one avian species, using just one of the two major Cry3Bb proteins.

Testing of impacts on non-target insects is also grossly inadequate. Cry3Bb toxins are active against insects in the Order Coleoptera (beetles, weevils and stylopids). This Order of insects contains far more North American species than any other – some 28,600 species (Arnett and Jacques, 1981). Fortunately within Coleoptera families, Cry3Bb toxins are known to mostly impact species in the family Chrysomelidae.

Of the eight studies assessing possible impacts on non-target insects and soil organisms, only one involved another insect in the Order Coleoptera – the convergent ladybird beetle, a member of the family Coccinellidae. The lack of a single study on one of the several dozens of insects in the most susceptible family suggests that the Monsanto concedes the point that non-target Chrysomelidae species will be adversely impacted.

There was essentially no experimental assessment of possible impacts on human health, other than what amounts to a sophisticated literature search of public databases on allergens and toxins. A single feeding study was carried out in catfish; there are no data submitted covering safety to cattle, poultry, swine or other farm animals likely to be fed large quantities of corn containing Cry3Bb proteins.

New Studies Submitted in Support of the Full Registration Application for Cry3Bb Transgenic Corn		
	Number of Studies	**Protein Tested or Corn Line**
Characterization and/or Expression of the Protein		
Molecular Analysis	1	Mon 853
Protein Levels	1	Mon 853 and 860
Protein Characterization	2	Protein 11098 and 11231
Total Characterization	4	
Health Oriented		
Acute Oral -- Mice	2	Protein 11098 and 11231
Digestibility --model study	1	Protein 11098 and 11231
Bioinformatics -- toxins*	1	Protein 11098 and 11231
Bioinformatics -- allergens*	1	Protein 11098 and 11231
Catfish Feeding trial	1	Mon 853 and 859
Total Health Oriented	6	
Non-Target Organisms		
Coleoptera (beetles)	1	Protein 11231
Honey Bees	2	Protein 11231
Water flees	1	Protein 11098
Other insect orders	4	Protein 11231
Total Non-Targets	8	
Ecological Impacts		
Bobwhite feeding	1	Protein 11231
Total All New Studies	19	

* Structural similarity of the Cry3Bb proteins were checked against public databases of known toxins and allergens. These were not feeding studies.

Questions Persist Over Levels of Expression

The July 1999 technical study submitted by Monsanto on the levels of protein expression in different tissues raises serious questions (Volume 3, MRID # 449043-02, "*B.t*. Protein 11231 and NPTII Protein Levels in Samples Collected from Corn Events MON 853 and MON 860 in the 1998 U.S. Field Trials"). It states that the level of Cry3Bb1 protein in MON 853 plants were similar in leaf and grain tissues (about 65 ug/g), were lower in forage (12 ug/g), and lowest in roots (4.5 ug/g). Protein levels declined during the growing season in leaf, whole plant and root samples of MON 853 plants. But in MON 860 plants protein levels actually increased through mid-season in roots and whole plants, but declined in leaf tissue.

These data show clearly that different transformation events lead to significantly different levels and patterns of protein expression. Such differences can be important in assessing efficacy, resistance management and non-target impacts, as well as changes in the micoflora in the digestive systems of livestock and humans.

In addition, the sparseness of data on Cry3Bb protein expression levels across various transformation events is startling. In the 1998 test season, Monsanto notes a shortage of seed and that some of the seed was of poor quality. Trials were carried out in seven locations. According to Monsanto's technical report (MRID # 449043-02), "Approximately fifty to seventy-five seeds were planted per plot of each event at each location," producing a stand count of between 11 and 37 plants per plot.

Accordingly, prior to submitting the current full registration application, Monsanto scientists had about 300 corn plants to work with in assessing protein expression levels across its two main transformation events. Such limited sampling provides no basis to estimate variability in expression across tissues as a function of agronomic systems, soil type, pest pressure and climatic variables. Knowledge of the degree of such variability is essential in assessing how management can impact efficacy, resistance management plans, and ecological and environmental risk outcomes.

Some of the critical measurements of expression levels were done on only two plants (for example, see the test for Cry3Bb1 and NPTII protein levels in roots and forage in Table 2, page 21). Less than a month after this technical report was completed, Monsanto submitted its application for full, unrestricted registration and in its August 19, 1999 cover letter wrote:

> "Please note that approval of this registration by May, 2000 would reduce the need for additional submissions and reviews for year 2000 field trials."

Recommendations

1. The EPA should reject the current application as both premature and incomplete. In its rejection letter the agency should provide clear guidance on the additional information and scientific documentation that must be generated and included prior to resubmission, as well as the protein expression, resistance management, and safety standards that Cry3Bb corn lines must met.

2. Additional field research should be carried out over several seasons. This will require EPA approval of a series of annual Experimental Use Permits (EUPs). Cry3Bb corn Experimental Use Permits should include several conditions and requirements --

- Agronomic provisions should specify where and how the hybrids should be planted to minimize problems, such as any adverse impacts on non-target organisms, while also maximizing the utility of refuges and other resistance management plan provisions.

- Pest management conditions should assure that Cry3Bb hybrids are planted only in conjunction with proven IPM practices. The NC-215 project (a land grant university consortium linking corn IPM specialists in the Midwest) should be called upon to recommend essential components of required IPM plans.

- Provisions setting out the agronomic and pest management system responsibilities of farmers should be clearly stated in the technology agreements signed by farmers or through some other legally binding mechanism.

3. The EPA should sponsor a public consultation process to seek guidance on how Cry3Bb studies should be designed and carried out. The Scientific Advisory Panel should ideally review priorities, projects and study protocols annually. The goal should be to assure that all opportunities are exploited to quickly broaden the knowledge base on the field performance, safety and sustainability of Cry3Bb transgenic corn hybrids.

4. In approving EUPs, the EPA should require Monsanto and its licensees to offer land grant university scientists an opportunity to carry out cooperative and/or independent assessments of:

- Resistance management plans;
- Transgene expression and toxin levels and environmental fate;
- Environmental and ecological impacts; and
- Livestock and other feeding trials with grain harvested from the plots.

Independent assessments of the technology are warranted because of the pressure on the agency for speedy approval and the likelihood of rapid adoption by farmers.

5. The EPA should sponsor an open process to determine how needed IPM, environmental and resistance management provisions should be crafted and enforced. The role and responsibilities of the registrant and its licensees in assuring compliance must be

clearly spelled out and made part of EUP and/or conditional registration requirements.

6. The EPA and USDA should jointly fund a program evaluating whether EUP and/or conditional registration requirements, including enforcement procedures, are achieving stated goals. Where and to the extent compliance or performance falls short of agreed upon goals, the provisions of an EUP or conditional registration would be violated and the EPA would, presumably, take appropriate steps to either curtail further use or correct underlying problems prior to the next crop season.

Over time this process will develop increasingly rich data on the performance, impacts and potential risks posed by this technology. If problems are shown to be modest, predictable and manageable, the EPA may incrementally relax provisions that it deems no longer necessary.

7. The EPA and USDA should assure that resistance monitoring efforts in the field are sufficient to detect resistance early enough so that proven remedial actions can be taken. The goal must be to prevent resistance to the full extent possible, detect and contain outbreaks when they occur, and eliminate resistance populations as quickly as possible.

8. The EPA and USDA should jointly design and fund a series of field studies on soil ecosystem impacts. The studies should go on for several years and begin with development of a complete profile of baseline soil quality and microbial community status. Such a baseline is needed to quickly identify possibly significant changes in soil quality and productivity.

9. While not under the jurisdiction of the EPA, we believe that before final approval of this technology, the Food and Drug Administration should sponsor and/or require additional research on the possibility of transgene flow and adverse impacts in the digestive systems of livestock fed *Bt*-transgenic corn, including Cry3Bb corn.

In cooperation with appropriate agencies, other possible routes of mammalian or human exposure should also be considered; for example, exposure to workers processing grain into livestock feed or people working in the seed industry.

The FDA, EPA, and USDA should cooperate to assure that qualified, independent research teams are given adequate resources to carry out this work in step with, if not in advance of, progress toward full approval and widespread use.

B. Environmental and Ecological Risks

1. Resistance Management

The registration application submitted by Monsanto states that fieldwork is ongoing on corn rootworm development, mating, movement, and feeding behavior – key variables in structuring a resistant management plan (RMP). The application acknowledges that there is insufficient information available now to design a plan and promises to come forward with a proposed plan by January 2000. Monsanto does anticipate, however, reliance on a high dose and refuge strategy (see Appendix 2, "Insect Resistance Management with Corn Rootworm Protected Corn Hybrids," Volume 1, "Administrative Materials in Support of Registration").

Hence, the application must include accurate data on protein expression levels in corn leafs, roots, pollen, silks and grain over time. In particular, attention must be paid to expression levels in roots, and possibly through root exudates early in season when first instar rootworm larvae are most vulnerable. It will also be vital to assess whether levels in leaf tissues and silk are high enough in mid-summer to reliably control adult rootworms that fly into Cry3Bb fields.

Expression levels may also vary within the architecture of the root system and/or over time as the plant grows, possibly as a result of variation in soil physical or chemical properties. Uneven expression in the root zone will make it much more difficult to devise effective resistance management plans. Hence the importance of data from field trials demonstrating the levels and evenness of protein expression in roots, leafs, pollen, silks, and grain under multiple combinations of soil types, tillage systems and climatic conditions.

The application states that survival of larvae and adult emergence "appears to be low but it remains to be accurately quantified this year." It also acknowledges that resistance is more likely to the extent surviving larvae emerging from a field of Cry3Bb corn also feed on Cry3Bb corn pollen and silks as adults. More work must clearly be done on expression levels to determine whether a high-dose is likely to be reliably delivered through the tissues rootworms are most likely to feed on as the season progresses.

Another serious concern is mentioned just in passing in the application – movement of adult rootworms that emerge in untreated fields into Cry 3Bb corn. Data in Volume 3 of the submission show that for MON 860 and MON 853 events, Cry3Bb1 protein levels decline in leaf tissues as the season progresses and hence may not deliver a true "high dose" to otherwise healthy adult corn rootworms. It is also probable that some previously unexposed adults will move into Cry3Bb fields for periods too short for them to ingest a lethal dose. Sub-lethal doses to adults would greatly increase the likelihood of resistance emerging.

Unique Challenges in Managing Corn Rootworms

Few insects are resistant to more insecticides than the corn rootworm. Populations are resistant to chemicals in all major classes of insecticides. Resistance management is a major concern whenever a new product is registered.

The challenge in managing resistance to Cry3Bb corn varieties will be significant. According to University of Illinois corn IPM specialist Dr. Michael Gray –

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

As an adult, corn rootworms are highly mobile. During its adult stage, the southern corn rootworm is also known as the spotted cucumber beetle -- a common vegetable pest that thrives on a wider variety of crops than northern and western corn rootworms. The Monsanto application cites dating showing that the western corn rootworm can survive on 21 species and the northern corn rootworm on 14 species.

While soil-applied *Bt.tenebrionis* products have not yet been commercialized for control of rootworm larvae, interest will grow in developing and marketing such products, especially if and as organophosphate and carbamate soil insecticides are driven off the market by the Food Quality Protection Act (FQPA).

Recently published research in *Nature Biotechnology* sheds light on *Bt*-transgenic crop resistance management plans (RMPs). The important paper by Shelton and colleagues shows clearly that refuges need to be separate from crop fields and that the spraying of refuges undermines their effectiveness (Shelton et al., 2000). This later 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. (The Appendix tables show that four out of 15 insecticides applied to corn in 1998 reduced populations of both ECB and corn rootworms).

Accordingly, the EPA and registrant will have to take into account the need to avoid the spraying of refuge acres with some insecticides targeted primarily against other corn insects.

Corn rootworm behavior poses another quandary in the design and placement of refuges. According to the Monsanto application, “Adult flight behavior consists of long distance flights soon after emergence that can carry insects for miles, as well as many shorter flights that occur largely within a field of host plants.” It remains unknown what percentage of adult females fly significant distances prior to mating, but even a small percentage would undermine the effectiveness of a resistance management plan based on refuges in or surrounding fields planted to Cry3Bb varieties.

2. Soil Health

Healthy soil is a universal 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.

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).

Soil Persistence

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 that express *Bt* in roots and through root exudates will assure even slower breakdown of *Bt* proteins.

Important work of 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.

Monsanto is proposing a high dose strategy for resistance management, so that the level of Cry3Bb in roots, and perhaps root exudates will be perhaps an order of magnitude higher than the LD-90 for corn rootworm larvae. As a result, there will be elevated levels of Cry3Bb proteins in the root zone, surely far more than under any natural conditions.

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. The environmental fate and movement of such bound *Bt.t*. residues will require careful field research to document. Conditions will arise periodically leading to a release of the *Bt.t*. as organic matter in sediment breaks down, possibly producing a short-term flush of *Bt.t*. entering species-rich and fragile ecosystems during critical periods when microbial activity is most active and food webs are at peak levels.

Possible Alterations in Soil Ecosystems

If approved, Monsanto's Cry3Bb corn may be rapidly adopted on millions of acres. It may deliver into the root zone concentrations of Cry3Bb toxins that are orders of magnitude above what occurs naturally. The application and supporting data provide little information on which to evaluate the impact of this large-scale experiment on the health of farm soils. As discussed below, scientific research suggests that large-scale planting of Cry3Bb crops could have ecologically significant effects on soils, especially if planted widely in a region over several years.

There is a vast diversity of *Bacillus* species within the largely unknown complex of organisms in soil, including considerable variability in *Bt* subspecies and strains. The diversity of *Bt* species may be a testament to the need of *Bt* species to continuously evolve in response to the defenses insects have evolved to combat them (Martin, 1994). There are over 100 known *Bt* strains, and no one knows how many are yet to be discovered or have become extinct.

Most *Bt* species emit proteins that are toxic to two or more orders of insects, while others appear non-toxic to virtually all insects. It is found all over the world in all soil conditions and both where insects are plentiful and rare. While population levels are known to fluctuate daily, they are generally higher in the spring and fall and lower in the summer. Phenotypes also can change dramatically over short periods and do so typically in response to an exogenous factor that triggers spikes or crashes in the population of certain organisms (Martin, 1994).

In a 1994 *American Entomologist* review article, Martin discusses a key field experiment involving foliar application of a *Bt* insecticide. Its application raised the population of the *Bt* strain in the product to a maximum level of 100,000 cfu/g of wet leaf weight. But just one day after application, the population had dropped to pre-treatment levels. As long as 21 days post-treatment, the applied *Bt* strain could still be detected at very low levels. But significantly, there had been an overall shift from *Bt* species to other bacteria that had displaced the indigenous *Bt* species on leaf tissues (Martin, 1994).

This study is one of the first to show that bacteria and microorganisms other than *Bt* species can react more quickly to fill ecological niches left by an exogenous shock impacting *Bt* species. The planting of Cry3Bb corn might prove to be just such a shock. Indeed, a series of experiments by a team of soil ecologists and microbiologists working principally at an EPA laboratory in Corvallis Oregon provides indirect evidence in support of this hypothesis.

Work carried out since 1994 by Katherine Donegan, Ramon Seidler, Gary Reed, Ramon Seidler, and other scientists have shown that transgenic plants can produce "changes in the population levels and composition of some soil and plant microorganisms" (Donegan and Seidler, 1999). Experiments with transgenic tobacco plants showed that transgenic plant tissues can increase nematode populations and alter tropic group composition, including a significant increase in fungal feeding nematodes compared to bacterial feeding species (Donegan et al., 1997). In contrast, Collembola populations were significantly lower.

Other experiments with cotton plants expressing *Bt.* subspecies *kurstaki* (*Bt.k.*) demonstrated surprising persistence of *Bt.k.* toxins in clay soils, especially those with high pH and salt content. In one experiment, 23 percent of the initial extractable *Bt.k.* toxin was recoverable after 23 days (Donegan and Seidler, 1999). A soil half-life of 40 days was computed from one dataset reflecting *Bt.k*. environmental fate in a silt loam soil. As expected, their experiments confirm that the more biologically active a soil, the more rapid the degradation of any *Bt* toxins within it.

Significantly, the EPA team in Oregon carried out some studies using transgenic potatoes expressing *Bt.tenebrionis* Cry3A protein. These transgenic potatoes were modified with a gene construct similar to the ZMIR14L vector used to engineer the Cry3Bb transgenic corn under review herein. The team found that "plant genomic DNA can persist in soil under field conditions for several months." Just less than 3 percent of the initial level of the marker gene was left after 84 days in one experiment. The team observed no significant differences in the persistence of the *Bt.t.* proteins in contrast to the persistence of *B.t.k* and Cry1Ab or Cry2A toxins.

Microcosm studies with purified *Bt.k* endotoxin from transgenic cotton plants led to some intriguing findings. Two of three transgenic lines tested produced short-lived "but significant increases in the levels of culturable bacteria and fungi" (Donegan and Seidler, 1999), despite the fact there were no observed direct impacts on soil microorganisms. This led the authors to suggest that "the effects were due to unexpected changes in plant characteristics, aside from the intended *Bt.k*. endotoxin production, that resulted from genetic manipulation or tissue culturing."

3. Impacts on Non-target Organisms

The need for a much more thorough assessment of non-target impacts is among the lessons learned from the EPA's review and approval of *Bt*-corn varieties engineered to control the European corn borer. Recent research has now shown just how different the new studies will need to be compared to most past studies assessing the toxicity of synthetic pesticides to non-targets.

Most pesticides, including formulations of traditional *Bt* insecticides, have been tested in standard laboratory assays involving a half-dozen to as many as ten indicator species. In the case of an insecticide sprayed onto a field that works largely through a toxic mode of action, such assays are reasonably useful as screening tools to determine whether there is much risk of adverse impacts on populations of beneficial or non-target 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 non-targets.

In terms of direct impacts, Monsanto's registration application states that the Cry3Bb proteins are active against species in "several families of Coleoptera." Still, Monsanto has submitted a bioassay on only one Coleoptera species in a family that is generally known not to be susceptible to Cry3Bb toxins.

Indirect Impacts Must be Assessed

In all likelihood, the adverse impacts of Cry3Bb on non-target 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 non-target 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 corn plants expressing *B. thuringiensis*. Similar morality 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).

Her most recently published work explores in greater detail observations from the earlier studies involving prey-mediated impacts. Such impacts can arise when a predatory insect like green lacewings feeds on a herbivore that has, in turn, been feeding on *Bt* corn plants. In both the earlier and new work, 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 some cases higher than expected. In general, 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 most 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 experimental 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).

The authors 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). [Most standard *Bt* foliar insecticides break down from exposure to sunlight in 24 to 48 hours, limiting the time non-target organisms are exposed to them].

C. Mammalian and Human Health Risks

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, an endotoxin from *Bt.tenebrionis*. Still, the studies on file are routine toxicological feeding experiments and were not designed to detect mammalian health hazards associated with gene flow or silencing, pleiotropic effects, the spread of antibiotic resistance, allergenicity, or the creation of more pathogenic strains of bacteria or viruses in the mammalian digestive system. In addition, the Ecogen studies were carried out with *Bt.t.* 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 registration application, Monsanto asserts that the "two Cry3Bb proteins described in this registration request share 99.2% and 99.1% amino acid sequence identity with the Cry3Bb1, the holotype Cry3Bb protein present in Raven." While reassuring on the surface, Monsanto's patent application covering the underlying technology also states that, in the case of Cry1 proteins, the "deletion of only 4 amino (pMON5448) acids resulted in a complete loss of activity" against Lepidopteran insects.

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

1. 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.
2. Similarity of Cry3Bb proteins to other Cry proteins and *Bt* insecticides, especially the Ecogen product Raven, which contains a mixture of two Cry3 proteins and

Cry1Ac.

3. A 40 year history of safe use of *Bt* insecticides and Cry proteins.

We concur that there are very wide margins of safety in terms of acute poisoning when mammals ingest Cry proteins, including in all probability Cry3Bb proteins. Acute toxicity should be investigated through dermal, inhalation and dietary routes of exposure. Except among people highly exposed occupationally, acute toxicity is not likely to be the human health endpoint of primary concern.

On the second point, not enough is known about the degree of similarity or differences between Cry3Bb proteins and other Cry proteins in terms of the health effects of greatest concern, for example allergenicity, digestive system impacts, gene silencing and likelihood and nature of gene flow. Even less is known about differences across the Cry3Bb proteins associated with different transformation events.

Third, the American population has not been extensively exposed to any Cry proteins over 40 years, and 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 foliar *Bts* as typically used on fruit and vegetable farms. Residues are long-gone by the time the produce reaches the consumer.

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

We know now that truncated *Bt* toxins in corn tissues and potatoes can behave differently in the environment and digestive system of mammals than foliar *Bts*. Most of the human health data supporting *Bt*-transgenic plants was conducted with foliar *Bt* products, and hence are not adequate, by themselves, in the assessment of the risks following ingestion of the form of *Bt* in transgenic plants.

Understanding the Cry3Bb Transgene

The registration application contains detailed information on the transgene that must be reviewed by experts in the relevant fields. Other key information is contained in the Monsanto patent application (Patent number 5,763,241, June 9, 1998). According to Monsanto's *Bt.t.* patent –

> "Although the Coleopteran-type toxins and the Lepidopteran-type toxins are derived from *Bacillus thuringiensis*, there are significant differences between the toxin genes and the toxin proteins of the two types…
>
> "…although [the] genes may be evolutionarily related, they are quite distinct in both nucleotide and amino acid sequence."

Moreover, the patent explains that the codon-amplification process is very different compared to Cry1 *Bt* transgenics, focusing on deletions in a different terminus and leading to "… substantially different properties." This is why EPA should insist upon further research and more complete data and why, at this point, EPA should discount claims that the safety of Cry3Bb transformed corn can be inferred from Cry1 *Bt*-corn varieties and testing.

1. Antibiotic Marker Gene Flow

Recent evidence has rekindled lingering concerns over the use of antibiotic marker genes in transgenic plants. While the assessment of antibiotic resistance markers falls under the FDA's jurisdiction, we raise here some of the scientific questions that require further research.

The possibility that Cry3Bb corn could lead to the emergence of new strains of antibiotic resistant bacteria, or trigger other problems through gene flow can be evaluated only with the benefit of detailed information on the gene construct used in producing the new corn hybrids.

The gene cassette used to produce Cry3Bb corn contains two antibiotic marker genes. The 0.79 Kb *nptII* gene is from a transposon isolated from *E. coli*, and confers resistance to kanamycin and neomycin[5]. The construct also includes the 0.15 Kb *ble* gene which confers resistance to bleomycin.[6] The ZMIR14L vector also includes the 35S CaMV promoter.

When the first *Bt*-transgenic plant applications were reviewed, there was little evidence that antibiotic marker genes might trigger resistance in bacteria of significance to human health or soil quality. Now the transfer of antibiotic marker genes in plant cells to soil bacteria has been demonstrated (Gerbhard 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 (a family of common soil bacteria). They found that "transformation of naturally competent bacteria by transgenic plant DNA, even with plant homogenates, was demonstrated for the first time…Recently, horizontal gene exchange between distantly

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[5] 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.

[6] Bleomycin is an antibiotic used as an anticancer drug (Berkow, 1992). The FDA has not approved the use of the *ble* gene for use in transgenic crops.

related bacteria as well as gene exchange from bacteria to yeast, mammalian cells, and plant cells has been reported." In more recent work, the team showed that A*cinetobacter* bacteria that had taken up the antibiotic resistance gene from transgenic plants could 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).

Moreover scientists have now shown conclusively that the ease of gene flow among bacteria led to the emergence of the highly pathogenic strain *E. coli* 0157, which has killed many people in the U.S. and abroad (Upton et al., 1996; Neill, 1997; Buchanan and Doyle, 1997). In a recent review article, Ho and several colleagues summarize the significance of the proliferation of antibiotic resistance genes in plants, bacteria and the environment by observing:

> "Studies of dozens of emergent species of pathogens showed that genes for antibiotic resistance and virulence often reside in the same regions of the bacterial DNA, in plasmids or transposons, which may be passed horizontally from one species to another, picking up extra genes, recombining and generating novel combinations in the process." (Ho et al., 1998).

Until recently it was also thought that foreign DNA, such as the gene constructs in *Bt*-transgenic 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). The authors speculate that "these findings suggest transport of foreign DNA through intestinal wall and Peyer's patches to peripheral blood leukocytes and several organs." 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).

D. Maximizing the Benefits of Cry3Bb Transgenic Corn

Corn is vulnerable to significant yield losses from several insects, but the corn rootworm complex is by far the most damaging. In addition to rootworms, cutworms, and other below-ground insects, the European corn borer (ECB) is a episodic corn pest that can cause serious losses in some years from above-ground feeding inside the corn plant. Corn borers tunnel inside the corn stalk and weaken it. This causes poor growth and lodging (plants fall over making harvest difficult and the drying process uneven), and can also make plants susceptible to a range of pathogens and other insect problems.

In recent years a critical change has occurred in corn insect pressure -- the behavioral adaptation of the Western corn rootworm (WCR) to the long-effective corn-soybean rotation. Rotating corn with soybeans has been recommended for decades and widely practiced. It has been one of the great success stories in the management of a major pest through a cultural practice. Rotations and corn insect IPM reduced corn insecticide use by half from 1971 to 1998, as shown in Appendix Table 2. This progress has helped lessen water quality problems and reduced grower cash costs (for an excellent overview, see Gray and Luckman, 1994). On continuous corn in the 1990s, 90 percent or more of corn acres are treated with a soil insecticide while less than 15 percent of rotated corn has been treated.

Ironically the success of the corn-soybean rotation in limiting WCR losses set the stage for trouble. Both farmers and scientists were lulled into thinking that no other steps were needed in managing this pest. Evolutionary forces, however, found an opening. According to University of Illinois corn IPM specialist Dr. Michael Gray, economically significant Western corn rootworm damage in first-year corn following soybeans was first documented in isolated fields in the mid-1980s.

Over the next 10 years the pattern of infestation was uneven and sporadic. Some seasons, like the wet crop year in 1998, do not favor WCRs and hence mask changes in pest behavior and damage potential. But it has become clear that the Western corn rootworm had developed what scientists call *behavioral resistance* to a management-based control strategy, in this case crop rotation.

Pesticide Alternatives

There are over 25 pesticides registered as corn insecticides and about 15 are used on significant acreage. Most fall in the organophosphate (OP) family of chemistry, but several synthetic pyrethroids are also used on sizable acres.

The most widely used OPs are terbufos and chlorpyrifos. Both were applied to about 6 percent of national corn acres in 1998 at a rate just a little over one pound of active ingredient per acre. Together these two insecticides accounted for over 75 percent of total insecticide use on corn in 1998. 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. The products rarely show up as residues in the food supply as a result of pre-plant soil applications. 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 alternative to these OPs is the relatively new pyrethroid, tefluthrin (Force). This product has gained sizable market share since 1991 when it was applied to 2 percent of corn acres. In 1998 use had risen to 5 percent of national acres, just below the market leaders chlorpyrifos and terbufos (see Appendix Table 1). The average rate of application of tefluthrin is just one-tenth of a pound per acre. Given that tefluthrin is far less toxic and that it is applied at one-tenth the rate of OP products, the environmental impacts triggered by an acre-treatment with tefluthrin probably are far less than an acre-treatment with an OP.

In addition, Rhone Poulenc recently received full registration for the reduced risk insecticide fipronil, which is beginning to gain market share. 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 much lower rates and many will meet EPA's reduced risk criteria.

IPM Alternatives Abound

Despite the behavioral change in western corn rootworms, crop rotations remain the backbone of corn IPM systems. Less than 10 percent of the acres planted to corn following soybeans or another crop require an insecticide treatment. These acres also would not benefit from the planting of Cry3Bb corn.

According to Mike Gray, "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).

Gray is among land grant university entomologists that recommend rotation to a crop other than corn when rootworm beetles average 0.75 or more per plant. If rotation is not an acceptable option to a grower, then a soil insecticide should be applied. He argues for the use of transgenic insecticidal corn varieties for corn rootworm through "prescriptive use." Core components of a prescriptive use program for Cry3Bb corn would include scouting and adherence to thresholds, crop rotations, proper placement and management of refuges, and adherence to other resistance management plan provisions.

He believes that the risk of resistance emerging to *Bt*-toxins is great enough to justify the costs of developing and implementing a prescription use program with a emphasis on limiting the planting of transgenic cultivars to just those acres where they are really needed. Long-term Cry3Bb corn efficacy would be preserved by careful adherence to resistance management plans and by alternating the major corn rootworm control tactic from one year to the next, in conjunction with area-wide control strategies. Unfortunately, steps likely to maximize and sustain the benefits of Cry3Bb corn to growers are incompatible with the goal of maximizing sales and market share for the manufacturer.

E. Decision Rules and Framework

The EPA seeks comments on what is clearly a major new biotechnology. Moreover, this application is the first of two closely related *Bt*-corn applications the agency will have to act on in just the next few months[7]. Within two years, there may be as many as four different transgenic corn technologies designed to control corn rootworms moving through the EPA review process. The same issues and questions will arise as subsequent technologies move through the EPA review process.

Independent scientists and other stakeholders will need a shared base of knowledge to intelligently participate in review and consultation processes. We recognize that providing such information may require changes in current EPA administrative policies and procedures and that such changes have been resisted in the past by registrants. Still, open, credible and convincing scientific review processes do not happen behind closed doors.

As the process moves forward, we urge EPA to insist upon good science and reliable answers to legitimate questions prior to granting unrestricted approvals. Once any new pesticide or transgenic crop variety is fully registered, EPA loses most of its leverage and assumes a heavy burden when new data demonstrates the need to impose additional restrictions.

In the review of this and other major and new plant-pesticide applications, the agency must take seriously the need for a more thorough and transparent stakeholder consultation and scientific review processes. The EPA and industry must develop mutually acceptable ways to modify current policies and scientific review processes so that necessary technical and scientific information is widely available ***before*** prior to reviews and key decisions.

We appreciate the chance to submit these comments and await the agency's decisions on the pending EUP and this full registration application.

Sincerely,

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Carolyn Raffensperger SEHN	Michael Hansen CPI/CU
Charles Benbrook Consultant to Environmental Defense	Joe Mendelson Center for Food Safety

[7] Dow AgroSciences is working with Pioneer Hybrid to bring to market *Bt*-corn varieties engineered to control the or rootworm complex. Commercial introduction is planned for crop season 2001, pending regulatory approvals.

Appendix Table 1. Percent National Corn Acres Treated with Insecticides by Target Pest ("ECB" is the European corn borer) [Cells are percents; 1.62 is 1.62%]						
Active Ingredient	**Likely Target Pest**	**1971**	**1982**	**1991**	**1995**	**1998**
lambda-cyhalothrin	ECB					2.00
permethrin	ECB			2.00	4.00	2.00
dimethoate	ECB		0.00	0.28		1.00
propargite	ECB			0.18		0.50
Bt	ECB			0.40	1.00	
carbaryl	ECB	1.62	0.17			
diazinon	ECB	2.50	0.18	0.20		
esfenvalerate	ECB			0.44		
fenvalerate	ECB		0.07			
malathion	ECB	0.20				
methomyl	ECB		0.40			
methoxychlor	ECB	0.08				
Subtotal ECB		**4.4**	**0.8**	**3.5**	**5.0**	**5.5**
One-half Acreage Treated with Rootworm + ECB Products		1.4	2.4	5.7	5.5	5.0
Total % Acreage Treated for ECB		**5.8**	**3.3**	**9.2**	**10.5**	**10.5**
terbufos	Rootworm		9.40	8.00	6.00	6.00
tefluthrin	Rootworm			2.00	5.00	5.00
cyfluthrin + tebupirimfos	Rootworm					3.00
carbofuran	Rootworm	4.97	6.66	3.00	2.00	1.00
chlorethoxyfos	Rootworm					1.00
fonofos	Rootworm		6.88	4.00	1.00	1.00
phorate	Rootworm	4.53	4.57	2.00	1.00	0.50
aldrin	Rootworm	10.18				
bufencarb (RE-5353)	Rootworm	5.98				
chlordane	Rootworm	0.72				
DDT	Rootworm	0.01				
endrin	Rootworm	0.10				
ethoprophos	Rootworm		0.84			
flucythrinate	Rootworm			0.15		
heptachlor	Rootworm	2.57				
isofenphos	Rootworm		1.15			
paraquat dichloride	Rootworm		0.25			
toxaphene	Rootworm	0.19	0.37			
trimethacarb	Rootworm			0.17		
Subtotal Rootworm		**29.2**	**30.1**	**19.3**	**15.0**	**17.5**
One-half Acreage Treated with Rootworm + ECB Products		1.4	2.4	5.7	5.5	5.0
Total % Acreage Treated for Rootworm		**30.6**	**32.5**	**25.0**	**20.5**	**22.5**
chlorpyrifos	Rootworm + ECB		4.13	9.00	7.00	6.00
bifenthrin	Rootworm + ECB			0.34	1.00	2.00
fipronil	Rootworm + ECB					1.00
parathion-methyl	Rootworm + ECB	0.06	0.19	2.00	3.00	1.00
disulfoton	Rootworm + ECB	0.70	0.01			
monocrotophos	Rootworm + ECB		0.07			

Active Ingredient	Likely Target Pest	1971	1982	1991	1995	1998
oxydemeton-methyl	Rootworm + ECB		0.47			
parathion	Rootworm + ECB	2.06				
Subtotal Dual Target		**2.8**	**4.9**	**11.3**	**11.0**	**10.0**
Sum of % Acres Treated with All Chemicals, All Pests*		**36.4**	**35.8**	**34.2**	**31.0**	**33.0**

* Total percent acres treated with all insecticides overstates the percent of total national corn acreage to a small degree since some acres are treated with an insecticide targeted for ECB control as well as a rootworm insecticide.

Source: Compiled by Benbrook Consulting Services from field crop agricultural chemical use data, National Agricultural Statistics Service, USDA, multiple years.

Appendix Table 2. Pounds Applied of Corn Insecticides by Year and Target Pest ("ECB" is the European corn borer)						
Active Ingredient	**Likely Target Pest**	**1971**	**1982**	**1991**	**1995**	**1998**
dimethoate	ECB		1,916	78,000		244,000
propargite	ECB			146,000		173,000
permethrin	ECB			207,000	237,000	129,000
lambda-cyhalothrin	ECB					23,000
Bt	ECB			3,430	6,410	
carbaryl	ECB	1,649,000	166,027			
diazinon	ECB	1,991,000	174,034	116,000		
esfenvalerate	ECB			12,000		
fenvalerate	ECB		8,505			
malathion	ECB	114,000				
methomyl	ECB		155,181			
methoxychlor	ECB	92,000				
Subtotal ECB Only		**3,846,000**	**505,663**	**562,430**	**243,410**	**569,000**
One-half Pounds Applied with Rootworm + ECB Products		828,000	2,085,992	3,771,000	2,797,500	2,229,500
Total Pounds Applied for ECB		**4,674,000**	**2,591,655**	**4,333,430**	**3,040,910**	**2,798,500**
terbufos	Rootworm		8,660,113	5,986,000	4,277,000	5,043,000
carbofuran	Rootworm	2,681,000	5,205,320	2,278,000	946,000	542,000
fonofos	Rootworm		5,101,382	2,890,000	786,000	499,000
tefluthrin	Rootworm			244,000	286,000	368,000
tebupirimfos	Rootworm					249,000
phorate	Rootworm	2,661,000	3,777,715	1,306,000	750,000	158,000
chlorethoxyfos	Rootworm					122,000
cyfluthrin	Rootworm					12,000
aldrin	Rootworm	7,759,000				
bufencarb (RE-5353)	Rootworm	3,575,000				
chlordane	Rootworm	842,000				
DDT	Rootworm	4,000				
endrin	Rootworm	30,000				
ethoprophos	Rootworm		675,705			
flucythrinate	Rootworm			6,000		
heptachlor	Rootworm	1,104,000				
isofenphos	Rootworm		1,329,418			
paraquat dichloride	Rootworm		73,826			
toxaphene	Rootworm	182,000	601,335			
trimethacarb	Rootworm			121,000		
Subtotal Rootworm		**18,838,000**	**25,424,814**	**12,831,000**	**7,045,000**	**6,993,000**
One-half Pounds Applied with Rootworm + ECB Products		828,000	2,085,992	3,771,000	2,797,500	2,229,500
Total Pounds Applied for Rootworm		**19,666,000**	**27,510,806**	**16,602,000**	**9,842,500**	**9,222,500**

Active Ingredient	Likely Target Pest	1971	1982	1991	1995	1998
chlorpyrifos	Rootworm + ECB		3,885,190	6,716,000	4,531,000	4,008,000
parathion-methyl	Rootworm + ECB	15,000	28,090	812,000	1,024,000	275,000
bifenthrin	Rootworm + ECB			14,000	40,000	113,000
fipronil	Rootworm + ECB					63,000
disulfoton	Rootworm + ECB	312,000	4,917			
monocrotophos	Rootworm + ECB		60,172			
oxydemeton-methyl	Rootworm + ECB		193,615			
parathion	Rootworm + ECB	1,329,000				
Subtotal Dual Target		**1,656,000**	**4,171,983**	**7,542,000**	**5,595,000**	**4,459,000**
Pounds Applied for All Chemicals, All Target Pests		**24,340,000**	**30,102,460**	**20,935,430**	**12,883,410**	**12,021,000**

Source: Compiled by Benbrook Consulting Services from field crop agricultural chemical use data, National Agricultural Statistics Service, USDA, multiple years.

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