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BIOTECHNOLOGY

RESEARCH PROGRAM

RESEARCH AND DEVELOPMENT

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Biotechnology Research Program

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Contents

Acronyms

ARS	Agricultural Research Service
BLM	Bureau of Land Management
BPPD	Biopesticides and Pollution Prevention Division
Bt	*Bacillus thurigeiensis*
CRADAs	Cooperative Research And Development Agreement
DOI	Department Of the Interior
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
GIS	Geographic Information System
GM	Genetically Modified
GMO	Genetically Modified Organism
GSF	National Research Center for Environment and Health
IPA	Intergovernmental Personnel Act
IRM	Insecticide Resistance Management
NIAID	National Institute of Allergy and Infectious Diseases
NAS	National Academy of Sciences
NCEA	National Center for Environmental Assessment
NCER	National Center for Environmental Research
NERL	National Exposure Research Laboratory
NHEERL	National Health and Environmental Effects Research Laboratory
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NPS	National Park Service
NRC	National Research Council
NRMRL	National Risk Management Research Laboratory
OPP	Office of Pesticide Programs
OPPT	Office of Pollution Prevention and Toxics
OPPTS	Office of Prevention, Pesticides, and Toxic Substances
ORD	Office of Research and Development
PCR	Polymerase chain reaction
PIP	Plant Incorporated Protectant
R&D	Research and Development
RFA	Request for Assistance
STAR	Science to Achieve Results
TSCA	Toxic Substances Control Act
USDA	U.S. Department of Agriculture

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Foreword

In accordance with the Environmental Protection Agency's (EPA's) mission to minimize risks to human health and to safeguard ecological integrity, the EPA Office of Prevention, Pesticides, and Toxic Substances (OPPTS) is committed to assessing and mitigating any risk posed by biotechnology-derived crops. Consequently, the Biotechnology Initiative Steering Committee prepared this plan to guide the implementation of an integrated Biotechnology Research Program. This plan was formulated with the goal of improving the science needed to inform Agency decisions about the risk and safety of the products of biotechnology.

Many of the scientific concerns about the risk and safety of genetically engineered crops were raised in three reports[1] from the National Research Council (NRC) of the National Academy of Sciences (NAS). These scientific concerns served as a departure point for the development of this implementation plan. There are three main areas of concern: (1) risks to human health of the allergenicity of biotechnology products, (2) risks to natural ecosystems of gene transfer from engineered organisms to natural species in the wild, and (3) mitigating the development of resistance and of gene transfer. The breadth of scientific issues related to the safety of bioengineered crops, as well as limited resources, make it imperative to have a focused, integrated, cross-Office of Research and Development (ORD) research program that coordinates with ongoing research programs in other federal agencies. We also needed to ensure that the work funded under this research initiative complements work underway in other sectors.

The Biotechnology Initiative Steering Committee convened a workshop to meet with colleagues from OPPTS and key senior officials from other federal agencies, the National Academy of Sciences, and the European Union to discuss the EPA's proposed Biotechnology Research Program. During our discussions, we wanted to determine the following: (1) Is our proposed program scientifically sound and relevant? (2) Does it complement related efforts elsewhere? and (3) Is the proposed work appropriate for EPA? The conclusion by non-EPA attendees was that our proposed research program met these objectives. We also discussed opportunities for inter-agency collaboration.

The Plan was then peer reviewed by the Board of Scientific Counselors May 13, 2004. This document reflects their comments.

[1]NRC, 2000. Genetically Modified Pest-Protected Plants: Science and Regulation, Washington, DC: National Academy Press.
NRC, 2001. Ecological Monitoring of Genetically Modified Crops: A Workshop Summary, Washington, DC. National Academy Press.
NRC, 2002. Environmental Effects of Transgenic Plants: The Scope and Adequacy of Regulation, Washington, DC: National Academy Press.

Evaluating Potential Risks Associated with Biotechnology Products

Background

Biotechnology presents a wealth of opportunities to improve crop productivity, nutritional value, and resistance to pests and other stresses. However, there are potential risks to human health, natural ecological systems, and existing agricultural systems that need to be evaluated so that the products of biotechnology can be properly regulated. Currently, EPA regulates several biotechnology products (e.g., pesticides, either produced by plants or by microorganisms, and non-pesticidal substances such as industrial enzymes, biosensors, and bioremediation agents produced using microorganisms). While discussions continue about whether EPA's scope of regulation should be broadened to include animals (e.g., insects) that produce pesticidal substances and plants and/or animals that produce non-pesticidal substances, no such products are currently under review by EPA.

From a human-health perspective, a major concern is the potential toxicity and allergenicity associated with genetically modified foods. Potential adverse effects can arise from intended modifications (i.e., from the pesticidal substance) or from unintended effects resulting from the production of an unexpected metabolite. To date, the products approved by EPA for use in human food have all been proteins that degrade rapidly and from which no chronic effects would be expected. This approach has been accepted by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel. However, some members of the public have raised the concern that proteins not previously part of the food supply could be allergens. It is, however, well accepted that the genetic material itself will not cause an acute or chronic toxic effect; thus DNA has been exempted from tolerance.

The regulation of biotechnology products is also intended to minimize the risks to human-managed (i.e., agricultural) and natural ecosystems. Such risks are associated with the consequences of unintended release of genetically modified plants or their bioengineered genes. For example, there are concerns about the ecological impacts resulting from replication and persistence of transformed organisms that could out-compete native species in a given environment. In terms of the risks to agricultural systems, there are potentially adverse long-term consequences of evolved resistance to the biotechnology product. Pest resistance could

render related conventional products (e.g., the spores of *Bacillus thurigiensis* [*Bt*] used as pesticidal sprays) ineffective, reducing crop productivity or necessitating increased usage of conventional pesticidal applications (which also threatens ecosystem health).

Goal

The goal of the Biotechnology Research Program is to provide the scientific information needed to assess and manage the risks of biotechnology. The research program will accomplish this by providing the tools needed to generate information about biotechnology products, by generating the knowledge needed to understand the nature and magnitude of potential risks and benefits resulting from the use of biotechnology products in commerce, and by providing the means to prevent or control such risks. At this time the focus is on the risk from plant incorporated protectants (PIPs).

Research Approach

The Agency Challenge

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) evaluates the environmental risks posed by pesticides and chemicals to safeguard all Americans, including children and other vulnerable members of the population, as well as our most threatened species and ecosystems. Within OPPTS, the Office of Pesticide Programs (OPP) regulates the use of all pesticides in the United States and establishes maximum levels for pesticide residues including genetically engineered pesticides. While it is not anticipated that biotechnology products will pose new types of risks, these new products are often on the cutting edge of science and regulatory policy; and research is needed to ensure that their safety can be

appropriately evaluated. Also, within OPPTS, the Office of Pollution Prevention and Toxics (OPPT) regulates the use of industrial chemicals and certain biotechnology products, such as microorganisms used in the manufacture of specialty chemicals and bioremediation agents. OPPT also implements the Pollution Prevention Act and hence has an interest in biotechnology product stewardship that would lead to "green" chemicals. OPPT has an emerging interest in certain transgenic plants for uses such as phytoremediation and enhanced wood production although OPPT does not implement regulatory oversight in this area at this time. Currently, most biotechnology risk assessment research concerns in OPPTS are affiliated with pesticidal products.

In assessing safety, the basic framework for pesticide regulation provides guidance as to the nature of any new risks. EPA has recognized that PIPs (or genetically engineered plants which produce their own pesticides) represent potentially different risks from traditional, chemical pesticides. For example, while there is very low worker exposure and no spray drift, there are issues regarding gene flow to wild relatives and pollen movement spreading the new pesticides to non-altered crops. In addition, the level of protein produced is very small; but, because proteins can be allergens and even low levels of a new protein might lead to sensitization and eventual allergic reactions, special emphasis on allergenicity is given to evaluation of these products.

With respect to environmental risk, effective tools and methods are needed to minimize the likelihood of negative ecological effects such as the following:

Ecosystems

(a) harm to non-target species, such as soil organisms, non-pest insects, birds, and other animals;
(b) disruptive effects on specific biotic communities;
(c) irreparable loss of changes in species diversity and genetic diversity within species.

Agri-Systems

(a) creating new or more vigorous pests and pathogens;
(b) exacerbating the effects of existing pests through hybridization with related transgenic plants or microorganisms.

Both

(a) pleiotropic or epistastic effects on plant physiology due to emerging metabolic engineering approaches. [These manipulations, found in current commercialized transgenic organisms, may result in unintended effects in host plants or non-target plants that may inadvertently receive the transgene.];
(b) rapid development of resistance to the engineered crop by target pests that may result in greater use of more harmful pesticide products over the long term.

With respect to protecting human health, EPA must assess whether pesticides derived through biotechnology are at least as safe as their conventional counterparts; and the EPA must ascertain that any levels of additional or unique risk are clearly defined. A significant challenge may occur in the future if transgenic technology results in more substantial and complex changes in exposures and/or if such technology results in compounds that are more toxic. It is important to note that many pesticidal substances such as phenols and aldehydes occur naturally in plants.

Progress also needs to be made in developing definitive methods for the identification and characterization of protein allergens. EPA needs to be able to estimate accurately the levels of exposure to the genetically engineered products that are released in the environment, and EPA needs the means to evaluate whether such exposures are potentially harmful. Finally, EPA needs to find and evaluate ways to prevent or mitigate identified risks.[2] Contributing to the effectiveness of this program are the integration of science activities across the risk assessment paradigm and strong interaction with OPPTS. The proposal also includes the use of workshops involving scientists with appropriate expertise from academia, industry, and other government institutes. These workshops are designed to develop broad consensus with respect to research needs and strategies and to coordinate research efforts not just within EPA, but with the National Institutes of Health (NIH), the Food and Drug Administration (FDA), the U.S. Department of Agriculture (USDA), and the scientific community at large.

Key Science Issues with Respect to Biotechnology Products

A problem-directed research program has been developed that focuses on five key issues: (1) the potential allergenicity of proteins introduced into the food supply by gene transfer; (2) the potential ecological effects of biotechnology products on non-target species; (3) the spread of transgenes to the natural environment via seed dispersal or gene flow to sexually compatible relatives; (4) the development of pesticide resistance in the target species; and (5) strategies for identifying the risks of concern and effective risk management technologies to mitigate these risks when monitoring studies indicate unintended adverse consequences are likely to occur.

[2]Under both FIFRA and TSCA, EPA also has an obligation to consider the potential benefits of biotechnology pesticide products.

1. Potential Allergenicity of Proteins Introduced into the Food Supply by Gene Transfer

There are no valid animal models for predicting allergenicity and the long-term effects from consuming crops containing PIPs and other biotechnology products. EPA and FDA are currently using several screening assays designed to compare new substances to the properties of known dietary allergens. However, current screening criteria will not identify some substances using this approach.

Research Need:
Develop an understanding of the basis for human sensitization to dietary allergens, develop methods to assess dietary allergenicity, and apply such methods to monitor human populations exposed to genetically modified (GM) foods for increased allergenicity.

2. Potential Ecological Effects of Biotechnology Products on Non-Target Species

(a) Questions have been raised about the effects of PIPs on non-target species. Besides potential direct toxicity to insects, concerns have been raised that PIPs are so effective at controlling insect pests that bird populations might be adversely affected because of a lack of food. However, recent data indicate that the number of song birds increase in areas where *Bt* cotton use is high. The data suggest *Bt* cotton may increase available food supply as well as reduce avian mortality (Brandt, et al., EPA Public Interest Finding and Review of the Benefits for Monsanto Company's Corn Rootworm-Protected Field Corn Product [Event MON 863], February 19, 2003).

Research Need:
Baseline and monitoring studies to assess the structure and dynamics of populations of beneficial organisms and insect pests in and around crops grown using conventional, organic, and biotechnology pest-management tools.

(b) There are few standardized, validated, affordable field assays for assessing exposure and effects on non-target species. This is a significant gap in our understanding of potential unintended consequences.

Research Needs:
(i) Develop methods to evaluate the scale over which biologically meaningful exposures to non-target species may occur and the mechanisms (e.g., pollen, insect movement, predation) that mediate this exposure;

(ii) Develop an understanding of the mechanisms that lead to adverse effects in non-target species (e.g., honeybees) as a basis to develop standardized, validated, affordable field assays for testing for effects on important species; and
(iii) Develop an understanding of the effects of metabolism in genetically altered plants as a basis to assess risk and to develop methods for testing genetically altered plants for such changes.[3]

(c) Questions have been raised about the effects of genetically engineered microorganisms on non-target species. Recombinant bacteria have been reviewed by OPPTS for use in biosensors and bioremediation applications as they apply to hazardous wastes such as polynuclear aromatic hydrocarbons and polychlorinated byphenyls.

Research Need:
Genetically engineered microorganisms have raised issues of non-target effects and opportunistic pathogenicity for aquatic species, wildlife, and humans. Better methodologies to detect such effects prior to release and to monitor for ecological effects in the field are needed.[4]

[3]Increasingly, transgenics are being developed with altered metabolic or signaling pathways, and such constructs may have secondary effects on plant physiology due to pleiotropy or epistasis. For instance, plants are being developed for altered metabolism (chemical production, altered wood production, salt tolerance, and phytoremediation), as well as for novel pest resistance.

[4]OPP has reviewed genetically engineered bacteria for use as pesticides. OPP has relied on the existing data requirements for microbial pesticides although additional research was conducted by ORD in the past. Future research in this area would be of value. This need is not being addressed in this research plan.

3. Escape of Altered Plants to the Natural Environment and the Likelihood and Effects of Gene Transfer

(a) The ability of some crops to transfer introduced genes through hybridization to wild and/or weedy relatives can make the assessment of effects on non-target organisms very difficult. There is one report on *Arabidopsis thaliana*, which does not normally outcross, in which a transgenic plant "... showed a dramatically increased ability to donate pollen to nearby wildtype mothers compared with *Aribidopsis thaliana* mutants expressing the same mutant allele as the transgenic plants" (Bergelson, et al.,1998, Promiscuity in Transgenic Plants, *Nature* v. 395, p. 25).

Research Need:
Explore the factors influencing gene transfer rates to provide a basis for better assessments.

(b) Some transgenes may have a limited persistence due to their insert locations, characteristics of the genetic cassette, or the plants or microorganisms themselves. This could result in limiting exposure to the gene product.

Research Need:
Develop methods to evaluate the persistence/maintenance of transgenes in plants and microorganisms, the exposure to those gene products, and evaluate whether environmental conditions or common stressors influence this process.

4. The Development of Pesticide Resistance in the Target Insect Species

Laboratory and small-scale field testing has been the basis for evaluating the likelihood of resistance development. Long-term, extensive monitoring has not been conducted to determine whether the effects predicted in such tests actually occur in the field.

Research Needs:
(i) Develop models to estimate the likelihood of the development of insect resistance that incorporate detailed biological information for pest species, including gene flow and mating patterns in the wild, geographic and chromosomal distribution of resistant alleles, and their additive and non-additive effects on resistance under selective pressures in the field; and
(ii) Perform monitoring studies of gene transfer, the development of resistance to PIPs by target pests, and effects on non-target species (as noted in 2b) to allow field validation of conclusions regarding transgenic plants with new pesticide traits.

5. Risk Management

The effectiveness of management strategies to avoid key risks for extended periods and the effectiveness of risk management technologies to mitigate risks associated with unintended adverse consequences must be evaluated and expanded for new PIP crops.

Research Needs:
(i) Improve strategies to identify the key risks of concern, develop evaluation schema to understand the effectiveness of management strategies, and develop effective risk management technologies to mitigate these key risks when monitoring studies identify the presence of unintended adverse consequences; and
(ii) Explore the application of socioeconomic methods such as benefit-cost analysis and life-cycle analysis to better understand issues related to the public acceptance of genetically altered products and to evaluate whether the genetic alterations produce new organisms that are not substantially equivalent to currently existing ones.

Performance Results and Expected Benefits

By FY 2008, this research program will result in an improved capability to assess the risks of allergenicity from GM food, improve the capability to assess the ecological risks associated with genetically modified organisms (GMOs), and develop tools to understand and better manage gene transfer and resistance. Program performance will be measured in the following ways:

(a) the use of research products by OPPTS in the registration and re-registration process, both in hazard identification and risk assessment, and in setting risk management requirements for registration;
(b) general acceptance of these methods by other regulatory agencies; and
(c) public acceptance of EPA's approach to regulating GM crops.

Projected Outputs

The budget for the biotechnology program allows EPA to begin to address these important issues, but it is not sufficient to address them all. Consequently, ORD used the priorities provided by the program office and a scientific-program view to bring together the research plan described here. The research will be limited to PIPs. It will not cover genetically engineered microorganisms and plants genetically engineered for non-pesticidal purposes. Those areas which are not accommodated at this time may be revisited as a part of the regularly scheduled progress reviews and incorporated in the program as resources allow.

The identification of the specific projects that will be performed by each ORD Laboratory and Center, as well as those that will be identified for funding by the Science to Achieve Results (STAR) program, have been developed by the Biotechnology Initiative Steering Committee and are included in Appendix A. The anticipated outputs include the following:

Endpoint	NAS 2000	NAS 2002A	NAS 2002B
HEALTH EFFECTS			
Allergenicity Detection Methods	X		X
ECOLOGICAL EFFECTS			
Non-target Testing Field Surveys & Monitoring	X	X	
Metabolic changes (plieotropic or secondary effect) impact on non-targets and/or human health	X		X
Impacts of gene flow--beyond "it occurs"	X		
Resistance management strategies	X	X	
Monitoring (long-term ecological)	Need For	Strategies For	
Risk Management	X	X	

NRC (National Research Council). 2000. Genetically Modified Pest-Protected Plants: Science and Regulation, Washington, DC. National Academy Press.
NRC (National Research Council). 2002A. Environmental Effects of Transgenic Plants: The Scope and Adequacy of Regulation, Washington, DC. National Academy Press.
NRC (National Research Council). 2002B. Animal Biotechnology: Science-Based Concerns, Washington, DC. National Academy Press.

1. Models to predict dietary allergenicity from consuming crops containing PIPs and other biotechnology products will be developed and verified as a basis to assess the potential allergenicity of proteins introduced into the food supply by gene transfer;
2. Standardized, verified, and affordable assays to test for exposure and effects of PIPs on non-target species as a basis to assess the potential ecological and other effects from the use of biotechnology;
3. Explicit information on pest genetics and ecology that will validate or improve existing models to predict development of resistance;
4. The identification of factors influencing gene transfer rates to better understand the potential for altered plants to escape into the natural environment and methods to evaluate the persistence/maintenance of transgenes in plants as a basis to understand the likelihood and effect of gene transfer;
5. The likely safety of biotechnology products will be evaluated by long-term monitoring to determine whether the effects predicted in the laboratory and small-scale tests actually occur in the field as a basis for evaluating the likelihood of pesticide resistance development; and
6. Risk management strategies to provide means to mitigate risks when unintended adverse consequences occur.

Appendix A. Project Sheets

This Appendix contains project sheets which describe the work we plan to accomplish in order to address the three major concerns facing EPA: (1) allergenicity, (2) gene transfer, and (3) mitigating resistance and gene transfer. We have developed five major research areas. As the needs outpace available resources, it will not be possible to meet all the needs, nor will it be possible to fully cover each need that is addressed under this plan. The five major research areas and associated research need(s) are listed followed by project sheets that describe the related priority work EPA will cover under its initial biotechnology efforts.

Potential Allergenicity of Proteins Introduced into the Food Supply by Gene Transfer

There are no valid models for predicting dietary allergenicity and the long-term effects from consuming crops containing PIPs and other biotechnology products.

Research Need:
Develop an understanding of the basis for human sensitization to dietary allergens, develop methods to assess dietary allergenicity, and apply such methods to monitor human populations exposed to genetically modified foods for increased allergenicity.

There are two projects addressing this key research area:

1. Potential allergenicity of genetically modified organisms.

2. Solicitations through the STAR extramural program to support EPA's Biotechnology Research Program.

Project Title: **Potential Allergenicity of Genetically Modified Organisms**

Laboratory/Center: NHEERL

Program Contact: MaryJane Selgrade, (919) 541-1821, selgrade.maryjane@epa.gov

Primary Research Objective/Goal: The goal is to develop an animal model suitable for assessing potential allergenicity relative to other food proteins and for testing hypotheses regarding conditions (e.g., age, genetics) that contribute to susceptibility. This project focuses on the development of an animal model to predict allergenicity and will not address the need to monitor human populations.

Background: Biotechnology presents a wealth of opportunities to genetically engineer crops to improve productivity, enhance resistance to pests and other stressors, and provide nutritional value. However, there is growing concern that there may be risks to human health that have not been adequately explored. The biggest concern is that, as a result of the introduction of novel proteins into the food supply, biotechnology may unwittingly introduce a potent food allergen that could seriously affect the health of susceptible individuals. Currently, there is no animal model that can be used to test proteins for potential allergenicity following oral exposure, nor are there other means to readily identify proteins that might be potent allergens. Furthermore, the mechanisms underlying the development of food allergy and the factors that contribute to individual susceptibility are poorly understood.

Project Description and Critical Path: EPA sponsored a workshop jointly with FDA and NIEHS entitled "Assessment of the Allergenic Potential of Genetically Modified Foods" in December 2001 to review the state of the science and to develop research needs. The development of an animal model to assess the potential allergenicity of orally administered proteins was determined to be a major research need. Such a model or models are needed to answer the following questions:

(1) Does dietary exposure to transgenic pesticide proteins induce immune, inflammatory, and histopathology responses typical of food allergy?

(2) Is the degree of digestibility inversely related to risk of allergenicity?

(3) Is early life the most vulnerable time for dietary allergy sensitization?

(4) Does the food matrix make a difference in allergic responsiveness?

(5) How potent is the transgenic pesticide protein in the induction of dietary allergic responses (i.e., what is the dose-response relationship relative to known food allergens)?

(6) Where there is potential for both respiratory and oral exposure, what are the risks when an individual sensitized by the respiratory route ingests the protein; and what are the risks of respiratory exposure in an individual sensitized by the oral route?

The following critical path will be followed in order to address these questions:

(1) Develop a dietary allergy model in a laboratory rodent using a modification of the respiratory allergy protocols.

a. Suckling, weanling, and adult rodents (BALB/c mice or Brown Norway rat) will be exposed by gavage or fed multiple times with various doses of a known food allergen to establish the ability to induce food-allergy responses. Allergic responsiveness will be judged based on the induction of antigen specific IgE and IgA in addition to gut mucosal eosinophil influx and respiratory responses. The lung and skin are the most frequent target organs even when the route of exposure is ingestion. Experimental conditions that most closely mimic food allergy in humans will be used in subsequent studies.

b. Once the model is developed, rodents will be fed or gavaged multiple times with various doses of a prototype transgenic pesticide protein. The most likely candidate is the *Bt* toxin. Various forms of this toxin with varying degrees of digestibility will be tested. Allergic responsiveness will be assessed based on results obtained from the above studies. Appropriate positive and negative controls will be incorporated into the model.

(2) Assess the responses to the transgenic pesticide protein allergen in both a purified form and in a food matrix. The food matrix is the way in which most human ingestion will occur, and it may provide an adjuvant effect. Therefore, exposure to the purified protein alone may not be adequate to assess its potential allergenicity. Using the model protocol, rodents will be gavaged with both the purified protein and an equivalent amount of the protein in a food matrix. Comparison of the responses should provide insight into the role of the food matrix in dietary allergy.

(3) Assess the relative potency of transgenic pesticide proteins when compared to known food allergens. Using the model protocol, responses to transgenic pesticide proteins will be compared to the responses of a range (strong to weak allergy inducers) of established food allergens.

(4) Use the model to assess effects of respiratory exposure following oral sensitization and oral exposure following respiratory sensitization.

Schedule:

Publish Workshop Proceedings:	February 2003
Begin work to develop animal model:	Spring 2003 (Depends entirely on when the animal facility in the new building opens)
Hire first post-doc:	By end of 2003
Develop animal models	2005
Test *Bt* toxin & digestibility theory	2006
Demonstrate vulnerability of newborns	2008

Projected Outputs/Impacts:

FY 2008 APG:	Improved capability to assess the risks of allergenicity from genetically altered food.
FY 2003 APM:	Publish results of jointly sponsored (FDA & NIEHS) workshop.
FY 2005 APM:	Develop models and methods for assessing potential allergenicity.
FY2008 APM:	Demonstrate the vulnerability of newborns/ identify windows of vulnerability.

Project Title: **Solicitations through the STAR Extramural Program to Support EPA's Biotechnology Research Program**

Laboratory/Center: NCER

Program Contact: Elaine Francis, (202) 564-6789, francis.elaine@epa.gov

Primary Research Objective/Goal: Engage the external research community to assist EPA's understanding of the basis for human sensitization to dietary allergenicity and to develop methods to assess dietary allergenicity.

Background: EPA has developed a problem-driven Biotechnology Research Program that focuses on five key issues of most importance to the Office of Prevention, Pesticides, and Toxic Substances (OPPTS): (1) potential allergenicity of proteins introduced into the food supply by gene transfer; (2) potential ecological effects of biotechnology products on non-target species; (3) escape of altered plants to the natural environment and likelihood and effects of gene transfer; (4) the development of pesticides resistance in the target species; and (5) strategies for identifying the key risks of concern and effective risk management technologies to mitigate these key risks when the monitoring studies indicate unintended adverse consequences are likely to occur.

The extramural grants program through the Science to Achieve Results (STAR) program will issue a solicitation(s) for proposals from scientists in academia and not-for-profit organizations for research that will complement our intramural research program. Those proposals that are of highest quality, of greatest relevance to the Agency, and that provide a balance to EPA's Biotechnology Research Program's portfolio will be awarded.

Project Description and Critical Path: The Biotechnology Research Program framework has identified a number of research needs under each of the five key issues identified above. ORD's intramural program does not have the capacity nor the capability to address all of these needs. ORD will use its STAR program to engage the academic and not-for-profit community to help address the critical data gaps. USDA has been issuing solicitations for extramural proposals for the last several years in four of the five key areas in which EPA is interested. The issue not covered in the USDA solicitations is the one dealing with potential allergenicity. This is also an area which has limited intramural capacity and is an area of extremely high importance to OPPTS. It seems most appropriate, therefore, to consider this issue as the highest priority for which a solicitation will be developed. NCER will work with USDA, NIAID, and NIEHS to determine whether they are interested in issuing a joint solicitation. If they are not, NCER will issue an Request for Assistance (RFA) by itself. The specific topics of interest related to the key issue of allergenicity will be developed working with the Biotechnology Research Planning Committee to ensure that the solicitation does not duplicate what can best be done intramurally and that it targets specific topics of highest priority that would complement the intramural research.

Schedule:

Potential partners from other federal agencies who are interested in leveraging resources and issuing a joint solicitation will be sought starting in FY03. A solicitation supported by either EPA alone or by EPA in conjunction with other federal agencies will be issued in FY04. Awards of grants will be made in FY04-05. If EPA issues a solicitation by itself, then three years' worth of resources will be needed to support a reasonable extramural program. Therefore, only one solicitation would be issued in the FY04-06 period. If however, other federal partners are interested in leveraging resources, it may be possible to issue a second solicitation during this period of time.

Projected Outputs/Impacts:

FY 2004 APM:	Issue a solicitation for research proposals.
FY 2008-2009 series of APMs:	Reports on individual grant results of extramural research.

Potential Ecological Effects of Biotechnology Products on Non-Target Species

Questions have been raised about the effects of PIPs on non-target species.

Research Need:
Baseline and monitoring studies to assess the structure and dynamics of populations of beneficial organisms and insect pests in and around crops grown using conventional, organic, and biotechnology pest-management tools.

There is one project addressing this key research need:

1. Non-target and ecosystem impacts from genetically modified crops containing plant-incorporated protectants (PIPs).

There are few standardized, validated, affordable assays for assessing exposure and effects on non-target species.

Research Needs:
(i) Develop methods to evaluate the scale over which biologically meaningful exposures to non-target species may occur and the mechanisms (e.g., pollen dispersal, insect movement, predation) that mediate this exposure;
(ii) Develop an understanding of the mechanisms that lead to adverse effects in non-target species (e.g., honeybees) as a basis to develop a standardized, validated, affordable assay for testing for effects on important species; and
(iii) Develop an understanding of the effects of metabolism in genetically altered plants as a basis to assess risk and to develop methods for testing genetically altered plants for such changes. [Increasingly, transgenics are being developed with altered metabolic or signaling pathways, and such constructs may have secondary effects on plant physiology due to pleiotropy or epistasis. These plants are being developed for altered metabolism (chemical production, altered wood production, salt tolerance, and phytoremediation), as well as for novel pest resistance.]

Questions have been raised about the effects of genetically engineered microorganisms on non-target species.

Research Need:
Genetically engineered microorganisms have raised issues of non-target effects and opportunistic pathogenicity for aquatic species, wildlife, and humans. Better methods to detect such effects prior to release and to monitor for ecological effects in the field are needed. [OPP has reviewed genetically engineered bacteria for use as pesticides. OPP has relied on the existing data requirements for microbial pesticides although additional research of value was conducted by ORD in the past, and future research in this area would be of value.]

One project was developed to meet needs 2bi and 2bii. No discrete projects were developed to meet needs 2biii and 2c. However, the results from other research areas (e.g., 2a and 3) will likely include or necessitate the development of the needed assays for the experimental approach.

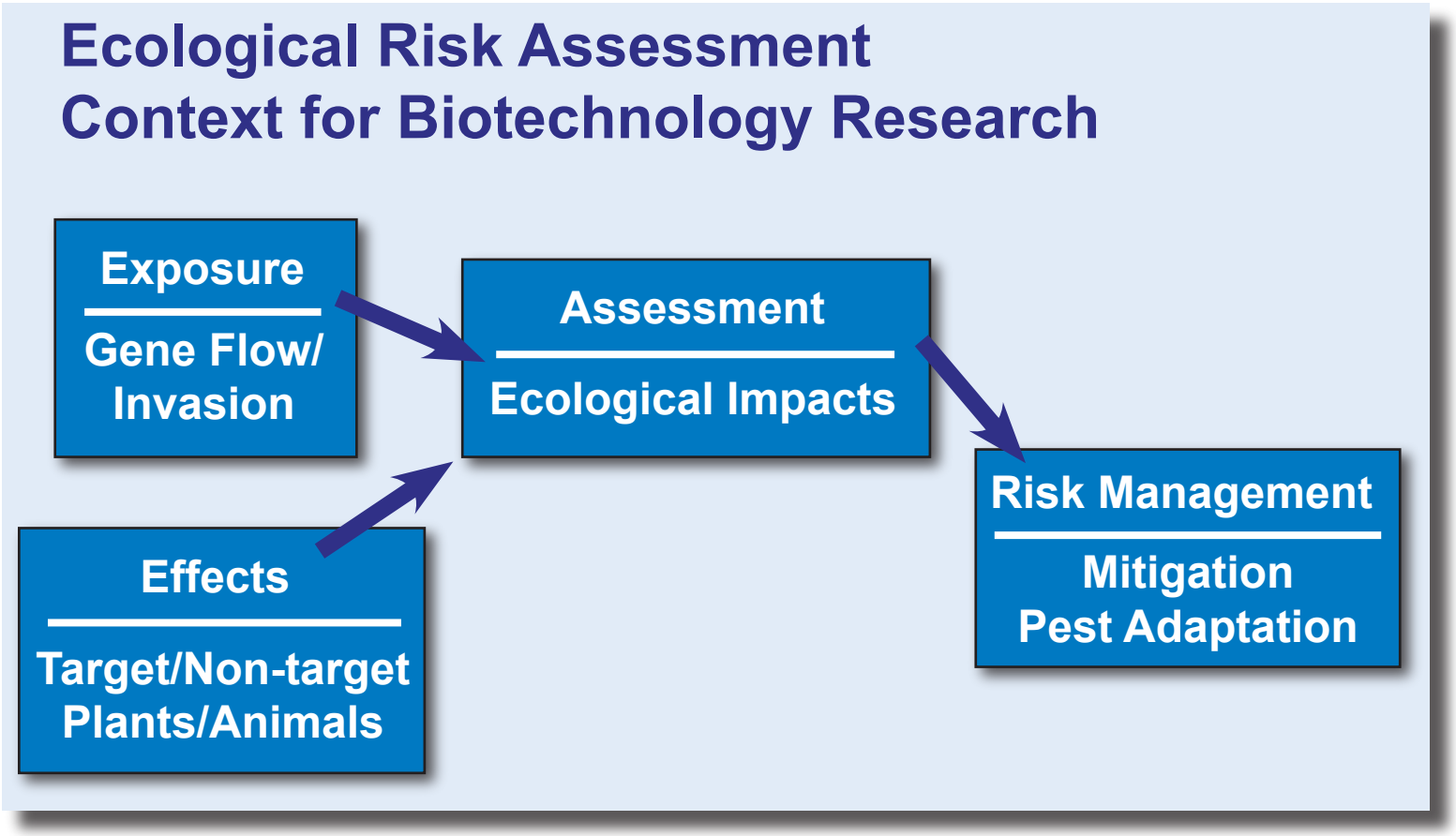
Project Title: **Non-Target and Ecosystem Impacts from Genetically Modified Crops Containing Plant-Incorporated Protectants (PIPs)**

Laboratory/Center: NCEA

Program Contact: Bob Frederick, (202) 564-3207, frederick.bob@epa.gov

Primary Research Objective/Goal: The goals are to develop methods to measure direct effects and secondary trophic level effects on non-target organisms, to characterize assessment endpoint(s), and to develop predictive strategies to evaluate potential ecosystem-level effects.

Background: The risk of unintended and unexpected adverse effects on non-target organisms and ecosystems is a key issue in environmental risk assessment of PIP crop plants. While there has been considerable examination of the effects of *Bt* crops on certain non-target organisms, particularly using species-specific laboratory testing, more work is needed to examine effects (or lack of effects) at the field level. Field censuses documenting species diversity and abundance are important, but they require appropriate baseline studies against which to compare results from agro- and other-ecosystems containing PIP crop plants. This research will be structured to answer the questions (1) What are the potential ecological and other effects from the use of biotechnology products on non-target species? and (2) What are the effective strategies for identifying the key risks of concern and effective risk management technologies to mitigate these key risks when the monitoring studies indicate unintended adverse consequences?



Project Description and Critical Path: NCEA will develop standardized and streamlined methodologies to conduct baseline assessments of agricultural and near-field ecosystems non-target species diversity and abundance. In addition to broad field censuses, particular plant and animal species may serve as indicators of environmental impacts of PIP crop plant releases. Bioindicators may be efficient and sensitive tools to predict adverse impacts during product evaluation as well as to measure the long-term impacts of environmental releases. NCEA will identify a suite of ecologically significant indicator species at different trophic levels in, for example, *Bt* corn and cotton agro-ecosystems.

While species presence and/or abundance could offer valuable indicators of non-target effects, potential effects of PIP crop plants should also be examined in terms of ecosystem functions. Relevant ecosystem functions could include nutrient cycling, predator-prey interactions, and the provision of non-target wildlife habitat. We plan to develop methods and conduct field assessments of these potential ecosystem-level effects in PIP crop plants but expect the results will be relevant to environmental releases of other modified crop plants as well.

Schedule:

FY06 APM:	Report on a conceptual framework for assessing the ecosystem scale effects genetically modified crops (NCEA).
FY08 APM:	Report on effects of *Bt* crops on agro-ecosystem functions as a risk assessment tool (NCEA).

Projected Outputs/Impact: The baseline survey methodology and suite of bioindicators will provide a needed framework for on-going research requirements to registrants and will be valuable in regulatory decision-making and long-term environmental monitoring of PIP crop plants by the EPA Office of Pesticide Programs and possibly other offices. Results of the ecosystem function studies will allow more meaningful interpretation of monitoring results and will support more accurate assessment of ecosystem impacts from PIP crop plants.

Project Title: **Genetic Evaluation of Long-Term Risks of Plant-Incorporated Protectants: Exposures and Effects on Non-Target Species**

Laboratory/Center: NERL

Program Contact: Mark Bagley, (513)569-7455, bagley.mark@epa.gov

Background: EPA has been given the mandate to assess the environmental risks of GM crops based on the best available information. Presently, however, the information available to make meaningful decisions about long-term environmental risks of GMOs is limited. As crops with new traits and stacked transgenes head through the development pipeline, both potential benefits and potential risks may increase. It is important that EPA continue to evaluate the usefulness of its current data to ascertain long-term ecological risks and to develop new types of data where necessary. A long-term risk of special concern is unintended collateral effects of PIPs on non-target species. A thorough assessment of the effects of GM crops on surrounding ecosystems is urgently needed, including research into the types of baseline data required for effective monitoring of ecosystem health.

Project Description and Critical Path:

Exposure of PIPs to non-target organisms. Based on the phylogenetic relationships between the target pest species and non-target species, as well as the mode of action of the PIP transgene, we will identify indicator species for exposure monitoring in and around *Bt*-corn and *Bt*-cotton agro-ecosystems. Building on our experience in developing targeted gene expression assays as indicators of endocrine disrupting compounds and other environmental contaminants, we will develop quantitative PCR assays for exposure to *Bt* compounds. Target loci for gene expression analysis will be identified by a combination of differential display, screening subtractive cDNA libraries, and identification of homologous genes from well-described insect species, including *Drosophila melanogaster*. General stress response genes and PIP-responsive genes will be differentiated, and specific assays will be developed.

Genetic monitoring of non-target organisms. We will evaluate the population genetic structure of indicator species (identified as part of the gene expression assessment) in and around sets of *Bt*-corn and *Bt*-cotton test plots in order to establish the baseline conditions for monitoring ecosystem changes due to localized *Bt* toxin exposures. Gene flow and genetic diversity parameters within and among populations will be evaluated by microsatellite analysis in relation to planting histories for GM and non-GM crops.

By taking similar measurements over multiple insect generations and plant cropping cycles, we will be able to monitor changes in genetic diversity, effective population sizes, and effective numbers of migrants through time. In conjunction with the exposure assays, we will evaluate changes in population sizes over a period of at least four years as an initial assessment of the utility of this methodology for long-term monitoring.

Schedule:

FY03 1. Review and report on scientific literature related to genetic monitoring.
2. Begin screening for candidate genes for gene expression assays for non-target insects.
3. Put contracts in place and begin pilot-scale assessments of non-target insect populations around corn agro-ecosystems.

FY04 1. Produce report on development of PCR-based indicators of resistance evolution.
2. Complete development of microsatellite markers for three non-target insect species.
3. Continue development and assessment of gene expression assays for non-target organisms.
4. Continue and expand monitoring near corn agro-ecosystems.
5. Initiate full-scale monitoring of non-target insect populations near cotton agro-ecosystems.

FY05 1. Collect year 2 (cotton) and year 3 (corn) population genetic data for non-target insect populations.
2. Complete development and evaluation of gene expression assay for non-target insects.
3. Coordinate and host ORD/OPP workshop.

FY06 1. Collect year 3 (cotton) and year 4 (corn) population genetic data for non-target insect populations.
2. Initiate planning for expert panel meeting on genetic monitoring.

FY07 1. Collect year 4 (cotton) and year 5 (corn) population genetic data for non-target insect populations.
2. Complete development and evaluation of a long-term monitoring strategy based on collection of population genetic and gene expression data.
3. Convene expert panel to develop and refine recommendations for implementation of a genetic monitoring program.

Outputs/Impacts: This work will provide the Office of Pesticide Program, Biopesticides and Pollution Prevention Division (OPP/BPPD) with significant new data and tools to evaluate long-term ecological effects of genetically modified crops. Results of this research will be communicated through a number of annual products.

APM 2003: Review of the scientific literature on the use of genetic monitoring for long-term ecosystem surveillance with special attention to its use in agro-ecosystems.

APM 2005: Joint ORD/OPP workshop on the analysis of population genetics of invertebrates in agro-ecosystems.

APM 2006: Report on the development and evaluation of a gene expression assay of PIP exposure to non-target insects.

APM 2007: Development and evaluation of a genetic monitoring program to assess long-term effects of PIPs on non-target organisms.

APM 2008: Report on expert panel recommendations for a genetic monitoring program to assess long-term effects of PIPs on non-target organisms.

Escape of Altered Plants to the Natural Environment and Likelihood and Impact of Gene Transfer

The ability of some crops to transfer introduced genes through hybridization to wild and/or weedy relatives can make the assessment of effects on non-target organisms very difficult.

Research Need:
Explore the factors influencing gene transfer rates to provide a basis for better assessments.

Some transgenes may have a limited persistence due to their insert locations, characteristics of the genetic cassette, or the plants or microorganisms themselves.

Research Need:
Develop methods to evaluate the persistence/maintenance of transgenes in plants and microorganisms, the exposure to those gene products, and whether environmental conditions or common stressors influence this process.

No discrete project was developed to address research need 3a. One project was developed to meet research need 3b:

Evaluating gene flow from genetically modified crops and its potential ecological effects.

Project Title: **Evaluating Gene Flow from Genetically Modified Crops and its Potential Ecological Effects**

Laboratory/Center: NHEERL (WED lead), Lidia Watrud, (541) 754-4567, watrud.lidia@epa.gov

Primary Research Objective/Goal: There are three specific outcomes for these studies: (1) an understanding of the potential for transfer of novel genetic material from GM crops to non-target plants and the associated ecological consequences of such exchange; (2) methods for determining and minimizing amounts and circumstances of gene transfer from proposed GM crops that can be provided by registrants when applying to OPP/BPPD for registration of new PIPs; and (3) identification of inputs for probabilistic risk assessment models of gene flow from GM crops.

Background: Currently, engineered crops are planted on tens of millions of acres in the U.S. alone. Pollen from transgenic crops may hybridize with related crops or weeds, potentially transferring the transgene to crop-crop or crop-weed hybrids. The resultant F1 hybrids may in turn self- or out-cross to other compatible species or may backcross to the transgenic or non-transgenic parent. In addition, the transgenic genes may move via feral plants or seeds; i.e., over-wintering transgenic plants or seeds that escape cultivation or via seeds that have fallen from planters, combines, trucks, or railroad cars during routine planting, harvesting, and shipping activities. While it is commonly argued that cultivated crops would not persist well outside of agronomic situations due to their need for high soil-fertility levels, little information is available on the survival, fertility, and out-crossing potential of hybrids formed between crops and compatible weedy or native species. Many species in each of the two latter categories (weedy and native species) commonly thrive in low fertility soils. It also is not known how exchange of transgenes will affect the overall fitness of non-crop plants, either enhancing or decreasing their ability to compete within the natural plant community. Methods are needed to allow such questions about ecological risks to be adequately addressed during development and deployment of crops with novel PIP transgenes.

Project Description and Critical Path:

Six major scientific questions will be addressed by this research:

(1) How far and with what frequency do transgenes move from GM crops into other plants?

(2) Which biological and non-biological factors affect gene flow?

(3) How long do transgenes persist in non-target host populations?

(4) What are the unintended ecological consequences to plant communities of gene transfer and/or dissemination of feral crop plants?

(5) Can unintended ecological effects of genetically modified crops be reduced by designing strategies to minimize gene flow?

(6) Can probabilistic risk assessment models be developed using parameters identified in our studies?

In order to answer these questions, four lines of research will be conducted.

1. Develop gene tracking methods – qualitative and quantitative molecular methods or other cytological, biochemical, or morphological markers will be developed to track transgenes (or components thereof) from GM crops to other crops or non-crop plants. Molecular methods (e.g., PCR) will be developed to facilitate detection of stress response at the genomic level in support of ecological effects studies.

2. Select compatible crop/non-crop species – compatible plant species will be selected for greenhouse or field studies of gene transfer. Rates of transfer will be compared between novel and conventional crop protection genes to provide information on stability and persistence of genetic material. Differences due to life-history traits (e.g., pollination methods) will be assessed, as well as effects of type of genetic construct (e.g., nuclear vs. chromosomal inserts; single vs. multiple engineered traits; crop protection vs. crop nutrient quality traits; protein vs. non-protein metabolically engineered traits, etc.).

3. Evaluate ecological effects – studies will be conducted on the consequences of genetic transfer on fitness (i.e., survival, yield, biomass production) of recipient plants. The potential for persistence of the gene through succeeding hybrid and backcross generations of the parental and other compatible species also will be studied. Differences due to transgene insert locations, characteristics of the genetic cassette, or the species involved will be evaluated. Fitness consequences of gene expression will be studied in multispecies communities subjected to various environmental stressors (e.g., herbicide application; temperature/humidity fluctuations; etc.). Molecular methods such as microarrays will be used to study genomic level stress responses in relation to fitness parameters.

4. Develop probabilistic risk assessment models - risk assessment methods to evaluate potential adverse effects of gene transfer from GM crops will be developed using probabilistic methods building on similar work currently underway for conventional herbicides. This includes estimates of exposure (e.g., probability of gene transfer for a given crop location, environmental factors, etc.) and effects (e.g., probability of ecological effects as a consequence of novel genetic material moving into non-crop plant species).

Projected Outputs/Impacts:

APG (FY08): Understand the factors influencing gene transfer rates from GM crops and the potential for altered plants to escape into the natural environment and provide methods to evaluate the persistence maintenance of transgenes in plants as a basis for assessing the likelihood and ecological impact of gene transfer.

APM (FY04): Quantitative measures for tracking transgenes in crop and non-crop plants.

APM (FY06): Methods for estimating frequency of gene transfer from GM crops to non-crop plants.

APM (FYOS): Molecular methods (e.g., microarrays) applied to plant genomes for assessing genetic change and environmental stress.

APM (FY07): Ecological consequences of movement of transgenes from GM crops to non-crop plants.

APM (FY08): Probabilistic methods for assessing ecological risk of genetic transfer from GM crops.

Projected Schedule: See table next page.

Timeline/Outputs Gene Flow Research

FY 2002	FY 2003	FY 2004	FY2005	FY 2006-2007
Review literature	Contribute to APM on strategy for updated test guidelines and finalize research plan	Continue R&D intramural studies in laboratory, chambers, the field, and model inputs	Continue intramural and extramural R&D	Complete short-term R&D
Identify resources: plants, traits, people, and organization	Approved QA plan in place	Initiate extra-mural R&D via co-ops, IAGs (USDA-ARS, DOI-NPS, DOI-BLM), contracts, and CRADAs with the private sector	Convene meeting of investigators to review findings, methods, problems, and to define model parameters	Continue long-term R&D
Attend workshop; identify data gaps; and select crops, traits, and geographies	Initiate lab and chamber studies with transgenic and parental plants	Identify collaborators for international ecological effects and molecular tracking collaborations in multi-year field studies with a wind and an insect pollinated crop	Convene workshop to develop national and international data collection network	Produce protocols, publications, and test model
Draft research plan	Initiate DNA analyses of plant and soil materials from field sites in US region(s) where selected crop(s) are grown			Produce Agency reports: findings, methods, and white paper on strategies to minimize gene flow effects
Pilot on site studies of DNA characteristics, persistence, expression, and transforming ability	Hire NRC post-doc			
Hire NHEERL post-doc	Formulate and issue RFA			
Identify potential IPA (academic and federal agency) and GSF collaborators	Identify US and international collaborators			

The Development of Pesticides Resistance in the Insect Target Species

Laboratory and small-scale field testing has been the basis for evaluating the likelihood of resistance development. Long-term, extensive monitoring has not been conducted to determine whether the effects predicted in such tests actually occur in the field.

Research Needs:
(i) Develop models to estimate the likelihood of the development of insect resistance that incorporate detailed biological information for pest species, including gene flow and mating patterns in the wild, geographic and chromosomal distribution of resistance alleles, and their additive and non-additive effects on resistance under selective pressures in the field.
(ii) Perform monitoring studies of gene transfer, the development of resistance to PIPs by target pests, and effects on non-target species (as noted in 2b) to allow field validation of conclusions regarding transgenic plants with new pesticide traits.

There are two projects addressing this key research need:

1. Genetic evaluation of long-term risks of plant-incorporated protectants: evolution of resistance in targeted pests.

2. Field assessment of insecticide resistance management (IRM) for PIPs.

Project Title: **Genetic Evaluation of Long-term Risks of Plant-Incorporated Protectants: Evolution of Resistance in Targeted Pests**

Laboratory/Center: NERL

Program Contact: Mark Bagley, (513)569-7455, bagley.mark@epa.gov

Background: The long-term environmental risks posed by GMOs are generally less well understood than the short-term risks. Thus, it is important that EPA continue to evaluate the usefulness of its current data to ascertain long-term ecological risks and to develop new types of data where necessary. Adaptation to PIPs by targeted pests is a long-term risk of special concern. Additional information is needed to evaluate the biological assumptions of models used to develop EPA's required strategy for resistance management (i.e., high dose, structured refuge) for different species and traits.

Project Description and Critical Path: Prediction of the likelihood and rate of adaptation by targeted pests depends on a number of factors, including variation in the toxicity of the plant product over time, the fraction of each population that is exposed to PIPs, gene flow between exposed and unexposed populations, the number of genes and amount of genetic variation influencing the trait, dominance and epistatic interactions, and genetic correlations with other fitness traits. Many of these factors are complex and poorly understood for most species.

We will evaluate genetic parameters for one model plant-pest system: *Bt*-corn and western corn rootworm, *Diabrotica virgifera*. This system has been identified by OPP as a concern for development of *Bt* resistance. The work will include laboratory assessments of genetic variances and covariances between fitness traits at several levels of exposure to *Bt* toxin. In addition, we will examine the population genetic structure of pest populations using microsatellite DNA markers. This analysis of neutral genetic markers over several generations and from several populations will provide information on effective population sizes and will quantify migration/gene flow.

This information will be used to parameterize models of pest adaptation that will be tested under laboratory-controlled gene flow scenarios. Using information on target genes of action (e.g., from gene expression studies) and homologous resistance-conferring genes (e.g., *Bt R-4* in tobacco budworm and bre-5 in nematodes), we will design targeted PCR assays for allele frequency changes at candidate resistance loci. Frequencies of these resistance markers in field populations will be estimated. This approach will be evaluated as an early warning system for detecting the evolution of resistance by the target pest. If successful, these approaches may also be applied to other *Bt* crops and pests such as the *Bt*-cotton and the tobacco budworm.

Schedule:

FY03 1. Begin development of PCR-based assays for *D. virgifera* resistance development.
2. Begin development of microsatellite DNA markers for *D. virgifera*.
3. Begin field collections of *D. virgifera* for population genetic analysis.
4. Begin in-house cultures of *D. virgifera* for laboratory genetic analyses.

FY04 1. Produce report on development of PCR-based indicators of resistance evolution.
2. Complete development of microsatellite markers for *D. virgifera* and non-target insects.
3. Initiate full-scale laboratory and field analyses of *D. virgifera* genetics.

FY05 1. Continue laboratory analyses of *D. virgifera* genetic characteristics, including mapping and QTL analyses.
2. Complete field analyses of *D. virgifera* population structure.

FY06 1. Complete laboratory analyses of *D. virgifera* genetics.
2. Incorporate genetic information on *D. virgifera* into improved models of resistance evolution and evaluate their implications for alternative resistance management strategies.

Outputs/Impacts: This work will provide the Office of Pesticide Programs, Biopesticides and Pollution Prevention Division with significant new data and tools to evaluate long-term ecological impacts of genetically modified crops. Results of this research will be communicated through a number of annual products.

APM 2004: Report on the development and evaluation of a PCR-based "early warning" assay for adaptation by target pest populations.

APM 2006: Assessment of pest genetic architecture in order to inform optimized resistance management plans.

Project Title: **Field Assessment of Insecticide Resistance Management (IRM) for Plant Incorporated Protectants (PIPs)**

Laboratory/Center: NCEA

Program Contact: Bob Frederick, (202) 564-3207, frederick.bob@epa.gov

Primary Research Objective/Goal: The goal is to develop field methods to assess and monitor the effects of the high-dose/structured refugia IRM strategy on the long-term susceptibility of target pests to *Bt* endotoxins.

Background: The development of target pest resistance to the *Bt* transgene[s] used as plant-incorporated protectants is a serious risk both to the sustainability of *Bt* crops and to the wider utility of environmentally "soft" microbial *Bt* pesticides. Therefore, EPA requires growers of *Bt* crops to follow the high-dose/structured refugia strategy to delay or prevent resistance development. This marks the first time EPA has required resistance management as part of a pesticide registration. Effective management requires sensitive tools for detecting resistance in field pest populations while the resistant alleles are still sufficiently rare to allow for corrective action.

Project Description and Critical Path: The research is composed of two parts, each involving the development and refinement of field-based methodologies to assess and manage *Bt* resistance in the field. One component will focus on field testing and validation of the high-dose/structured refugia strategy for *Bt*-resistance management. Key assumptions of the models upon which this strategy is based still have not been tested in field populations of the target pests. Significant data gaps exist regarding pest biology, ecology, and population dynamics, particularly with respect to dispersal and use of alternate hosts. Target pests include key lepidopteran cotton pests, *Helicoverpa zea* and *Pectinophora gossypiella*; *Helicoverpa zea*, a pest of both cotton and corn; and, on corn specifically, the lepidopteran pest, *Ostrinia nubilalis* and two beetle pests, *Diabrotica barberi* and *D. virgifera*. We propose to address these ecological data gaps in a series of field and regional-scale studies.

A second research component will focus on developing appropriate tools to identify and measure *Bt* resistance in field populations of the target pests. These tools will include both functional screens or bioassays and molecular markers. Preliminary markers have been developed for *H. virescens* and *P. gossypiella* but have not yet been tested extensively in field populations. We plan to develop and test additional markers as well as develop more streamlined bioassay techniques.

Desired Outcomes: The research results will inform regulatory decision-making by OPP and will provide critical tools to Regional Offices involved in promoting grower compliance with IRM requirements. EPA will develop tools capable of identifying the evolution of *Bt* resistance at sufficiently early stages to allow corrective action to prevent loss of *Bt* crops as effective and least toxic alternatives to conventional pesticides.

Projected Outputs:

FY06 APM: Final report outlining appropriate tools for monitoring resistance development in the field and the use of target pest ecology to refine IRM strategies as they are determined in risk assessment practice.

Risk Management

The effectiveness of management strategies to avoid key risks for extended periods and the effectiveness of risk management technologies to mitigate risks associated with unintended adverse consequences must be evaluated and expanded for new PIP crops.

Research Need:

(i) Improve strategies to identify key risks of concern, develop evaluation schema to understand the effectiveness of management strategies, and develop of effective new technologies to mitigate these key risks as when monitoring studies identify the presence of unintended adverse consequences.

There are three projects addressing this key research need.

1. Development of strategic monitoring programs for ecological impact from plant-incorporated protectants (PIPs).

2. Development of management and field-scale tools to manage the risks of gene transfer and non-target effects from PIP crops to the environment.

3. Development of management and field-scale tools to manage and delay the development of insect resistance to PIP crops by extending crop life.

Project Title: **Development of Strategic Monitoring Programs for Ecological Impact from Plant-Incorporated Protectants (PIPs)**

Laboratory/Center: NCEA

Program Contact: Bob Frederick, (202) 564-3207, frederick.bob@epa.gov

Primary Research Objective/Goal: The goal is to determine effective strategies to identify the key risks of concern and appropriate risk management technologies to mitigate these key risks when the monitoring studies indicate unintended adverse consequences.

Background: Historically, monitoring programs in association with field releases of genetically modified organisms have been, explicitly or implicitly, called for as a part of risk assessment/management schemes or regulatory agenda. However, it is often not clear what should be monitored, why, or for how long. Recommendations of objectives and methodologies have been made with little understanding of their scientific basis due to a lack of information. Monitoring for the development of insect resistance to pesticides–identified as early as 1991–provides the single best example of science-based monitoring program development. This is, however, only one of many potential ecological concerns associated with GM crops; and often the decision as to what to monitor for has depended as much on what was possible to monitor as it has on the identified concern.

While wide-ranging, non-specific monitoring programs to detect new or unique effects of genetic engineering are being suggested, such monitoring may be quite expensive and inefficient. Surely most studies of this nature will find nothing at great expense even if a previously unknown problem eventually turns up. It will be most helpful to decision makers and those who will be charged with the design and implementation of monitoring programs to know explicitly what should be monitored, the reason behind the concern(s) that generated the need for monitoring, the appropriate methods to conduct the monitoring study, and the purpose for which the data are to be collected.

Project Description and Critical Path: NCEA will convene a meeting of science experts to discuss the state of the science in environmental monitoring efforts, particularly those related to the determination of ecological impact from PIP crop plants. The most promising areas for comparison analysis monitoring will be developed. The comparison approach was described in a recent NRC workshop summary on Ecological Monitoring of Genetically Modified Crops. The goal is to determine what in-field condition(s) might prove to be indicative of change (as an early warning indicator) or impact (evidence of a environmental impact, e.g., decreasing insect populations). By focusing on the agro-ecosystem condition, it may be possible to ameliorate the spatio-temporal problems associated with large-scale planting of PIP crop plants and the natural variability inherent in identifying and tracking ecosystem change. This research will be conducted for a minimum of three growing seasons. If and where appropriate, the data resulting from the research will be used to test the power of population change models as predictive tools for ecosystem impact or to assist in development of cost-effective monitoring efforts.

Projected Outcomes: The research results will be useful both in regulatory decision-making by OPP; and more generally, they will provide information critical to the risk assessment of PIPs. EPA will have an evaluation of current and future ecological monitoring methods of potential use for post-registration surveillance. Results from the field component will lead to constructive evaluation of assumptions made in risk assessments of GM crops.

Projected Outputs/Impacts:

FY06 APM: Conference report on monitoring strategies for determining ecological impacts as they are considered in risk assessment from GM crops.

FY07 APM: Report on the field comparison approach to risk-based monitoring for ecological impacts from genetically modified crops.

Project Title: **Development of Management and Field-Scale Tools to Manage the Risks of Gene Transfer and Non-Target Effects from PIP Crops to the Environment**

Laboratory/Center: NRMRL

Background: Biotechnology presents a wealth of opportunities to improve crop productivity, nutritional value, and resistance to pests and other stresses. However, there are potential risks to human health and ecological systems that need to be evaluated for the proper regulation of transgenic pesticidal crops. Currently, EPA regulates biotechnology products that are pesticides produced by plants or by microorganisms. It is important to continue to develop supporting information to correctly identify the risks of concern associated with PIP crops and the associated risk management strategies or tactics to mitigate any unintended adverse consequences connected with these crops. The management portfolio to meet the challenges needs to be expanded. There are no strategies for identifying the key risks of concern, nor are there effective risk management technologies to mitigate these key risks when monitoring studies indicate unintended adverse consequences. It is also important to explore the application of socio-economic methods such as benefit-cost analysis and life-cycle analysis to better understand issues related to the public acceptance of genetically altered products and to evaluate whether the genetic alterations produce new organisms that are not substantially equivalent to currently existing ones.

Project Description and Critical Path: Techniques that have been developed for full field evaluation of non-target effects in Europe will be evaluated for their applicability to the U.S. Standardization and testing of the techniques will be undertaken to understand the directive force and reliability of data collected through the use of these techniques. Field testing of selected techniques will be undertaken to ensure their reliability and the integrity of information derived from them. The standardized and tested techniques will be assembled for use by future seed registrants and public dissemination.

Scrutinizing possible non-target and gene-transfer effects will continue to be an integral part of the evaluation strategy used by the Office of Pesticide Programs to register PIP crops. The information database supporting this inquiry is very small and largely devoted to specific issues. The proposed research attempts to close the known information gaps specifically in the area of management tools.

Remote sensing and other forms of sensing technology will be tested for their ability to assist the understanding and modeling of pollen distribution that is the major pathway for gene transfer for the PIP crops. Management tactics and strategies designed to avoid adverse effects associated with pollen transport will be developed and evaluated. Certain crops are known for their wide pollen distribution patterns so this detection technique will be selectively used as a function of crop characteristics.

Projected Outputs/Impacts:

APG (2009): Establish and deliver for future use by Office of Pesticide Programs, EPA regions, and state and local governmental agencies gene-transfer mitigation and non-target effects tools and strategies to aid the management of environmental risks associated with PIP crops to help maintain the biological integrity of the environment.

FY 04 APM: Develop and deliver review of current practice for the management/mitigation of gene-transfer and non-target effects from PIP crops and tools to assist risk management of adverse effects of PIP crops.

FY 05 APM: Develop and deliver for future use by EPA regions and state and local agencies preliminary methods for gene-transfer detection and non-target effects detection to assist the risk management of the potential adverse effects of PIP crops.

FY 06 APM: Develop and deliver for future use by EPA regions and state and local agencies tools for gene transfer and non-target effects management to assist the risk management of the potential adverse effects of PIP crops.

FY 07 APM: Conduct and deliver for future use by EPA regions and state and local agencies preliminarily tested and validated tools for gene transfer and non-target effects management to assist the risk management of the adverse effects of PIP crops.

Project Title: **Development of Management and Field-Scale Tools to Manage and Delay the Development of Insect Resistance to PIP Crops by Extending Crop Life**

Laboratory/Center: NRMRL

Program Contact: John Glaser, (513) 569-7568, glaser.john@epa.gov

Background: Pest resistance is the major threat to the technology sustainability of PIP crops. Implicit in the control of resistance is the ability to monitor the incidence of resistance and to formulate management plans to intercept any pest resistance at the earliest date possible. Vast crop sizes of > 20 million acres are not amenable to discrete sampling practices that have the desired statistical power for the prediction of the incidence of critical resistance levels.

Project Description and Critical Path: Part of this research will focus on the use of remote sensing to discriminate a reflected light signature for pest infestation. Coincidences such as a signature arising from a bioengineered crop planting could serve as a tool to direct land-based inspection teams to investigate potential pest infestations.

There are several laboratory-based techniques that use field-collected insects to determine resistance. These methods are used in part to support the OPP registration decisions. It is incumbent upon EPA to ensure that the data derived from these techniques and methods are of the highest information content to support OPP decisions. The methods will be subjected to standardization and tested for verification and validation of results. Simulation models have been used in similar ways by OPP and will be subjected to standardization followed by verification and validation scrutiny.

The remote sensing of pest infestation will begin at the "proof-of-principle" stage by studying a subset of the corn crop in the north central part of the Corn Belt (Minnesota, South Dakota, Nebraska, and Iowa) where bioengineered corn can account for a large percentage of the corn crop. The first stage will be to discern the utility of remote sensing imagery to identify conventional from bioengineered corn.

This information will be useful in observing the compliance of growers to the mandated refuge requirements. Using satellite imagery and field "ground-truthing," the reflected light frequencies indicative of pest infestation will be selected for lepidopteran insects (European corn borer and others). A composite of several spectral wavelengths will be explored as indirect information related to pest information. Proof-of-principle evaluation will follow to evaluate the use of imagery for the earliest identification of field conditions that are indicative of pest infestations. This scrutiny will push the technology to the level of determining the importance of different noise contributions to the signal. It may also be possible to initiate the imagery of new corn events that protect from corn root worm in the first year of large field planting. It is anticipated that the reflected spectral signatures could be significantly different for these pests. Once the desired signatures are selected they will be tested. The remote-sensing system will be tested at a "proof-of-practice" level at which time an attempt to image the entire crop will be undertaken. Similar applications to the transgenic cotton crop will also be pursued. At each stage of development, reports will show the direction and extent of success that has been accomplished. Operational manuals and data inspection tools will be developed and published separately.

The scrutiny of resistance detection methodology and simulation modeling will be undertaken initially to standardize performance and communication related to use. The standardized methods will be tested in a variety of practitioners' hands to ensure verification and validation of reported results.

The bioengineered crop registration conducted by OPP rests squarely on the reliability of the available data relating to resistance management. The continued reliance on unstandardized methodology may lead to assumptions of crop performance that may not be met in the field. While not anticipated, crop failures are within the realm of potential outcomes. This research strives to develop a firm information basis from which reliable decisions can be made regarding the resistance management of PIP crops.

Field tools to assess the compliance of resistance management requirements will also be undertaken for use by EPA regions and states. Toxin detection technology will be evaluated and incorporated in field guidance for its use in the compliance monitoring.

Projected Outputs/Impacts:

APG (FY2009): Establish and deliver for future use by Office of Pesticide Programs, EPA regions, and state and local governmental agencies basic guidelines and tools to mitigate the development of resistance in targeted pest populations so as to extend the useful lifetime of PIP crops to minimize the use of chemical pesticides in agriculture.

FY 04 APM: Develop and deliver survey of current practice in management tools for delaying the development of resistance to PIP crops in targeted insects including modeling, remote sensing, and laboratory assays to help minimize pesticide use.

FY 05 APM: Conduct/deliver for future use by Office of Pesticide Programs, EPA regions, and state and local agencies verified/validated resistance management models for delaying resistance to PIP crops in target insects minimizing pesticide use.

FY 05 APM: Establish/deliver for future use by Office of Pesticide Programs, EPA regions, and state and local agencies principles for using remote sensing and GIS to detect pest infestation to delay insect resistance to PIP minimizing pesticide use.

FY 05 APM: Establish/deliver for future use by Office of Pesticide Programs, EPA regions, and state and local agencies single laboratory standardization of lab assays to detect pest insect resistance to PIP crops to minimize pesticide use.

FY 06 APM: Develop/deliver for future use by Office of Pesticide Programs, EPA regions, and state and local agencies data mining tools for models and methods to help manage and delay insect resistance to PIP crops to minimize pesticide use.

FY 06 APM: Establish/deliver for future use by Office of Pesticide Programs, EPA, and state and local agencies proof of application of remote sensing and GIS to detect pest infestation to delay insect resistance in PIP crops minimizing pesticide use.

FY 06 APM: Develop/establish/deliver for future use by Office of Pesticide Programs, EPA, and state and local agencies multi-laboratory standardization of lab assays to detect pest insect resistance to PIP crops to minimize pesticide use.

FY 07 APM: Establish/deliver for future use by Office of Pesticide Programs, EPA, and state and local agencies proof of practice for using remote sensing and GIS to detect pest infestation to delay insect resistance to PIP minimizing pesticide use.

Demonstration Project

A demonstration project is being designed to facilitate inter-laboratory participation and cooperation.

Project Title: Biotechnology Demonstration Project

Background: In planning the Biotechnology Initiative Research Program, the Biotechnology Initiative Steering Committee determined that a demonstration project was needed as a means to foster cross-laboratory collaboration, to create the opportunity for a productive synergy, and to effectively illustrate the connectedness of individual laboratory efforts.

Project Description and Critical Path: A demonstration project to assess ecological risk is being formulated with cross-laboratory cooperation from NCEA, NRMRL, NHEERL, and NERL. This project provides a means to demonstrate the tools and approaches developed through the larger program. With the close coordination and involvement of scientists from the Office of Pesticide Programs, the success of this effort will be measured by the extent to which the tools and approaches are integrated across the risk assessment/risk management paradigm to inform EPA decisions.

Projected Outputs/Impacts: Conceptually, the project will have two stages. The first will be to prepare a detailed white paper that (a) reviews the current state of the science in biotechnology risk assessment and how it is currently used; (b) analyzes expectations from emerging scientific inquiry both within and outside EPA; and (c) proposes a strategy for the evaluation of new and existing, science-based assessment tools. The second stage will be to implement the strategy infield experiment(s) designed to incorporate all components of risk analysis (problem formulation, conceptual model development, risk assessment, risk characterization, and risk management) and collect data to inform the process.

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