UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JAN 25 2005

OFFICE OF
RESEARCH AND DEVELOPMENT

Dr. James H. Johnson, Jr.
Chair, Board of Scientific Counselors
College of Engineering, Architecture, and
Computer Sciences
Howard University
2366 Sixth Street NW
Washington, DC 20059

Dear Dr. Johnson:

The Office of Research and Development (ORD) would like to take this opportunity to thank you and the rest of the members of the Board of Scientific Counselors (BOSC) Subcommittee on Biotechnology for your May 2004 review of the EPA ORD Biotechnology Research Program. Enclosed with this letter is our response to the comments raised in your letter of August 20, 2004. Please feel free to contact me if further information is needed.

We are pleased that the BOSC is supportive of this important research program and the direction in which it is moving. Again, thank you for your advice to ORD.

Sincerely yours,

William H. Farland, Ph.D.
Acting Deputy Assistant Administrator for Science

Enclosure

RESPONSE TO BOSC PEER REVIEW COMMENTS ON ORD's BIOTECHNOLOGY RESEARCH PROGRAM

General Comments:

1. **The areas of research that ORD has identified (risk to human health, risk to non-target organisms, the potential for gene flow, and insect resistance and management plans) are important and proper.**

   No comment needed

2. **The BOSC believes that the greatest emphasis in the ORD research portfolio at the present time should address genetically modified crops that contain genes for production of *Bacillus thuringiensis* toxin (Bt) because these could conceivably cause all four effects of concern: allergenicity, resistance in target populations, risks to non-target organisms, and conveyance of a selective advantage to cross-hybridized wild-type plants.**

   No comment needed

3. **Risks to non-target organisms and the environment remain as one of the largest potential problems of GM crops in the minds of the public.**

   No comment needed

4. **The Framework document outlines a good program to study gene flow from transgenic plants to non-crop hybrids using genomic techniques to confirm expression of transgenes and to evaluate the fitness and ecological effects on crops and non-crop hybrids.**

   No comment needed

5. **The insect resistance and management plan of ORD is an important component in the research portfolio of its biotechnology program. The idea to work with NASA on remote sensing of crops for risk management is excellent**

   No comment needed

6. **The BOSC asks that EPA and ORD be aware and sensitive to social issues as they develop research products, interact with other Federal agencies, and communicate risks to the public, including the public beyond our Nation's borders.**

   EPA's sensitivity to social and scientific concerns related to biotechnology is partly why

ORD launched its biotechnology research initiative, and it is why the topics for research were chosen. There are scientific uncertainties regarding allergenicity of novel proteins. Gene flow and pollen movement to other crops has become more important as the adoption of biotechnology crops has increased, and farmers are concerned whether outcrossing could impact their ability to market their conventional (non-engineered) crops. Also insect resistance management for the *Bacillus thuringiensis* toxin (Bt) crops is highlighted because of the importance of Bt pesticides as safe products worthy of extra protection of their efficacy.

EPA works closely with other Federal agencies involved with biotechnology, especially the US Department of Agriculture, the Food and Drug Administration, and the National Institutes of Health (related to allergenicity). EPA is guided by the Coordinated Framework for the Regulation of Biotechnology published in 1986 by the Office of Science, Technology, and Policy; this document lays out a cooperative process among the Federal agencies. We have discussed with other Federal agencies the advantages of joint research and grant programs and will continue to explore more opportunities in the future.

EPA also participates in many international activities aimed at harmonizing risk assessment standards and procedures for biotechnology, capacity building, and information sharing. The US Government recently launched a new web site to provide information on all approved agricultural biotechnology products including links to EPA's risk assessments for plant-incorporated protectants (PIPs). EPA's risk assessment (called a Biopesticide Registration Action Document or BRAD) for each active ingredient is on the web site. The URL for the list of active ingredients is http://www.epa.gov/pesticides/biopesticides/ingredients/index.htm. There is a section on plant-incorporated protectants, and one of the headers is the currently registered PIP products http://www.epa.gov/pesticides/biopesticides/ingredients/index.htm.

EPA is committed to sound science and transparency for biotechnology. To meet these objectives, EPA calls on its FIFRA Scientific Advisory Panel as well as the Scientific Advisory Board and the BOSC for peer review and guidance. Unlike other pesticides products going through the registration process, virtually all of the scientific studies are available for public review during the review time. All risk assessments are available on the web (see for example http://www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet_plant_pesticides.htm) which will also include ORD's biotechnology research plan. In 2005, EPA will initiate a redesign of EPA's biotechnology information on the web to improve public access.

Specific Comments

Allergenicity:

1. **The BOSC recommends that not all research on allergenicity be related to digestibility. Respiratory allergenicity also should be included, and SAR-based approaches should be considered such as epitope mapping**.

With respect to the in-house research program, digestibility is only one of the areas we intend to include in our research. (This is an important area because, at the present time, it is assumed that readily digestible proteins are not allergenic. Although there is not rigorous science to support this premise, it is currently used to make decisions about the safety of biotechnology products, because it is frequently the only available information relevant to allergenicity.) As indicated in the Biotechnology Research Program Document (April 2003), with respect to the intramural program, once we develop an animal model we intend to use it to address the following questions: 1) Does dietary exposure to transgenic pesticide protein 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?

We have considered SAR approaches and epitope mapping. However, it should be noted that the portion of the protein that is recognized by antibody (epitope or antigenic determinant), is the same whether the antibody is IgG (non-allergic) or IgE (allergic), so the structure of the epitope will not distinguish immunogenic from allergenic proteins. Epitopes are usually not linear (i.e. made up of amino acids lined up in a row when the protein is stretched out). Most epitopes are made up of non-contiguous amino acids brought together by protein folding. No amino acid sequences that would label a protein as an allergen have been identified, even for respiratory allergens. There are, however, data bases of known allergens and as these grow some trends may emerge. Certainly, if certain Bt toxins elicit allergic responses in our animal model, we will investigate whether the epitope responsible for these reactions is structurally related to peanut or other allergens known to cause food allergy in humans. This information might then suggest some hypotheses relative to SAR that could be tested.

The capacity of the in-house program is limited and the need for animal model(s) is great. Thus, we are reaching out to the larger scientific community through a biotechnology RFA to be offered by the Science to Achieve Results (STAR) grants program in FY05. It will focus on research to develop an animal model to evaluate the potential allergenicity of GM crops in order to explore underlying mechanisms and issues of susceptibility. Some understanding of underlying mechanisms is needed before SAR studies can be attempted. We anticipate close communication between the in house and grants program to insure that the two programs are complementary (not redundant). We will also be continuing to pursue opportunities for partnerships with NIEHS, NIAID, and USDA for joint RFAs.

2. Developing an animal model (mouse model) for identifying mechanisms of allergenicity is believed to be important by the BOSC, as stated in the Framework document.

No comment needed

3. ORD should consider how its approach on the potential allergenicity of BT-products can be extrapolated to other GM crops that may be developed in the future, such as herbicide-resistant spring wheat and others. Collaboration that already has been established with NIAID and NIEHS, in matters of human allergenicity is encouraged, and use of the pharmaceutical models of FDA for safety testing (levels 1, 2, and 3) may prove helpful.

Certainly we would want to use our animal model to test other GM products. The plan is for this model to be incorporated in the FAO/WHO decision tree or some improved version of that decision tree. We have made efforts to partner with NIEHS and NIAID (but have had little success with the latter), and we are keeping the lines of communication open. Once the animal model is developed we will seek out collaborators who can help us bridge the gap between animal data and human health effects. With respect to FDA, in consulting Dr. Dan Weaver (a founding member of FDA's immunotoxicity committee), FDA has no tiered testing approach for allergenicity. The tiered immunotoxicity testing that FDA has is for immune suppression and beyond the general concept of a tiered approach is not appropriate for allergenicity testing. The FAO/WHO decision tree is a tiered approach and at present the only one proposed for food allergy.

The FAO/WHO decision tree represents a type of tiered approach for food allergy. It is currently a theoretical concept because some of the tools we need (such as an animal model) are not available. A big question, which we, along with the rest of the scientific community are trying to address, is whether this is the right decision tree. A tiered approach would be much easier to devise if we had a better understanding of the mechanisms underlying food allergy. We will do our best with the resources we have to explore those mechanisms in our animal model.

Risks to non-target organisms and the environment:

1. Risks to non-target organisms and the environment remain as one of the largest potential problems of GM crops in the minds of the public.

No comment needed

2. The findings so far by EPA ORD, that Bt-proteins have narrow spectrum insecticidal activity and that no serious effects are anticipated on non-target organisms, seem justified, but ORD should be ever-vigilant to the possibility of subtle, long-term effects that are difficult to measure without an extensive monitoring program.

We agree with this observation and have taken it into consideration in the design of the research program. Long-term environmental impacts from stressors with no known acute effects have proven extremely difficult to anticipate, let alone amenable to cost-effective monitoring programs that quantitatively describe them if they were to occur. This issue was a major discussion point at the recent monitoring symposium "Development of Strategic Monitoring Programs for Ecological Impact from Plant-Incorporated Protectants"sponsored and organized by ORD's National Center for Environmental Assessment (August 3-5, 2004; Washington, DC). The result of the discussions from this symposium will be used to identify approaches to long-term monitoring that might be possible to initiate in the near-term and that will provide the greatest probability for environmental protection.

We also recognize that there is a great deal to be learned from the collective research done on potential impacts from biotechnology over the past twenty years. Accordingly, we have initiated a cooperative agreement with the University of California, Santa Clara to establish a meta-database and analysis system for evaluating the results of relevant laboratory and field experiments. The goal is to develop a database that will incorporate not only the published literature, but also the supporting data included in the product applications submitted to EPA and USDA. This approach has a high probability of identifying potentially impacted components of ecosystems and of suggesting appropriate environmental endpoints for long-term monitoring.

3. The BOSC agrees that monitoring a few key indicator organisms and habitats over a long period of time is the proper approach to this problem.

No comment needed

4. The BOSC further recommends that the ORD Biotechnology program include the recent ecological field studies of GM crops in the United Kingdom in their research database. These studies indicated that ecological diversity could either increase or decrease due to the use of GM crops, depending on the crops, non-target organisms, and cropping practices considered. It would be prudent to learn whatever can be gleaned from these studies and not to reproduce them.

The ORD Biotechnology program is cognizant of the farm-scale evaluations (FSE) that looked at the effects of GM crops on non-target organisms. As the BOSC indicated, one of the main conclusions from the FSE was that non-target effects depend on the type of crop used, the non-target organisms involved, and the cropping practices considered. Since the FSE looked only at herbicide tolerant crops, and did not look at Bt crops, we do not consider the research performed by ORD to be duplicative. In addition, ORD’s research is focused on development of monitoring methods to evaluate long-term effects at larger scales than the immediate farm. We believe that it is the proper role of EPA to be protective of this wider ecosystem. Nonetheless, we have taken the lessons of the FSE to heart and will be attempting to more directly compare non-target effects of Bt crops to non-target effects of conventional cropping systems.

5. The Agency should track closely the overall use of pesticides (both herbicides and insecticides) on GM-cropland in the United States, although the BOSC realizes that this responsibility is not a research activity.

Both USEPA and the USDA National Agricultural Statistics Service (NASS) collect annual data on the sale and use of pesticides at the state level. USDA pesticide use surveys conducted by NASS since 1993 provide on-farm chemical use data for eleven crops: corn, cotton, soybeans, wheat, rice, grain sorghum, peanuts, fall potatoes, other vegetables, citrus, and apples. Under the sponsorship of USEPA, USDA and other agencies assembled a comprehensive database of pesticide use for the years 1992 and 1997. It covers 220 active ingredients and 87 crops at the state level, and provide information on: (1) the percent of a crop's acreage in a state treated with an individual active ingredient, and (2) the average annual application rate of the active ingredient per treated acre. Because these data represent the average application and treatment rates by state, they do not yield precise estimates of use at the county or farm level. The USDA NASS developed county use levels based on agricultural land use acreages to apportion the state-level pesticide use values to the counties. California has had full pesticide use reporting data since 1990 at the section level.

The California Pesticide Use Reporting database was designed specifically to assess pesticide risk, that is to estimate the likelihood of an adverse health or ecological effect from pesticide exposure(s) due to spray drift. Detailed information is collected from pesticide users, including date and hour of application, type and amount of crop acreage treated, and site identification (in mapping coordinates). This comprehensive database allows analyses to be made of type and amount of pesticide applied to particular crops, including differentiation of genetically engineered and conventional varieties.

The Agency receives some of this information for herbicide tolerant crops (because we regulate the chemical herbicide). The Agency also receives registrant reports on the acreage planted to Bt crops by county and State (although much of this information is confidential business information and not publicly available). The Agency does not track which individual farmers are using a GM crop and on how much of their acreage although the National Agricultural Statistic Service does survey for this information. Although the pesticide use practices were not specifically documented for Bt crop acreage, the Office of Pesticide Programs looked at insecticide use practices in areas where Bt corn and Bt cotton had been adopted as part of its 2001 reassessment of Bt crops. The information shows dramatic use reduction for Bt cotton where chemical insecticide use was common and less reduction for the first generation of Bt corn products where only a small percentage of the corn crop was treated with chemical insecticides, because it was difficult to be effective with the insect living inside the corn stalk. Newer Bt corn products aimed at controlling corn rootworm are predicted to show larger use reductions.

Gene Flow:

1. **Canola and creeping bentgrass are model plants being utilized. Gene introgression (retention of the genetic characteristics in subsequent generations of the non-crop hybrids) and gene expression after 3-5 years in mesocosms is a good approach for studying these important ecological questions**.

   No comment needed

Insect Resistance and Management:

1. **The idea to work with NASA on remote sensing of crops for risk management is**

excellent.

No comment needed

2. The provision for creation of refuges is logical, but there should be some social/behavioral research considered that measures how and under what circumstances farmers actually perform the recommended practices.

EPA considers the comments from farmers, grower organizations, and the Extension Service on what refuge and other IRM practices are reasonable for farmers to actually implement for Bt crops. Grower education with extensive materials and specific training is included as a condition of the registration for these product. Indeed, any resistance management method will only be effective if the premise is adopted by the farmers. In an effort to address this, EPA registrations require compliance monitoring to be completed by the registrants with growers who will not comply losing their right to buy the product and in extreme cases, a company could lose their registration if compliance is not addressed. We are aware of and will continue to monitor the progress of related social science research being supported by other federal and private funding (see for example the work of Terrance Hurley at the University of Minnesota).

One reason for research on the effectiveness of the high dose/refugia strategy is to determine whether alternatives such as insect management plans may be more acceptable (e.g., be easier to implement or require smaller set asides for non-Bt crop) to farmers and which would consequently increase compliance. As a part of the EPA program and complementary to other like research currently underway, some projects are attempting to better understand the implications of mixed food resources (e.g., different crop plants--corn and cotton--and natural vegetation) by polyphagous pests on the requirements for refugia. Such projects will necessarily use the principles of social science to properly direct the research and, because farmer cooperation is often a prerequisite to successful completion of these projects, they will provide useful insight into what practices are acceptable.

3. The rapid PCR screens and modeling strategy to assess resistance management is a good approach by ORD, but what are the markers being used to detect insect resistance?

ORD's efforts to assess resistance evolution in the Western corn rootworm (WCR) utilize a combination of laboratory and field-based approaches. The first step to understanding how resistance alleles will spread is to understand the population genetic structure of the species. We are studying the population genetics of this pest across North America in order to understand the dynamics of resistance evolution and to improve genetic models used to predict resistance evolution. This work relies mainly on analysis of selectively neutral microsatellite loci that we and USDA collaborators have developed.

Assessment of putative resistance alleles is more difficult because no strains of WCR are known to exhibit resistance to the Bt toxin as yet. Thus, we are pursuing multiple approaches to identify resistance markers. The first approach is to identify and screen candidate genes based on knowledge of resistance development in Lepidoptera. A second approach is to experimentally cross strains in genetic mapping studies to identify chromosomal regions that confer some level of resistance. Markers from these regions will be compared to candidate genes and will be used for field evaluations. We are also working with USDA to artificially select for resistance in the lab. The genetics of these resistant strains will be evaluated through similar mapping studies to help identify the major genes and pathways involved in Bt resistance in WCR.

4. "In the high dose, structured refuge resistance management research, the BOSC recommends that a population genetics study of resistance genes in root worm populations under Bt-crops be considered. However, because there are only two organisms that have ever been reported to become resistant to Bt-toxin in 30 years of use as a pure insecticide, the BOSC wonders whether proportionally there are too many resources overall being allocated to the resistance question."

We agree that a population genetics study of corn rootworms is important. This pest is responsible for one out of every seven insecticide applications on all agricultural crops in the USA. The predicted reduction in pesticide use due to anti-rootworm Bt corn is expected to provide a significant benefit to the environment and the public. If rootworms evolve resistance to Bt corn, however, the benefits of reduced pesticide use will be lost because farmers will have to return to using traditional chemical insecticides. Some of the current chemical and cultural control measures have already begun to fail because of resistance. For example, the western corn rootworm has already evolved resistance to several traditional chemical pesticides (requiring the use of even more toxic broad-spectrum insecticides). Furthermore, the western and northern corn rootworm have independently evolved resistance to a highly effective cultural control method, crop rotation of corn with soybeans. Thus, two main rootworm control methods have already started to fail, making it very important that Bt-resistance in rootworms is delayed as long as possible. We are collaborating with the USDA and academic researchers to conduct population genetic studies on rootworms to improve the models used to predict resistance development, and to ensure Bt crops are grown in a manner to minimize the likelihood of pest resistance evolution by taking into account the population genetics of the pest. This research will help determine the conditions necessary to prevent or delay resistance evolution, and lead to design strategies that ameliorate environmental impacts if resistance does evolve.

Regarding resistance research in general, a significant portion of EPA's biotechnology R&D resources is devoted to the issue of insect resistance development. The decision to invest heavily in this area was based on the immediacy of the need for information by the Office of Pesticide Programs and field management of Bt crops. Bt-resistance evolution would require a return to traditional chemical insecticides for the major corn and cotton pests.

EPA has implemented strict requirements for the use of resistance management as a part of their registration approval in order to delay or prevent resistance. Bt corn that targets rootworms contains a lower Bt-dose than previous lepidopteran Bt crops, and thus the likelihood of resistance is greater. Field data are required to test the models that are being used to predict resistance evolution. Our research includes populations genetics, molecular genetics, and remote sensing data that will be integrated to test the current models and test their assumptions. In other words assumptions about the pest populations (population genetics), the genetics resistance (molecular genetics), and the scale of refuge compliance (remote sensing) will all be critical elements in assessing and improving the models used to prevent resistance development. These areas of research involve rather sophisticated technologies. While the research investment is significant, the needs are also significant and the opportunity for an even greater return on that investment justifies the expenditure. It should be noted that EPA will be conducting annual reviews of it's program and adjusting the research focus and budget allocations using the same criteria established for the initiation of the program.