MULTI-YEAR PLAN FOR

SAFE PESTICIDES/SAFE PRODUCTS
2007-2015

Office of Research and Development

U.S. Environmental Protection Agency

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Administrative Note

The Office of Research and Development's (ORD) Multi-Year Plans (MYPs) describe what research ORD proposes to conduct over the next 5-10 years in specific high priority areas. The MYPs serve four principal purposes, to: 1) describe the future directions of the research programs, 2) present the anticipated significant outputs of the research, identifying which laboratory/center will be responsible and the timeframe in which it will be completed, 3) communicate the research plans within ORD and with stakeholders and clients, and 4) identify the significant accomplishments and outcomes of previously conducted research. Multi-year planning permits ORD to consider the strategic directions of the Agency and how research can evolve to best contribute to providing the scientific underpinnings for the Agency's mission of protecting human health and the environment.

MYPs are intended to be "living documents." ORD updates MYPs on a periodic basis to reflect the current state of the science, resource availability, and Agency priorities. This MYP was reviewed by ORD's Science Council in October, 2006, and approved in November, 2006. The research directions and past accomplishments described in the MYP will serve as the foundation for an external peer review by the Safe Pesticides/Safe Products Subcommittee of ORD's Board of Scientific Counselors (BOSC) to be held February 7-9, 2007.

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I. INTRODUCTION

The US Environmental Protection Agency's (EPA) Office of Research and Development's (ORD) Multi-Year Plan (MYP) for Safe Pesticides/Safe Products (SP2) describes the research program that is specifically designed to address the problem-driven science needs of the Office of Prevention, Pesticides and Toxic Substances (OPPTS). The purpose of the SP2 Research Program is to provide OPPTS with the scientific information it needs to reduce or prevent unreasonable risks to humans, wildlife, and non-target plants from exposures to pesticides, toxic chemicals, and products of biotechnology. The SP2 Research Program specifically addresses OPPTS' high priority research needs that are not addressed by any of ORD's other research programs. The research program is focused on: 1) providing OPPTS with predictive tools for prioritization of regulatory data requirements; enhance the interpretation of data submitted as part of the regulatory process in order to improve human health and ecological risk assessments; provide targeted, multidisciplinary research in response to OPPTS requests on filling critical data gaps for specific individual or classes of pesticides and toxic substances that are of high priority; 2) developing the scientific underpinnings necessary to transform ecological risk assessments to a more realistic, spatially-explicit probabilistic basis where effects on wildlife and non-target plants can be evaluated as to their impacts at the wildlife population and plant communities levels; and 3) providing the tools necessary for OPPTS to update its requirements for submissions of registrations for products of biotechnology and the scientific foundation to help OPPTS interpret data submitted.

The scope of the SP2 research program has been developed in partnership with OPPTS. OPPTS has the responsibility of carrying out the mandates of numerous laws including the Toxic Substances Control Act (TSCA), Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), and Food Quality Protection Act (FQPA) As such, OPPTS plays a leading role in regulatory risk assessment in EPA and, consequently, a number of its research needs are similar to those of other EPA Offices. Therefore, to a certain extent, research conducted in direct support of other EPA Offices (e.g., Office of Water) as well as research conducted under ORD's core human health, human health risk assessment, and ecological protection research programs are providing many of the scientific methods and models needed by OPPTS.

In order to avoid duplication of research, ORD asked the senior OPPTS leadership to articulate the major scientific uncertainties in implementing their regulatory programs. ORD did a cross walk of OPPTS' strategic needs with the research directions of all of its research programs articulated in MYPs. Figure 1 illustrates the alignment of OPPTS needs with ORD research. This led to the identification of major areas that were not addressed by other research programs and where SP2 resources for research should be focused. In instances where research is being conducted through several research programs on a particular need, the research is coordinated and complementary, and not duplicative. Descriptions of the research that complements the SP2 program can be found in the MYPs for the following research programs: Endocrine Disruptors (www.epa.gov/osp/myp/edc.pdf), Drinking Water (www.epa.gov/osp/myp/dw.pdf), Human Health (www.epa.gov/osp/myp/eHH%20MYP%20Final.pdf), Human Health Risk Assessment (www.epa.gov/osp/myp/HHRA.pdf), and Ecological Research (www.epa.gov/osp/myp/eco.pdf). (See Section VII for more details on relationship of SP2 research with that in other ORD programs).

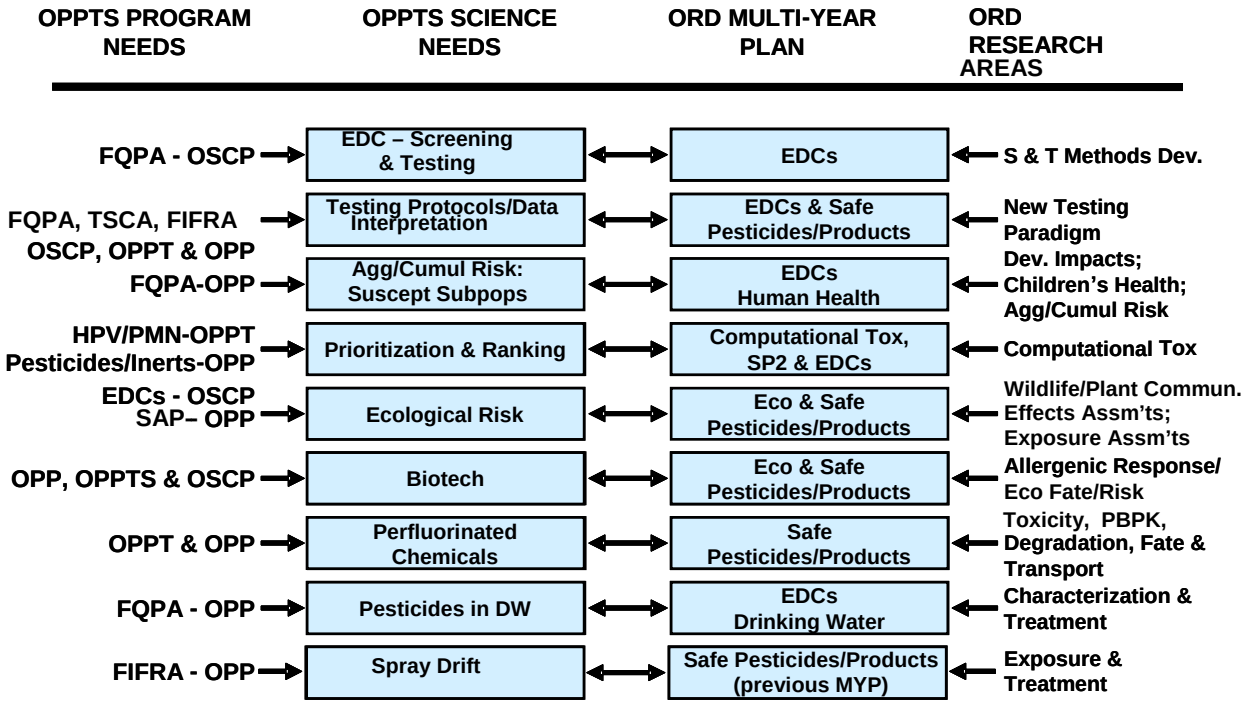


Figure 1. Alignment of OPPTS Needs with ORD Research

The research under the SP2 program is consistent with the recommendation of the National Research Council (NRC, 1997) report *Building a Foundation for Sound Environmental Decisions,* that the Agency should maintain a balanced program of "core and problem-driven research." The NRC indicated that problem-driven research is targeted at understanding and solving particular, identified environmental problems. The SP2 program consists mainly of problem-driven research and provides the balance to, and is coordinated with, the "core" research in the complementary programs.

ORD's SP2 research falls under EPA's Strategic Plan (2006-2011) Goal 4 Objective 4. **Goal 4, Healthy Communities and Ecosystems**, commits the Agency to protect, sustain, or restore the health of people, communities, and ecosystems using integrated and comprehensive approaches and partnerships. Objective 4 commits the Agency to **Enhance Science and Research**, by pledging the following: through 2011, identify and synthesize the best available scientific information, models, methods, and analyses to support Agency guidance and policy decisions related to the health of people, communities, and ecosystems. EPA's Strategic Plan further commits the Agency to focus research on pesticides and chemical toxicology; global change; and comprehensive, cross-cutting studies of human, community and ecosystem health.

As noted in the Agency's Strategic Plan (www.epa.gov/ocfo/plan/2006/entire_report.pdf), a key component of protecting the health of people, communities, and ecosystems is identifying, characterizing, and reducing any unreasonable risks presented by the thousands of chemicals on which the US population depends. For example, chemical and biological pesticides help meet national and global demands for food; provide effective pest control for homes, schools, gardens; and control animal vectors of disease. Every day the general public in the US comes into contact with industrial and commercial chemicals that are in products throughout our homes and workplaces. The SP2 research program is providing OPPTS with the tools it needs to make decisions about these chemicals.

The SP2 program is one of a few ORD research programs that include diverse multi-disciplinary efforts in the areas of human health, wildlife, and plants and cuts across the risk assessment/risk management paradigm. The complexity of the research in this program is reflected in the key science questions it is addressing. In several specific areas (e.g., perfluorinated chemicals, biotechnology), researchers are working in partnerships across disciplines to address the complex critical science needs. Such concerted multi-disciplinary efforts will enable us to achieve the Agency's Goal 4.4 Objective, as it relates to SP2, to "conduct research that contributes to the overall health of people, communities, and ecosystems."

The SP2 MYP arrays ORD's research program for the period 2007-2015 and revises and updates the previous MYP prepared in 2003 (US EPA, 2003). The SP2 MYP provides a focused research framework and direction that reflects available ORD scientific capabilities and capacity. The research described in this MYP assumes annual intramural and extramural resources of approximately 126 FTEs and $24.8 million, including payroll, travel and operating expenses.

Decisions regarding the conduct of specific research efforts under SP2 are based on the ORD's strategic and annual planning processes, which involve input and prioritization of research by the ORD and Program and Regional Office members of research planning teams as well as other Agency (e.g., OPPTS, ORD) senior managers, risk assessors, and stakeholders. The methods, models, and data developed through the SP2 research program are externally peer-reviewed and widely disseminated. The SP2 research program will undergo periodic external review by the Safe Pesticides/Safe Products Subcommittee of ORD's Board of Scientific Counselors (see Section VIII for additional details).

II. BACKGROUND

OPPTS' priorities and regulatory programs

The authorities and responsibilities of OPPTS are mandated primarily by TSCA (15 USC (C. 53) 2601-2692, 1976 http://www.access.gpo.gov/uscode/title15/chapter53_.html), FIFRA (7 U.S.C. s/s 136 et seq., 1996, http://www.access.gpo.gov/uscode/title7/chapter6_.html), FQPA (http://www.epa.gov/pesticides/regulating/laws/fqpa/), and the Pollution Prevention Act (42 USC 13101, 1990, http://www.epa.gov/opptintr/p2home/p2policy/act1990.htm). These statutes direct OPPTS to perform a wide variety of activities with the goal of protecting human health and the environment. OPPTS is authorized by TSCA, FIFRA, and FQPA, for example, to request data in order to: 1) evaluate the potential effects of industrial chemicals, pesticides, and products of biotechnology on human health and the environment, 2) discern when additional data are needed, 3)

set allowable levels of exposure or releases to the environmental that are protective of human health an the environment, and 4) determine whether risk management approaches are needed, and if so, which ones.

It follows, from their regulatory mandates, that there are several generic key areas of scientific needs that OPPTS has had historically: 1) the development of scientifically sound methods and models according to which OPPTS would in turn require the chemical and pesticide industries to submit data in compliance with TSCA and FIFRA, 2) the necessary data and tools needed by OPPTS to interpret the data submitted by industry and from other sources to evaluate the risks of chemicals, pesticides, and products of biotechnology, 3) frameworks under which assessments for human and ecological risks would be conducted, and 4) targeted data gaps on a single chemical or class of chemicals needed to complete an upcoming risk assessment and/or inform risk management decisions.

The first three areas are being met by multiple research programs through ongoing longer-term research efforts that are focused on OPPTS' greatest needs that evolve over time, as priorities shift and science advances. Some of the research to address these areas, especially if it has broad applicability to other Program and Regional Offices, is conducted under other research programs mentioned previously (also see Section VII). On the other hand, research that is of specific value to mainly OPPTS, such as developing methods and models that it would implement under TSCA and FIFRA, targeted research to support their specific risk assessment/risk management decisionmaking are conducted under the SP2 research program.

The fourth area, by the nature of the more immediate need to meet a deadline for a particular assessment, is met through shorter-term research efforts, and is largely conducted only through the SP2 research program. This requires the Research Program to be sufficiently flexible to adapt and accommodate the periodic urgent specific requests while maintaining the ongoing longer-term efforts.

For the last three decades, ORD research, under the SP2 Research Program and its predecessors, has provided OPPTS with continually improved testing methods that OPPTS, in turn, uses to produce guidelines for the industrial chemical and pesticide industries to follow in generating toxicity and exposure data. Therefore, for the relatively small investment that ORD has made in developing test methods, the Agency has received hundreds of millions of dollars' worth of data. Also historically, ORD research has been providing OPPTS with the needed tools to assist them in interpreting the data once they are submitted and incorporating the data into their risk assessments. The research described in this MYP continues to provide the same type of commitment and support.

Long Term Goals. Briefly, research is used in:

1 - prioritization of testing requirements, enhanced interpretation of data to improve human health and ecological risk assessments, and decisionmaking regarding specific individual or classes of pesticides and toxic substances that are of high priority.

2 - probabilistic risk assessments to protect natural populations of birds, fish, other wildlife, and non-target plants.

3 - decisionmaking related to products of biotechnology.

The three identified overarching long-term science needs have been structured as the long-term research goals for ORD's SP2 Research Program. The first goal is to build on the decades of test method development for assessing the risks of chemicals, to develop genomic and computational methods for prioritization of regulatory data requirements, to facilitate the interpretation of submitted data in risk assessments, and conduct short-term research to address targeted needs for upcoming specific risk assessment/management decisions. The second goal is to develop the scientific underpinnings necessary to transform ecological risk assessments to a more realistic, probabilistic basis where effects can be judged by their impacts at the population level and plant community level. The third goal is to provide the underlying science needed to evaluate products of biotechnology. See Section V for a more detailed description of the SP2 Long Term Goals (LTGs).

Figure 2 provides a conceptual framework for the SP2 research program, which links the resources to the outputs and programmatic outcomes to reduce or prevent risks to humans, wildlife, and non-target plants from pesticides, toxic substances, and products of biotechnology. The principal client for the SP2 research program is OPPTS. However, the research from this program is also of value to scientists in other ORD research programs, ORD's National Center for Environmental Assessment, and risk assessors in other Agency Program and Regional Offices, the states, other federal agencies, international organizations, the regulated community, and the academic community. These stakeholders use the products of this research, in line with the LTGs, as the scientific foundation for: 1) Prioritization of testing needs, enhanced interpretation of data, and decisions on targeted chemicals or classes of chemicals; 2) Probabilistic risk assessments for better informed decisionmaking in protecting natural populations of birds, fish and other wildlife, and plant communities; and 3) Decisionmaking related to products of biotechnology. Progress is measured by the extent to which methods, models and/or data from the SP2 research program are actually used in peer-reviewed risk assessments and other decisionmaking. The use of SP2 research products by OPPTS and others will contribute to decisionmaking related to reduction or prevention of exposures or releases of potentially harmful pesticides, toxic substances, and products of biotechnology into the environment.

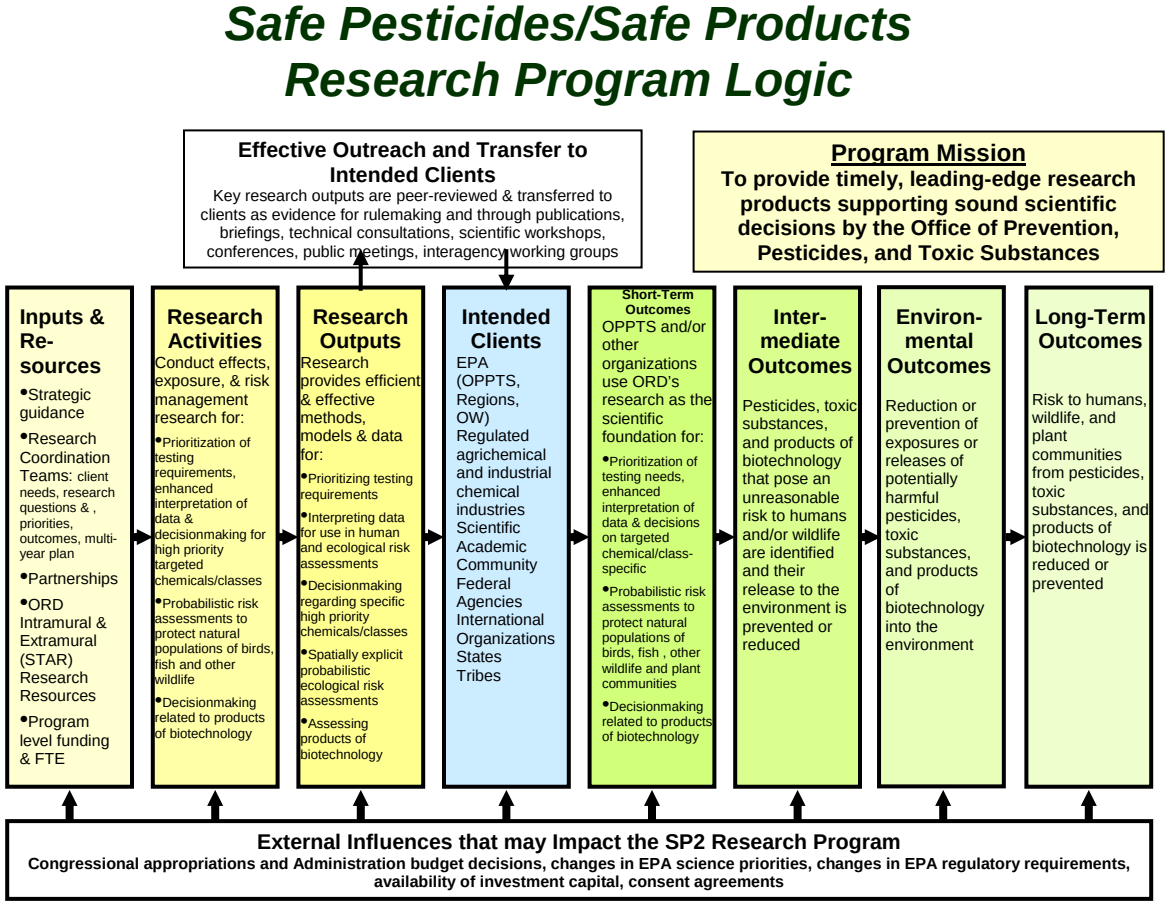


Figure 2. Logic Model for SP2 Research Program

III. RELATIONSHIP OF EPA'S RESEARCH TO THAT OF OTHER ORGANIZATIONS

Research outside of EPA

While several US Federal Agencies either conduct or support research in the area of test methods' development, their scope or approach differs from EPA's (see Long Term Goal 1). For example, the National Toxicology Program (NTP) coordinates toxicology testing within the federal government, conducts an interagency validation process for alternatives to whole animal tests, and convenes panels to develop risk assessments on targeted chemicals/classes of chemicals. The National Institute of Environmental Health Sciences (NIEHS) conducts and supports laboratory-based research to reduce the burden of human illness from environmental causes and has a Small Business Innovation Research (SBIR) program that supports the development of new toxicological testing approaches and promotes the technology transfer of new knowledge about mechanism for toxicity into applied testing methodologies (www.niehs.nih.gov/external/resinits/ri-11.htm;www.niehs.nig.gov/oc/factsheets/analt.htm). There is an interest both within NTP, specifically its Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and NIEHS, as there is at EPA, for the development of test methods that use fewer animals. ICCVAM evaluates and validates new test methods for implementation. NIEHS is using their intramural and extramural programs (e.g., National Center for Toxicogenomics) to develop

alternative methods and models including computerized modeling and predictive systems, tissue cultures, transgenic cells and animals, invertebrate species, and non-mammalian vertebrate species such as fish. Scientists from ORD's Comp Tox and SP2 Research Programs are collaborating with NIEHS on some of these efforts.

Various Agencies and scientific organizations conduct research to develop probability-based exposure models for ecological risk assessments complementing ORD's research (see Long Term Goal 2). For example, researchers at the Department of Energy's Argonne National Laboratory and Oak Ridge Laboratory have developed a number of probabilistic fate and transport, food-web, and aquatic models for evaluating potential risks to wildlife and aquatic receptors, for a range of environmental contaminants, at or near contaminated sites. The US Army sponsored the development of two wildlife exposure models (Spatially Explicit Exposure Model and FISHRAND-Migration) for improving the realism of terrestrial wildlife exposure models and predicting organic chemical uptake based on prey consumption and food web dynamics, respectively. The National Oceanographic and Atmospheric Agency is conducting research into tools for expressing ecological risk probabilistically. The United Kingdom's Department for Environment, Food, and Rural Affairs sponsors research to develop probabilistic approaches to reduce uncertainty in pesticide fate modeling. German researchers are collaborating on a research project (XPROB) to evaluate the use of human exposure factors in exposure modeling and provide guidance on probabilistic modeling. Arysta LifeSciences Corporation sponsored the development of the Probabilistic Exposure and Risk model for FUMigants (PERFUM) to address bystander exposures to fumigants following agricultural applications. CANTOX Environmental and Bayer Crop Science developed the probabilistic exposure model Granular Pesticide Avian Risk Assessment Model (GranPARAM) to estimate bird exposure to granular pesticides.

ORD participates in several interagency activities relevant to SP2 research. ORD co-chairs the Committee on the Environment and Natural Resources and its Toxics and Risk Subcommittee under the auspices of the Office of Science and Technology Policy (OSTP). ORD is a member of the Biotechnology Steering Committee under OSTP's Committee on Science. ORD is also a member of the Biotechnology Research Working Group (BRWG) and the Agricultural Biotechnology Risk Analysis (AGRA) Task Group, both working groups under the Steering Committee. Through these panels, research activities are shared across the agencies to facilitate collaborations and avoid redundancy. AGRA has developed a framework for federal research on agricultural biotechnology risk assessment and an inventory of ongoing federal research (the public document is in the process of being cleared for release). As a result of overlaying these two efforts, high priority research gaps have been identified. ORD's SP2 research program addresses several of these areas (see Long Term Goal 3). In addition, ORD participates on the United States-European Union Biotechnology Task Force. All of these efforts help ensure that ORD's SP2 research program is not duplicative of those conducted elsewhere.

The Organization for Economic Cooperation and Development (OECD) consists of 30 member countries and plays a prominent role in fostering "good governance in the public service and in corporate activity" (www.oecd.org). Under the OECD, EPA (mainly OPPTS and ORD) participates on many of their working groups aimed at promoting the development and harmonization of chemical testing guidelines and risk assessment approaches. For example, ORD has taken the lead within the OECD in revising the testing guidelines on developmental neurotxocity (TG 426). While the OECD itself does not conduct research it promotes scientific

innovation and encourages cross-member collaboration on research leading to the development of new testing guidelines and paradigms; and improved risk assessment and risk management tools. For example, ORD is participating in collaborative efforts through OPPTS with the OECD and the International Programme on Chemical Safety (IPCS) of the World Health Organization (WHO) on protocol development and the application of toxicogenomics in chemical assessments. The OECD's and IPCS' main areas of specific interest in the application of toxicogenomics include: 1) development of effective, increasingly efficient, and rapid approaches for the hazard screening of large number of chemicals, 2) improving understanding of cross-species sensitivity to facilitate cross-species extrapolation, 3) development of biomarkers, 4) harmonization of acceptance requirements among across member countries. These efforts reflect ORD's current interests and activities in the SP2 research program as well. ORD's participation in these efforts demonstrates the international recognition accorded to its scientists.

The chemical and pesticide industries develop the chemical-specific data that are submitted to OPPTS in accordance with regulatory requirements. In addition, the industry conducts other research. The American Chemistry Council (ACC) is a trade association of more than 190 member companies that represents the majority of the manufacturers of industrial chemicals in the US. ACC coordinates the chemical industry's research and testing programs. In 1999, the ACC initiated the Long-Range Research Initiative (LRI) to sponsor research aligned with health and environmental issues. Their latest five year strategy (2005) identifies three focus areas: improved methods, susceptible populations, chemicals in the environment Commonalities exist among these topics with the general objectives of the research in SP2, the Human Health, Human Health Risk Assessment, Endocrine Disruptors, and Ecological Protection Research Programs. In 2005-2006 the LRI is supporting 55 projects (www.uslri.org). They have ongoing research activities in many areas that complement EPA's intra- and extramural programs, e.g., studies on testing and testing methodology, susceptible populations, mechanisms of action, epidemiology, animal toxicology, wildlife studies, aquatic toxicology, environmental exposure, and environmental chemistry.

CropLife America (CLA) is the national trade association representing the plant science industry. One aspect of their activities is the Crop Protection Research Institute (www.croplifefoundation.org). They provide funding for the economic analysis of agricultural pests, pest management, and pesticide use and regulation. In particular they support the National Pesticides Use database.

Research conducted in EPA

No other programs have similar goals, in terms of scope and mission, as the SP2 research program that provides OPPTS with the tools it needs to carry out its regulatory mandates. EPA's SP2 research is multi-disciplinary, including: 1) research across all aspects of the risk assessment/risk management paradigm, i.e., in effects, exposure, risk assessment, and risk management; and, 2) as related to humans, wildlife, and plants. No other single organization has such an extensive portfolio of ongoing research in providing methods, models, and data for reducing scientific uncertainty regarding pesticides, toxic substances, and products of biotechnology. Comparison of potential benefits is conducted from a scientific perspective through coordinating with other research programs, participation at national and international scientific fora, and keeping abreast of state of the science. EPA's SP2 program includes many areas that are of unique importance in helping the

OPPTS meet its legislative mandates, such as requiring industry to submit data on pesticides, toxic substances, and products of biotechnology. The SP2 program also includes other research areas that serve to improve the basic scientific understanding regarding these agents that OPPTS and other parts of the Agency need to evaluate data submissions, conduct risk assessments, and to make informed management decisions. Furthermore, ORD's intramural program is complemented by an extramural program implemented through the Science to Achieve Results (STAR) program. ORD participation on interagency and international fora provides an opportunity for scientists to stay aware of research ongoing at other agencies/countries, help to ensure that work is not duplicated, and help to find potential collaborators.

Focus of EPA's contribution

Priorities for the SP2 research program were assigned based upon an assessment of the importance of the research to OPPTS, and to a much lesser extent any other Agency Offices, on the magnitude of the uncertainties in the knowledge base, the sequence of research needed to obtain the final answer, the possibility that the research would result in a significant product for hazard identification, risk characterization or risk management, the scientific and technical feasibility of conducting a successful project, and, finally either legislatively-mandated or Agency-set time frames.

ORD has significant expertise in the areas of toxicology, model development, engineering, and environmental exposures, relating to both humans and ecological systems, and in providing solutions to environmental problems. ORD scientists are respected members of the scientific community and leaders in their fields of concentration. Therefore, ORD can make/is making a significant contribution in the areas of development of more efficient and effective methodologies, models, and frameworks to evaluate chemicals, pesticides, and products of biotechnology and development of approaches to manage any unreasonable risks they pose. In addition, through the STAR program, ORD is engaging academic institutions to conduct research in areas that complement our intramural capability and capacity.

IV. Progress to Date/Changes from Previous Version

Progress to Date

Major accomplishments of the SP2 research program are described in Appendix VI. Accomplishments have been aligned by Long Term Goals (LTGs) which are defined in Section V. Where available, a website, where more detailed information can be found, has been provided.

Changes from Previous Version

The SP2 MYP has undergone some changes from the previous (2003) version. Most of the major changes that have been made to the SP2 MYP deal with the how the research program is organized for presentation. In addition, the content of the research program has evolved, as it has built upon its previous accomplishments and, through closer collaborations with OPPTS, has refined the specific research needed. Other changes reflect shifts of resources from extramural to intramural research support for the principle investigators, as well as significant decreases to the overall SP2 budget that have taken place since 2003. A list of significant changes in the current version of the SP2 MYP is as follows:

- The LTG structure has been modified and redefined to make it outcome-oriented; that is, it now describes how and by whom the results of the research will be used.
- LTG 4 has been eliminated due to budget decreases. Some of the remaining research critical to OPPTS has been moved to other LTGs.
- LTG 1 has been expanded to include the flexibility to conduct multi-disciplinary shorter term research on high priority chemicals or classes of chemicals for OPPTS. This new area includes the remaining research from LTG 4 on the toxicity of perfluorinated chemicals. The perfluorinated chemicals research portfolio has been expanded to take on a multi-disciplinary approach and now includes exposure and risk management research because of an increased need to understand exposure pathways and fate of this emerging class of chemicals. Priority research conducted previously under LTG 3, e.g., pesticides in drinking water, has been shifted to LTG 1. LTG 1 has been expanded to include computational toxicology research on prioritization, testing, and enhancement of risk assessments that is being done through the National Center for Computational Toxicology and the complementary extramural STAR program.
- LTG 2 has been expanded to include high priority research on the effects of herbicides on plant communities, especially as those effects affect the suitability of the communities as wildlife habitat.
- The visibility of ORD's research on biotechnology guided the decision that it should comprise its own LTG (3). Furthermore, as the original research projects from the 2002 initiative come to a completion, the research shifts from laboratory/center-specific projects toward a cross-laboratory/center integrated project. Severe budget reductions to this research, however, have led to a much more limited program than described in the previous MYP.
- Across the board, there are changes to Annual Performance Goals (APGs) and milestones or Annual Performance Measures (APMs). Some have been added, some have been deleted, and some have been delayed. Deletions include those that have been met in previous years as well as those that will not be done because of budget decreases. Delays in timing are also the result of impacts to resources.
- This version includes an Appendix that highlights the Accomplishments of the SP2 research program.
- This version includes an Appendix that provides greater detail to the research theme areas.
- This version provides improved examples of cross-linkages to other MYPs.

V. LONG TERM GOALS

Long Term Goals

Long Term Goal 1: OPPTS and/or other organizations use the results of ORD's research on methods, models, and data as the scientific foundation for: A) prioritization of testing requirements, B) enhanced interpretation of data to improve human health and ecological risk assessments, and C) decisionmaking regarding specific individual or classes of pesticides and toxic substances that are of high priority.

The ultimate outcomes are the development of improved methods, models, and data for OPPTS' use in requiring testing, evaluating data, completing risk assessments, and determining risk management approaches. More specifically the outcomes are the development by ORD and implementation by OPPTS of more efficient and effective testing paradigms that will be better informed by predictive tools (chemical identification, improved targeting cost less, less time, and fewer animals); improved methods by which data from the more efficient and effective testing paradigms can be integrated into risk assessments; and that OPPTS uses the result of ORD's multidisciplinary research approaches, that it specifically requests, for near term decisionmaking on high priority individual or classes of pesticides and toxic substances.

Long Term Goal 2: OPPTS and/or other organizations use the results of ORD's research as the scientific foundation for probabilistic risk assessments to protect natural populations of birds, fish, other wildlife, and non-target plants.

Results of this research will help the Agency meet the long term goal of developing scientifically valid approaches to extrapolate across species, biological endpoints and exposure scenarios of concern, and to assess spatially-explicit, population-level risks to wildlife populations and non-target plants and plant communities from pesticides, toxic chemicals and multiple stressors while advancing the development of probabilistic risk assessment.

Long Term Goal 3: OPPTS and/or other organizations use the results of ORD's biotechnology research as the scientific foundation for decisionmaking related to products of biotechnology.

OPPTS will use the results from this research program to update its requirements of registrants of products of biotechnology and to help evaluate data submitted to them.

Science Questions

Addressing the following key science questions through the SP2 research program will give OPPTS the tools it needs to meet its mandates. Under the LTGs or subparts of the LTGs, the key science questions are as follows:

LTG 1 –Subpart A - Providing OPPTS with predictive tools for prioritization of regulatory data requirements:

- What methods are needed for priority setting and screening?
- How can existing *in silico* and *in vitro* techniques be harnessed to develop effective and efficient screening and prioritization tools?
- How can existing quantitative structure activity relationship (QSAR) databases be improved?
- What endpoints are amenable for the development of *in vitro* screens?
- How can improved understanding of pathways of toxicity lead to improved predictive tools?

LTG 1 – Subpart B - Enhancing the interpretation of data submitted as part of the regulatory process in order to improve human health and ecological risk assessments:

- What methods are needed that could enhance the interpretation of data from current guidelines?
- How can current guidelines be revised to enhance sensitivity and improve data quality and interpretation?
- Can hypothesis-driven approaches for testing chemicals for multiple toxicity pathways be developed?
- How can current databases be enhanced and applied to improve access to data for hypothesis formulation and test evaluation?

LTG 1 – Subpart C - Providing targeted, multidisciplinary research in response to OPPTS requests on filling critical data gaps for specific individual or classes of pesticides and toxic substances that are of high priority:

- What methods and tools are needed for characterizing the following for perfluorinated chemicals?
  - Effects - toxicity and pharmacokinetics
  - Exposures – pathways of exposure, environmental degradation, fate and transport
  - Risk management options
- What protocols are needed for information on the impact of drinking water treatment processes on pesticides?
- To what extent, if any, do deck coatings and sealants reduce dislodgeable residues on the surfaces of CCA-treated wood?
- How can exposure methods be improved for use in large scale human studies?
- What factors affect the releasability of asbestos?
- What chiral pesticides are good candidates for production of safer, single enantiomer products?
- Can fast, simple, inexpensive lead paint test kits be developed quickly?

LTG 2 - Developing the scientific underpinnings necessary to transform ecological risk assessments to a more realistic, spatially-explicit probabilistic basis where effects on wildlife and plants can be evaluated as to their impacts at the wildlife population and plant community levels:

- What methods are needed for extrapolating toxicological data across wildlife species, media, and individual-level response endpoints?
- What methods are needed for characterizing population-level risks of toxic chemicals to aquatic life and wildlife?
- What approaches are needed for evaluating the relative risks from chemical and nonchemical stressors on spatially structured wildlife populations across large areas or regions?
- How can methods to assess direct and indirect risks to non-target plant species and plant communities from the use of chemical herbicides be improved?
- What probabilistic tools can be used to characterize or predict the fate and transport of pesticides and other environmental contaminants?
- How do environmental contaminants move through environmental compartments and become available for human, aquatic, and wildlife exposures?

LTG 3 - Providing the tools necessary for OPPTS to update its requirements for submissions of registrations for products of biotechnology and the scientific foundation to help OPPTS interpret data submitted:

- What are the potential risks of allergenicity to biotechnology products and how can they be evaluated?
- What are the risks to natural ecosystems of gene transfer from engineered organisms to natural species in the wild?
- What methods are needed to mitigate the development of resistance and of gene transfer?

The degree of emphasis for each LTG is based on the following criteria: 1) an assessment of the importance of the research to OPPTS and other Agency Offices, 2) the magnitude of the uncertainties in the knowledge base, 3) the sequence of research needed to obtain the final answer, 4) the degree of impact the research product(s) are likely to have, 5) their degree of criticality for an OPPTS hazard assessment, risk characterization, or risk management decisions, 6) the intramural and/or extramural capability and capacity to ascertain the scientific and technical feasibility of conducting a successful project, and 7) legislatively mandated or other regulatory time frames. The following Table summarizes the relative emphasis of each LTG over the period of FY2007 through 2015.

LTG	Emphasis from 2007 through 2015
1	Level and then increasing, when resources are freed from LTG 2
2	Level until probabilistic tools are developed and implemented, then decreasing
3	Level and then increasing, when resources are freed from LTG 2

The rationale behind the projected level of emphasis over time is as follows. Within the next eight years, it is expected that ORD will have developed the needed tools under LTG 2 for OPPTS to use in their probabilistic ecological risk assessments. Once those tools are completed, delivered, and implemented, the level of effort in that LTG need no longer remain at its current level. At that time resources from LTG 2 will be shifted to LTGs 1 and 3 to address projected increasing needs in those areas. For example, under LTG 1 there will be an increased need for more sophisticated predictive tools for prioritizing and screening chemicals. Under LTG 3, additional resources will be needed to restore resources back to their original FY03 level to address the intended goals of the research program related to agricultural biotechnology needs and to expand to address the growing need to provide OPPT with tools needed to review products of biotechnology associated with TSCA mandates.

Successfully addressing these LTGs will require a highly coordinated effort. The efforts will be overseen by the research planning teams under the direction of the National Program Director for Pesticides and Toxics. The SP2 MYP, the NHEERL Implementation Plan, the Wildlife Research Strategy (www.epa.gov/nheerl/publications/files/wildlife_research_strategy_2_2_05.pdf), the

Biotechnology Research Strategy (www.epa.gov/nheerl/publications/files/biotechnology_research_program_4_8_05.pdf) , the Computational Toxicology Framework (www.epa.gov/comptox/publications/comptoxframework06_02_04.pdf) and any other strategies or plans that may be developed around the current topics should be considered collectively when trying to understand the overall SP2 research program, in order to determine what specific research the Laboratories and Centers will be carrying out, and when. The research planning teams recognize the dynamic nature of the SP2 research and, therefore, will assess the priorities and sequencing in the MYP and other related documents periodically, so that the overall research program can be modified as the knowledge base increases, new technologies become available, and resources shift.

VI. DESCRIPTION OF THE FLOW DIAGRAMS AND SP2 RESEARCH PROGRAM

The flow diagrams (Figures 1-3 in Appendix II) depict the Annual Performance Goals (APGs) that will support the LTGs, the time frame for their completion, and their interrelatedness. The research that ORD is committed to conducting under each of the LTGs is reflected in the APGs and their respective Annual Performance Measures (APMs) and is described in greater detail below and in attached Tables 1-3 in Appendix III.

The research planning team took the following into consideration when determining the APGs: 1) the key questions based on OPPTS' programmatic needs, 2) the research themes that were being identified within NHEERL's SP2 Implementation Plan the Wildlife Research Strategy, Biotechnology Research Strategy, and the Computational Toxicology Implementation Plan, and 3) an assessment of other ongoing and anticipated ORD research efforts. As a result, eleven discrete research areas (APGs) were identified in which ORD, between the intramural and extramural STAR grants programs, can make a significant impact in advancing the state of the science on SP2 within the next 8 years. Schedules for these research areas were estimated based upon knowledge of: 1) existing resources, 2) intramural capacity and capability, 3) projected timelines for awarded grants, 4) the complexity of the area, and 5) in some cases, regulatory-driven deadlines.

Each APG has a number of APMs that represent discrete segments of research to be completed within the defined schedule. The APMs (Tables 1-3 in Appendix III) will help to determine progress made towards completing the APG. The APMs in the Tables represent the expected product from a given research area. Therefore, the Tables, for the most part, do not necessarily show continual progress of a research area from start to finish, but rather just the major "milestones." Most of the APMs are attributed to one of ORD's Laboratories or Centers. However, in an effort to improve collaboration across laboratories and centers on a given environmental issue, some APMs may represent the products of multiple organizational efforts. It should be noted that, for the most part, those that are attributed to NCER are products of STAR grants. Some APMs appear in multiple MYPs (or are leveraged with resources from other MYPs). It is important to note that the Tables capture the APGs and APMs currently anticipated. The research planning team recognizes the need to update the matrix periodically, as new milestones are anticipated and as emphases shift.

Please note that the APMs described in this MYP should not be confused with or ever designated as ORD or Agency APMs used in cross-Agency planning and accountability activities. It is anticipated, that APMs from the MYP will be aggregated and integrated to derive "Annual Performance Measures" for Agency accountability activities, when required.

The eleven APGs for the SP2 MYP are described below. For each APG, the objective, the significance/impact, and the schedule of the research are described. It is assumed that the various APMs, when aggregated, will lead to the achievement of the APG.

A summary of the science issues and the research program objectives makes up most of the remaining body of this MYP. A compendium of what research would be done if there were more resources, the timeline for delivery of research products by laboratory/center, more detailed descriptions of the research themes (an aggregation of multiple research projects addressing common key science questions around a specific topic), a listing of significant accomplishments, and a list of acronyms can be found as Appendices.

Long Term Goal 1: OPPTS and/or other organizations use the results of ORD's research on methods, models, and data as the scientific foundation for: A) prioritization of testing requirements, B) enhanced interpretation of data to improve their human health and ecological risk assessments, and C) decisionmaking regarding specific individual or classes of pesticides and toxic substances that are of high priority.

Ultimate Outcomes: *Research will provide improved methods, models, and data for OPPTS' use in requiring testing, evaluating data, completing risk assessments, and determining risk management approaches. Specifically the research will result in the development by ORD and implementation by OPPTS of more efficient and effective testing paradigms that will be better informed by predictive tools (what chemicals, improved targeting cost less, less time, and fewer animals); improved methods by which data from the more efficient and effective and existing testing paradigms can be integrated into risk assessments; and that OPPTS uses the result of ORD's multidisciplinary research approaches, that it specifically requests, for near term decisionmaking on high priority individual or classes of pesticides and toxic substances*.

Synopsis: ORD intramural and extramural research is:

- developing and applying the latest molecular and computational approaches to produce the next series of chemical prioritization tools and toxicity testing approaches;
- enhancing data interpretation by evaluating the diagnostic value of data obtained from current toxicity testing guidelines in order to develop improved targeted test methods for major classes of pesticides based on defined modes-of-action and identification and characterization of genomic and proteomic biomarkers;
- characterizing toxicity profiles of perfluoroalkyl chemicals, examining the potential for selected perfluorinated telomers to degrade to perfluoroctanoic acid (PFOA) or its precursors,
- developing methods and models to forecast the fate of pesticides and byproducts from source waters through drinking water treatment systems and ultimately to the US population,
- providing exposure methods for large-scale human studies, and
- addressing specifically identified research needs by studying chromated copper arsenate-treated wood, asbestos, chiral pesticides, lead-based kits.

Protecting human health and the environment from harmful agents carries the challenge of developing the capability for assessing hundreds of possible hazardous effects for tens-of-thousands of important commercial chemicals. Establishing strategic priorities to focus available laboratory testing resources on those chemicals posing the greatest potential risks is essential to EPA in minimizing environmental risks from harmful agents. Over the last three decades, ORD has developed for EPA an extensive arsenal of test methods needed in all aspects of regulatory risk assessment. ORD will continue, through the SP2 research program, to refine many of these methods and to reduce the uncertainty with respect to interpreting the results of tests in EPA decisions. However, through the SP2 research program, ORD will also address the greater challenge of developing the science necessary for EPA to know when and how to apply those test methods to gain greatest insight into the potential risks of a specific chemical.

Over the years, collaborations between ORD and OPPTS have resulted in substantial advances in human health and ecological testing paradigms and improved risk assessment approaches. In order

to address future challenges to providing credible scientific information, timely and efficiently, to support risk assessment and risk management decisions for industrial chemicals and pesticides, in 2004, OPPTS and ORD developed a strategic plan for research in support of OPPTS program activities. This plan provided a general framework to guide the development and implementation of new scientific programs and specific advancements that will further effective regulatory decision-making. Thus, the long-term solution to meeting this challenge will not be the generation of more data faster, but rather determining what specific effects data, for which chemicals, and which exposures is essential to assess and manage risks appropriately. In this context, the Agency requires sufficient, targeted, credible information from which to make decisions. Consistent with this view is the consideration of time and cost efficiencies associated with the generation and interpretation of toxicity data and the sound and responsible use of animals.

OPPTS is responsible for regulating certain chemicals for which there are little or no toxicological or exposure data (e.g., Pre-Manufacture Notification (PMN) and High Production Volume (HPV) chemicals, inert pesticide ingredients, antimicrobial pesticides). Therefore, there is a need for creating ways to accurately predict the toxicity and levels of exposure for these chemicals. Predicting the potency, activity, and exposure to these chemicals will enable OPPTS to make better informed decisions as to whether or not empirical studies are required to further refine a risk assessment for regulatory decisionmaking. Current approaches for testing chemicals require extensive resources. Therefore, priority setting approaches must be developed to determine the sequencing of chemicals or classes of chemicals to assess for a specified toxicity endpoint. Additionally, while extensive data sets are generated for many toxicity endpoints currently used in risk assessment, efficiency can be gained in using targeted testing to reduce critical uncertainty while minimizing resource utilization. The current inability to estimate endpoints sufficiently to set hypothesis-driven risk-based priorities is the result of a lack of understanding of pathways of toxicity and how they can be initiated by chemicals, as well as by a lack of methods to model the complex behavior of chemicals. By having an understanding of the initiating events of critical toxicity pathways OPPTS and ORD will be able to use credible *ex vivo* techniques to estimate the toxic potential of chemicals and allow them to be ranked/prioritized for their potential to elicit adverse outcomes. With the development and application of new computational and molecular tools, it is anticipated that *in silico* and *in vitro* techniques for prioritization and screening of chemicals for toxic effects resulting from exposure to PMNs, HPV/inerts and antimicrobial chemicals is highly feasible over the next seven years. The determination of possible levels of exposure to these chemicals will also need to be included into any screening or prioritization program. Thus, of the issues facing OPPTS, the need to develop more efficient ways to screen and prioritize chemicals for testing to acquire sufficient, targeted, credible information for decision making is of high priority.

To overcome these gaps, and to move toward a more sustainable risk assessment paradigm to support TSCA, FIFRA, and FQPA decisions, the SP2 research conducted under LTG 1 will provide EPA with predictive tools for hypothesis-driven prioritization of testing requirements and enhanced interpretation of exposure, hazard identification, and dose-response information. The research is complementary to and is coordinated with ORD's Computational Toxicology (Comp Tox) Research Program.

In 2002, EPA began a new research program in Computational Toxicology (www.epa.gov/comptox) to better understand the relationships between sources of environmental

chemical exposures and adverse outcomes. Computational toxicology is defined as the integration of modern computing and information technology with the technology of molecular biology and chemistry to improve EPA's prioritization of data requirements and risk assessment s for toxic chemicals. Three strategic objectives of the initiative are to: 1) improve understanding of the linkages in the continuum between the source of a chemical in the environment and adverse outcomes, 2) provide predictive models for screening and testing, and 3) improve quantitative risk assessment. While ORD has a separate Comp Tox program, many other ORD core and problem-driven research programs are also developing and applying comp tox tools to address their objectives. These research activities are coordinated with those of the Comp Tox Research Program and are linked under the Comp Tox Framework (www.epa.gov/comptox/comptox_framework.html). This is the case with the comp tox related activities in SP2, where as apparent, the objectives of both Research Programs have commonalities, especially in the area of developing/applying methods for the prioritization and screening of chemicals. LTG 1 of the SP2 Program not only includes intramural comp tox research but also the extramural STAR component of the Comp Tox Research Program. Through issuing targeted requests for application, ORD is engaging non-profit organizations, to conduct research in areas that complement our intramural activities. The STAR Comp Tox program, as noted below, is providing a unique opportunity for academic scientists to work cooperatively with EPA scientists on advancing the development and application of computational and molecular methods.

The research in SP2 LTG 1 also builds upon the screening and testing efforts underway in the Endocrine Disruptors (www.epa.gov/osp/myp/edc.pdf) and Human Health (www.epa.gov/osp/myp/HH%20MYP%20Final.pdf) MYPs by applying tools, techniques and knowledge to problem-driven research in support of the major OPPTS needs.

The development of research strategies and MYPs in ORD serves to keep research activities focused on the frontiers that cause the greatest uncertainties in risk assessment and risk management. In designing the strategic framework for the SP2 research program, it became clear that the broad spectrum of regulatory responsibilities in OPPTS presented special problems for research planning. While there was general agreement that ORD should focus more resources on fewer and more central research needs of the many OPPTS programs, there was also the concern over having all available resources committed to longer-term research and not having the flexibility or resources to respond to special scientific needs that arise. For this reason it, part of LTG 1 is devoted to addressing these shorter term targeted needs. It is anticipated that as these needs are met, that they will be replaced with other emerging needs of priority at that future time. The compilation of work described further under LTG 1 represents the high priority shorter-term research being conducted to address the currently identified targeted needs by OPPTS. The shorter-term research falls under two separate APGs. Research on the perfluorinated chemicals (PFCs) began several years ago focused only on characterizing their potential adverse health effects. More recently ORD has been asked to conduct research on characterizing their environmental releases, fate and transport, and degradation. Therefore, the body of research on the PFCs is large enough to merit a separate APG.

APG - Develop and validate virtual chemical and alternative methods for risk-based prioritization and screening of chemicals – FY 2015

High quality data are essential to the development of predictive models, such as QSARs, needed to prioritize chemicals for hypothesis-driven regulatory testing. Where large numbers of chemicals exist that need to be assessed based on little or no measured data, QSAR predictions can be used to prioritize which should be tested, when testing should occur, and which endpoints should be tested.

Such approaches are needed to form the basis for targeted regulatory testing to increase risk assessment efficiency, but are only possible where toxicity pathways are well defined and where assays are available for sufficient strategic testing to build applicable models. Strategies to systematically test within large chemical inventories are included in LTG 1 to minimize the collection of redundant information and to maximize the understanding of the attributes of chemical structural that initiate specific biological interactions. Targeted testing approaches in LTG 1 seek to incorporate new *in vivo* protocols that provide data with less uncertainty and/or to provide *in vitro* assays that have the potential to develop into high-throughput (HTP) systems when they are linked to the *in vivo* endpoint.

Tools needed for screening and prioritization follow a logical progression from early method/assay development, through the application of an assay or suites of assays in a diagnostic manner to elucidate toxicity pathways, to the systematic testing of multiple chemicals to identify groups that initiate toxicity in a common way. Once testing can be done within a pathway, chemical testing can be focused to determine features of chemical structural that facilitate chemical-biological interactions. Quantifying the structural requirements through which a chemical initiates a toxicity pathway allows the prediction of probable toxic interactions for a given chemical. As the knowledge base grows, it can be used as a basis to prioritize testing requirements based on likelihood of causing effects.

Short and intermediate-term research in this area focuses on providing better access to existing models, developing models where toxicity pathways are well understood, and providing tools and approaches that can be applied in the longer term as understanding of additional pathways allow. Long-term research in this area will attempt to integrate data generated from intermediate-term projects including *in vitro* screens and genomic and proteomic approaches with new knowledge of toxicity pathways to develop predictive models that specifically address data gaps in the risk assessment process. Systematically collected assay data in short- and intermediate-term projects will, using tools and approaches currently under development, serve as the basis for toxicity pathway-based QSAR prioritization protocols in the future for the major endpoints of regulatory concern. The gradual development of a library of pathway-specific models will eventually allow the prioritization of testing endpoints.

ORD research is:

- Determining the chemical structural requirements for initiation of distinct toxicity pathways by incorporating QSAR-based hypothesis generation, strategic chemical selection for hypothesis testing, *in vitro* assay optimization and targeted testing, and QSAR evaluation and improvement for mechanistic classifications for OPP pesticidal inerts and antimicrobials, chemicals for which data are lacking and predictions needed. Products include guidance on development of QSAR prioritization models for toxic effects endpoint in the context of well-defined toxicity pathways and demonstrated application of the methods for OPP chemical lists for which testing priorities are requested. (Project 1.1.1 – refer to more detailed description of each project in Appendix IV).
- Upgrading and expanding the capabilities of ASTER (Assessment Tools for the Evaluation of Risk) to rank large lists of chemicals based on data available in ASTER (e.g., ecotoxicity, environmental

partitioning, environmental persistence, and chemical bioconcentration in tissues), and searching the ECOTOX database for structural analogs. This will facilitate the identification of structural analogs and associated toxicity information to estimate potential hazard of untested chemicals or chemicals with limited toxic effects information. 1.1.1

- Refining an existing metabolism simulator to focus on metabolic transformations most likely to increase toxic potential for estrogenicity. The research helps in better understanding toxicity pathways from initiating event to response for metabolically activated chemicals. A computational tool is provided that allows prediction and prioritization of chemicals for which measured data are lacking. 1.1.1
- Developing and applying Nuclear Magnetic Resonance-based metabolomics for improving chemical exposure and risk assessments. ORD will produce: 1) validated markers of biologically relevant chemical exposure to important classes of pesticides and toxic chemicals, 2) information on the temporal and compensatory aspects of chemicals exposures, 3) information on similarities and differences xenobiotic metabolism and the impact of exposures across key species (small fish, rats, etc.), and 4) information on the linkage of exposure events to whole organism adverse outcomes. 1.1.2 (part of this project is linked to second grant described under 1.2.4 and 2.1.3)
- Developing *in vitro* cell culture models of the key events in brain development and methods to measure behavioral, morphological and neurochemical outcomes in a limited number of non-mammalian species. The database that results from data generated by testing the set of known developmental neurotoxicants using HTP methods will provide OPPTS with information on the utility and limits of the screening battery, and provide guidance for the interpretation and potential use of data from these alternative methods in a risk assessment context. 1.1.3
- Utilizing Sertoli cell cultures challenged with a panel of known reproductive toxicants to identify insult induced effects *in vitro*. Emphasis will be on identifying markers with the potential to be used in current one generation tier testing and research could result in the development of a HTP cell culture based screen for assessing testicular and epididymal insults. 1.1.3
- Examining protein expression profiling as a means to screen chemicals for their mode of action for pathway specific toxicity. Protein profiling is also being investigated to develop an "omics"-based approach to understanding differences in species sensitivity to chemical categories, and incorporated into existing *in vitro* and short-term *in vivo* assays needed to support hypothesis-based risk assessment and regulatory decisions. 1.1.3

In 2002, a request for applications (RFA) of proposals from non-profit organizations was issued through the Comp Tox STAR Research Program on new approaches to the development of HTP screening systems for identifying chemicals with estrogen, androgen, or thyroid hormone activities. In its initial phase, the Comp Tox Research Program used the endocrine system as a 'proof of concept.' The research awards made through this solicitation are expected to contribute to the development of HTP screening systems to assist in prioritization of chemicals for further screening and testing of their potential as endocrine disruptors. The methods generated from these studies will provide for multiple platforms for the HTP screening of potential endocrine disrupting chemicals, in some cases even allowing for remote, near real-time monitoring of potential endocrine disrupting chemicals in the environment. Under this program, ORD is supporting extramural research that is:

- Developing and applying a bioluminescent yeast-reporter system for screening chemicals for estrogenic and androgenic effects. 1.1.4
- Developing a mechanism-based, high-throughput screening assay for evaluating estrogen, androgen, and thyroid (EAT)-like activities in an invertebrate species that also can be used to evaluate interactive effects of endocrine-active compounds through receptor cross-talk. 1.1.4
- Investigating the proposition that perturbations in the normal amount or timing of a hormone-regulated gene product can be taken as evidence of chemical exposure and used as an endpoint in a screening assay using the zebrafish to detect potential endocrine disrupting activity. 1.1.4

- Developing a rapid, sensitive, biologically-integrated screening assay to identify endocrine-disrupting chemicals (EDCs) using a medaka fish model. 1.1.4

APG - Evaluate and provide guidance regarding the sensitivity and predictive value of current test methods and those under development for the improved identification and characterization of the potential of environmental chemicals to cause human health and ecological risks – FY 2013

SP2 research is developing hypothesis-driven risk assessment paradigm that moves in a logical and transparent manner from a process that requires extensive toxicity testing followed by the elimination of information not relevant to the assessment for all chemicals (e.g., food-use pesticides) to a paradigm where far fewer tests are needed but the experiments are designed to elucidate the toxicity pathways that are triggered by a given chemical/class of chemicals to identify what additional specific *in vivo* information will be most relevant to the assessment. OPPTS could apply such a paradigm to both new and existing chemicals including conventional food-use pesticides. The goal is to be able to continually make scientifically sound regulatory decisions but with significantly less required data generation. This process could be applied to chemicals from across OPPTS programs because the amount of data required to understand toxicity pathways from initiating events to adverse outcomes will be limited only to endpoints of interest which will greatly limit the number of *in vivo* tests needed to determine a chemical's toxic potency.

SP2 research on database development projects are making the most efficient use of existing OPP registrant data submitted under a wide variety of testing guidelines. The large quantity of guideline study data has the potential to advance the understanding of toxicity pathways by providing *in vivo* outcome information collected under standardized guidelines. However, to reach this potential the data need to be widely accessible and searchable. LTG 1 research is addressing these needs for toxicity endpoints, and chemical metabolism and degradation pathways. Outputs of the database efforts will contribute both to enhancing data interpretation and to prioritization and screening. The first step of the approach is to identify where data already exist and to make them more easily accessible for evaluation by both risk assessors and researchers. Thus, building and populating well-designed databases is a near-term emphasis because it is the key to making progress in the intermediate and longer-term. Searchable databases contribute in multiple ways to LTG 1 projects by:

- Greatly assisting data interpretation by allowing efficient access to information otherwise unevaluated due to inaccessibility or simply not knowing it exists
- Allowing records to be grouped by user-defined descriptors to facilitate evaluation of data in new ways and to discover associations previously not examined
- Providing better access to program office data to allow identification of critical linkages in toxicity pathways thus enhancing interpretability of *in vitro* and biomarker data linked to adverse outcomes
- Assessing where knowledge gaps exist, and
- Allow examination of where correlations can and cannot be made across endpoint measures, across chemicals, and across species

ORD is working with OPPTS risk assessors and others to design databases that will:

- Allow better understanding of existing data
- Allow systematic evaluations of some of the data resulting from newer guidelines and protocols

that have as yet not been evaluated
- Facilitate comparisons of information available in current tests with newly developed tests to guide the development of new testing approaches in a hypothesis-driven manner to target where efficiencies or enhanced interpretation can be gained.

Where a toxicity pathway is sufficiently understood, and where assays are available for that pathway, systematically harnessing the existing knowledge-base needed may provide all that is needed for the development of more realistic scientific basis to assess risk. In that vein, toxicity pathway-specific QSARs for prioritization and screening are under development for currently well-defined pathways building on the OPP historical database. In the mid- and longer- term research is underway to demonstrate the approach and build the tools needed which can be applied in the future as more key pathways are elucidated.

Efficient access to existing data to identify similar acting chemicals based on toxicological outcome can be achieved by developing searchable databases. Collaborative efforts between ORD and OPP risk assessors under LTG 1 will focus on outcomes that are of high priority to enhance interpretation of data and that optimally leverage existing ORD research approaches and expertise, so that both risk assessors and researchers maximally benefit from these efforts. LTG 1 database projects also support the need to enhance our current ability to interpret many types of data submitted under existing guidelines. In some instances sufficient data have only recently become available as relatively new guidelines were introduced over the last several years. Areas are identified where extensive evaluations of newer datasets and examination of test protocols by ORD and OPPTS collaborators will be undertaken to help inform current risk evaluations and to direct the development of hypothesis-driven predictive, diagnostic markers (biochemical, proteomic, genomic) for given risk endpoints (e.g., immunotoxicity, neurotoxicity, reproductive effects). Hypothesis-driven predictive, diagnostic markers will help us learn if we use the results to form the basis for further targeted testing. The goal is an increased understanding of toxicity pathways to guide testing and to generate high-quality datasets.

Where toxicity pathways are not sufficiently understood (e.g., where there are critical knowledge gaps in the continuum from chemical initiation of the toxicological process to its manifestation as a whole organism adverse outcome) research will be undertaken in concert with ORD's core research programs in Human Health, Endocrine Disruption and Computational Toxicology to assess current understanding of the events leading to adverse effects, to identify critical knowledge gaps, to understand commonalities in toxic pathways to develop integrative new methods that provide information on multiple effects, and to develop *in vitro* assay approaches for rapid screening.

Under database development, ORD researchers are collaborating with OPPTS scientists to:
- Design a database to provide access to currently non-accessible metabolic map information that exists in OPP files. Access to metabolism data in a searchable format is key to understanding the role of metabolic activation in toxicity pathways, and to being able to generating targeted hypothesis that will address the highest priority uncertainties for the types of chemicals of most concern to the program offices. 1.2.1
- Design a chemical degradate pathway database to provide ready access to pathways of degradation associated with identified reaction types, for specified bioassay conditions across all study types and chemicals of concern. The development of searchable databases is key to efficient use of existing information and moving toward a new paradigm based on hypothesis-driven testing and prioritization. 1.2.1

- Build the template to incorporate developmental neurotoxicity data from the literature and Office of Pesticide Programs (OPP) datasets into an inclusive searchable database. The database developed could serve as a model for similar databases for other endpoints (e.g., reproductive toxicity, immunotoxicity). 1.1.3

In conducting research to enhance interpretation of existing guideline data and those from new methods, ORD researchers are:

- Evaluating and providing guidance on major issues regarding the methods used in the current Developmental Neurotoxicity (DNT) Testing Guidelines. Project outputs will include guidance documents for data requirements, standardized protocols and data evaluation, for use both within and outside the Agency which should be of immediate use to OPPTS in terms of data interpretation, requirements for future testing, and guidance for test method development and refinement. 1.2.2
- Identifying sensitive *in vivo* and *in vitro* approaches for recognizing and screening alterations of rodent immune function through the application of genomics. A comparison of the sensitivity of *in vivo* and *in vitro* immune function results versus genomics should lead to the identification of the most sensitive metric(s) for immunotoxicity testing that could be incorporated into a single assay to assess for potential neurotoxic and reproductive effects and could lead to a more efficient use of animals in testing. 1.2.3
- Using *in vitro* and *in vivo* approaches to understand and discriminate the compensatory and toxicological responses of the highly regulated hypothalamic-pituitary-thyroid (HPT) system. Changes in gene expression in the pituitary, thyroid, and peripheral tissues under normal conditions and following exposure to chemicals known to interfere with TH synthesis will be linked with functional measurements of key hormones and enzymes that are part of the HPT pathway, all of which will be interpreted in the context of organismal-level effects. 1.2.3

In 2003, an RFA was released on the use of systems biology approach in hazard identification and risk assessment. Applicants were asked to develop, with or without cross species extrapolation, integrative, quantitative models of the function of the HPG or HPT axes with emphasis on the descriptions of the normal physiological processes and mechanisms of perturbation following exposure to endocrine disrupting chemicals in routinely used animal models. Through this RFA, ORD is supporting research that is:

- Developing a screening method to use molecular techniques to screen for effects of chemicals on the hypothalamic-pituitary-gonadal (HPG) axis with a special emphasis on steroidogenic pathways and hormonal control mechanisms along the HPG-axis in the Japanese medaka. 1.2.4
- Developing a computational model to evaluate molecular and protein biomarkers in relation to reproductive dysfunction in fathead minnows exposed to environmental estrogens. The model is incorporating a number of biochemical endpoints along the entire HPG axis, directly evaluating physiological changes and reproductive endpoints and the pharmacodynamics and kinetic distribution of the contaminants. Awardees and ORD scientists are working collaboratively on this project (see complementary research under 2.1.3) through cooperative agreements. 1.2.4
- Developing a computational model that will identify and predict critical estrogenic endocrine disruptor elicited changes in gene expression which play a central role in the observed physiological/toxic effects based on systematic and quantitative data obtained from comparative *in silico*, genomic, molecular and histopathological approaches using the rat uterus. 1.2.4

In 2004, an RFA was issued to establish an environmental bioinformatics research center. Two awards were made as cooperative agreements to enable close collaborations with scientists from

ORD and EPA program offices. The research is expected to contribute to development and application of dose-response information analysis and enhance current quantitative risk assessment practices and reduce uncertainties. The computational methods developed will link to data from molecular toxicology and other studies in order to move risk assessment from a hypothesis-driven science toward a predictive science. ORD is supporting research that is:

- Developing novel analytic and computational methods, creating efficient user-friendly tools to disseminate the methods to the wider community, and applying the computational methods to data from molecular toxicology and other studies by establishing the Carolina Environmental Bioinformatics Research Center. 1.2.5
- Addressing, in a systematic and integrative manner, multiple elements of the toxicant Source-to-Outcome sequence as well as developing cheminformatics tools for toxicant characterization by establishing the New Jersey Research Center for Environmental Bioinformatics and Computational Toxicology. 1.2.5

In late 2006, an RFA will be issued to solicit proposals with the goal of developing predictive environmental and biomedical computer-based simulations and models that address data gaps in environmental and human health risk assessment and will strengthen the ability of predictive scientific data to guide sound future scientific policy and decisions. 1.2.6

APG - Develop the scientific underpinnings related to the effects, exposures, and risk management of perfluorinated chemicals to inform Agency risk assessment/management decisions - FY 2013

A number of PFCs are increasingly being used for a variety of household and industrial applications. These include the surfactant coatings for fabrics and paper products, fire-fighting foams, non-stick cookware, electronic etching baths, and insecticides. EPA began an investigation of some of these chemicals because perfluorooctanoic acid (PFOA) is found at low levels both in the environment and in the blood of the general US population, is very persistent in the environment, and causes developmental and other adverse health effects in laboratory animals. EPA has summarized its concerns and identified data gaps and uncertainties about PFOA in a notice published in the Federal Register (http://www.epa.gov/fedrgstr/EPA-TOX/2003/April/Day-16/t9418.htm, OPPT-2003-0012).

EPA has negotiated with multiple members of the regulated community to develop data on PFOA through enforceable consent agreements, memoranda of understanding, and voluntary commitments. Information on these activities can be obtained through EPA's PFOA website (www.epa.gov/oppt/pfoa). One of these compounds, perfluorooctyl sulfonate (PFOS) has been withdrawn from the commercial market by the 3M company in 2000, but is still readily available from other overseas manufacturers. At present, little information is available concerning sources of environmental exposures, environmental fate and transport, and potential adverse health effects of these PFCs. Completing PFOA risk assessment actions is one of the Program Priorities of the OPPTS Assistant Administrator in FY06-07 (www.epa.gov/oppts/pubs/programpriorities.htm). While some data are being developed by the regulated community, there are other scientific needs that have been identified by OPPTS as critical to their being able to complete their risk assessments for the PFCs.

ORD research is, therefore, focusing on these unmet needs and is:

- Characterizing the developmental toxicity of PFOS and PFOA, determining to what extent data on these chemicals can be predictive of effects to other environmentally relevant PFCs, and identifying an alternative animal model to the rat. This research addresses OPPTS's immediate need to characterize the hazards of these chemicals for human populations and will help form the basis of other projects that focus on modes of action (MOAs) for PFCs toxicity. 1.3.1
- Elaborating on the already known pharmacokinetic profiles of PFOS and PFOA in laboratory rats by: 1) studying profiles in the mouse; 2) discerning differences between immature and adult rodents; and 3) evaluating the patterns tissue accumulation. The studies will be conducted in concert with a comprehensive investigation coordinated by NTP, OPPTS and ORD of 18 PFCs identified by OPPT. 1.3.2
- Evaluating the immunotoxicity, hepatotoxicity and the endocrine disruptive (thyroid and estrogen) potentials of PFCs and determining to what extent potential effects seen in rodent animals can be extrapolated to humans. Studies will also be conducted to assess the MOA of any adverse effects and whether developing animals are more sensitive to some of the effects than adults. 1.3.3
- Characterizing the underlying MOA responsible for the adverse effects caused by PFCs, including determining the involvement of peroxisome proliferator activated receptor (PPAR) signaling pathway in PFC toxicity. Results will provide information regarding the affinity of PFCs for PPAR isoforms, and comparisons of the affinities of the compounds in rodent versus human receptors. 1.3.4
- Developing sampling and analytical methods to characterize the distributions of PFOS, PFOA, and other PFCs in key environmental and biological matrices that are important for assessing environmental transport and fate and human exposure. 1.3.5
- Developing data on the stability and distribution of selected perfluorinated organic chemicals in real-world environments. Research is being performed to assess the potential for fluorotelomer-based polymer products (FBPPs) to degrade in a variety of soil and sediments and characterize the environmental distribution of perfluorinated compounds in soils. 1.3.6
- Determining fluorotelomer alcohol (FTOH) polymer product stability during wastewater (WW) treatment by: 1) developing analytical and experimental methods to characterize fluorinated surfactants in various environmental matrices, 2) describing the composition of FTOH polymer formulations released to the environment via down-the-drain disposal, 3) determining the environmental loadings of FTOHs and PFCs from WW treatment, and 4) determining the potential for FTOH polymer products to transform or degrade during WW treatment. 1.3.7
- Characterizing the source, transport, and fate of PFCs in the indoor environment and the factors that may affect PFCs release from articles of commerce (AOC) by determining the PFCs content in new AOC, characterizing the PFC emissions from aged AOC containing fluoropolymers or fluorotelomers by chamber studies, and identifying the major indoor exposure routes for the general US population. The findings will suggest potential risk management solutions. 1.3.8

APG - Develop the scientific underpinning related to the effects, exposures, and risk management of specific individual or classes of pesticides and toxic substances that are of high priority to the Agency to inform Agency risk assessment/management decisions – FY 2010

An area of critical concern to OPPTS and the Office of Water is the ultimate product that results and is available for exposure to the environment and public when a pesticide or toxic chemical enters a drinking water distribution system. Drinking water (DW) treatment often has a large

effect on pesticides and toxic chemicals that occur in source waters; and both OPP and OPPT have articulated their high priority need to incorporate these effects into chemical risk assessments. FQPA requires OPP to consider all anticipated dietary exposures when conducting risk assessments for pesticides. DW is considered a potential route of exposure because many DW sources contain detectable levels of pesticides and toxics. The majority of the US population consumes treated DW. It is anticipated that DW treatment will: a) partially remove some chemicals, b) transform some chemicals (perhaps to more-toxic byproducts), and c) have virtually no effect on some chemicals. Unfortunately, very little data has been collected, and very little mechanistic information is available, on the effects of DW treatment processes on pesticides or on any other chemicals.

OPP has announced a policy to systematically consider DW treatment effects on pesticides for FQPA risk assessments. For this policy to make risk assessments significantly more certain, pesticide-specific information on DW treatment effects is needed. Also, reliable tools to extrapolate effects across chemical classes are required. These needs, which have been articulated by OPP, are driving this research. Without the type of information and tools that ORD will provide, OPP will have to assume that DW treatment has no effect on a pesticide or toxic chemical. This may be overly- or under-conservative, depending on the pesticide or toxic chemical, and the treatment conditions. ORD has been partnering extensively with OPP and OW on this research. Addressing DW and pesticides interface issues is one of the Program Priorities for the OPPTS Assistant Administrator in FY06-07 (www.epa.gov/oppts/pubs/programpriorities.htm). To address these needs, ORD research is:

- Producing tools to address OPP's DW treatment research needs by: 1) developing/evaluating a protocol, 2) performing DW treatment studies, 3) providing chemical-specific information on the effects of water treatment on pesticide transformation pathways, 4) providing physicochemical parameters for transformation products, and 5) developing predictive models for forecasting treatment effects. This research will allow OPP to provide a protocol to pesticide manufacturers for their use in submitting data in a consistent manner and will provide OPP with DW treatment data and guidance on their interpretation for inclusion in their risk assessments. 1.4.1

Chromated copper arsenate (CCA) is a chemical wood preservative injected under high pressures to protect wood from decay and insect damage. In February 2002, the manufacturers of CCA-treated wood asked EPA to remove the registration of CCA for residential use including playground equipment, decks, and landscape timbers. The CCA-treated wood manufacturers phased out production and use of CCA in favor of alternative chemicals effective December 31, 2003 (www.epa.gov/oppad001/reregistration/cca). Of current remaining concern is the possible dermal exposure of children to the arsenic contained within the CCA-treated wood that has been used for playground equipment, decks, and outdoor furniture. Since there was little data available on efficacy of sealants and coatings to minimize dermal exposure, ORD and the Consumer Product Safety Commission (CPSC) conducted studies to identify and evaluate commercial coatings that can reduce or prevent exposure to arsenic from CCA treated wood.
To address these needs, ORD research is:

- Evaluating the ability of coatings available to consumers to reduce dislodgeable CCA residues on the surfaces of CCA-treated wood and demonstrating a methodology that industry can use to further develop, improve, and demonstrate product performance. 1.4.2

For the last 10 years, ORD has been a part of a large multifaceted interagency study, the Agriculture Health Study (AHS), to evaluate pesticide exposures and their potential to cause adverse health effects in pesticides applicators and their families. The ORD-led AHS Pesticide Exposure Study was an exposure-measurement field study for a subset of agricultural pesticide applicators and participating family members in the larger AHS cohort. It was conducted to provide information to assess and refine the exposure-classification procedures developed from the AHS questionnaire data and for the National Cancer Institute (NCI) and NIEHS to better understand factors affecting pesticide exposures for agricultural pesticide applicators and their families. ORD's contribution to this study is:

- Generating high quality exposure data for classifying pesticide exposures for agricultural applicators and assessing and refining the NCI/AHS exposure algorithms. Identifying and understanding key exposure factors can guide development of improved exposure reduction strategies and guidance developed by OPP and other organizations and increase the value of epidemiological study results in pesticide exposure and health assessments. 1.4.3

Through its existing chemicals program, OPPTS oversees the implementation of its asbestos program (www.epa.gov/asbestos). Asbestos is commonly used as an acoustic insulator, in thermal insulation, fire proofing and in other building materials. While much is known about the effects of and exposures to asbestos, there are still some remaining research needs. ORD research is:

- Providing scientifically sound sampling and analytical approaches for measuring asbestos fibers in various media and applying these measurements to assessments of human exposures to asbestos fibers. The research focuses on two key areas: 1) determining which types of measurements are needed to support asbestos exposure assessments in likely real-world scenarios, and 2) comparing the efficiencies and effectiveness of available asbestos sampling and analytical techniques used in exposure assessments. 1.4.4
- 1) Evaluating aerosolization of asbestos and related fibers from bulk materials to develop a framework for modeling asbestos breathing zone concentrations generated by activities of varying intensity on outdoor and indoor surfaces. 2) Obtaining asbestos fiber releasability data from soil and carpet for calculation of emission factors. These data and model(s) will allow EPA Regional Offices and others to make rapid decisions about whether a soil or other bulk material is contaminated with asbestos. 1.4.5

On January 10, 2006, EPA proposed new requirements in the Federal Register to reduce exposure to lead hazards created by renovation, repair, and painting activities that disturb lead-based paint (www.epa.gov/lead). The proposal supports the attainment of the Federal government's goal of eliminating childhood lead poisoning by 2010. Implementing targeted actions to meet the 2010 lead goals is one of the Program Priorities for the OPPTS Assistant Administrator in FY06-07 (www.epa.gov/oppts/pubs/programpriorities.htm). The research to date has shown that commercially-available paint lead test kits are not an effective means of identifying homes that do not contain regulated lead-based paint. To meet the new rule requirements, it is necessary that new efficient, cost effective technologies meeting the desired sensitivity within a specified range of false positive and false negatives rates be developed and made available commercially. ORD research is:

- Investigating available state-of-the-art analytical technology for its potential to be modified to meet OPPT's needs for the new lead rule. 1.4.6

The fact that over 25% of modern pesticides are chiral molecules provides an excellent opportunity for production of safer products and reduction of the load of unnecessary chemicals entering the environment. ORD research is:

- Addressing the importance of enantiomer selectivity in the fate, persistence, exposure, effects and risk assessment of chiral pesticides and other pollutants. This research provides data for OPP to use to better inform risk managers on the certainty/uncertainty and need for additional information for pesticides that are either single, enriched or racemic compounds. 1.4.7

Long Term Goal 2: OPPTS and/or other organizations use the results of ORD's research as the scientific foundation for probabilistic risk assessments to protect natural populations of birds, fish, other wildlife, and non-target plants.

Ultimate Outcomes: *Results of this research will help the Agency meet the long term goal of developing scientifically valid approaches to extrapolate across species, biological endpoints and exposure scenarios of concern, and to assess spatially-explicit, population-level risks to wildlife populations and non-target plants and plant communities from pesticides, toxic chemicals and multiple stressors while advancing the development of probabilistic risk assessment.*

Synopsis: Intramural research is:

- creating the scientific foundation for conducting probabilistic risk assessments for fish and wildlife populations and plant communities by developing: methods for extrapolation among species and exposure scenarios of concern; models for characterizing environmental exposures and population biology in spatially-explicit habitats; models to assess relative risk of stressors; and tools to define geographical regions/ spatial scales for risk assessment.

OPP is leading the way in expanding ecological risk assessments (ERAs) to provide probabilistic expressions of risk to aquatic and terrestrial wildlife populations and plant communities, including reducing uncertainties in all tiers of the risk assessment process as uncertainties that are extrapolated from limited data sets are better defined and put into context. For this purpose, methods are required to support population-level ERAs of increasing degrees of specificity, detail and realism; to determine the absolute /or relative (incremental) risk of chemical and non-chemical stressors; and at varying geographical regions/ or other areas of regulatory concern.

The research conducted under LTG 2 of the SP2 MYP is developing efficient methods, including models, for OPP to review, register, and regulate thousands of chemicals in a timely fashion. OPP's strategic direction is moving toward probabilistic assessments in response to recommendations from their Scientific Advisory Panel. ORD has developed the Wildlife Research Strategy (WRS,

www.epa.gov/nheerl/publications/files/wildlife_research_strategy_2_2_05.pdf) which describes a tiered approach using a series of wildlife risk assessments. A similar tiered approach is used with plant risk assessments. In addition, because neither stressors nor wildlife populations or plant communities are distributed uniformly within the environment, the interplay between spatial and temporal heterogeneity in wildlife population and plant community structure and spatial and temporal patterns of stressors is a major factor controlling the severity of effects on wildlife populations and plant communities. Thus, a critical feature of this research is the development of probabilistic models that deal explicitly with the spatial distribution of wildlife populations, plant communities and stressors over time. While steps provide general guidelines for wildlife population and plant community-level risk assessment, the level of accuracy and realism appropriate for each step varies with assessment needs and management goals. For example when applied in the context of site-specific risk assessment, these models can be applied to real landscapes, by interfacing with geographical information systems (GIS). For more generalized regional or national-level assessments (e.g., for pesticide registration), simulated (or constructed) landscapes can be used that mimic the general characteristics of the ecosystems of concern.

Data needs for each assessment differ depending upon the goals of the assessment and the desired level of confidence in the outcome. For example, screening level or "lower tier" assessments may involve a relatively simple evaluation of chemical fate and effects, including qualitative judgments about the likelihood of exposure and potential for bioaccumulation. In the case of compounds regulated under the PMN process, this may involve expert opinion about which class the chemical belongs to and selection of an appropriate analog or QSAR. A similar approach may be applicable to some "inert" ingredients of pesticide formulations, antimicrobials and HPV chemicals. More involved assessments are required for "active" compounds covered by FIFRA as well as some TSCA chemicals of special concern. These "higher tier" assessments are more likely to include quantitative evaluations of effects and exposure information and may involve model-based efforts to extrapolate data from surrogate species and chemicals. Currently, data available for these needs also differ considerably. Registrants of chemicals with pesticidal activity are required to provide a core set of data regarding toxicity to wildlife and vegetation. In contrast, under the TSCA Section 5 PMN review process, importers or manufacturers of chemicals are not required to submit any effects data; that is, the EPA can request toxicity test results, e.g., using mammalian (usually in support of human health questions), avian or aquatic species only if there is reason to believe that a specific adverse effect can occur.

To address these needs, LTG 2 research is directed toward improving current risk assessment processes used by OPP and OPPT for new and existing pesticide and chemical risk assessments, with specific focus on their needs to: 1) make better use of the current data that they receive during product registration or for existing substance review, and 2) move the science forward through the inclusion of more sophisticated data and analyses.

To address the needs of OPP, OPPT and other program offices, ORD's WRS describes critical research that is providing the scientific foundation for probabilistic risk assessments to inform decisions related to protection of natural populations of birds, fish and other wildlife.

Specifically, this research focuses on the development of those approaches and tools whose advancement is most needed to conduct spatially-explicit, population-level risk assessments. Similar research is necessary to protect native plant populations. The research conducted under LTG 2 focuses on three of the four critical steps identified in the WRS, which must integrate with the first step (exposure characterization) to complete this process. These three steps include the development of methods to improve the characterization of effects of chemical and non-chemical stressors on the fitness of individuals of various species, on the viability of populations of species with varying life histories, and on the dynamics of spatially-structured wildlife populations and plant communities inhabiting heterogeneous landscapes.

The goal of the LTG 2 research is to develop scientifically valid approaches to assess risks to wildlife populations and plant communities from multiple chemical and non-chemical stressors. This requires a means of mathematically integrating dose-response and habitat suitability relationships as well as computer platform for site-specific, spatially-explicit population modeling. To address these needs, the research is arrayed under the following four APGs.

APG - Provide methods for extrapolating toxicological data across wildlife species, media, and individual-level response endpoints – FY 2013

Research is emphasizing approaches for extrapolating toxicity data to a broader array of species, environmental media, and response endpoints - in particular, the endpoints required as input to population response models. Research is continuing to refine the ECOTOX database, Acute to Chronic Estimation (ACE), and Interspecies Correlation Estimations (ICE) programs. Several areas within the model have been identified for further development including: 1) identification and expansion of appropriate surrogate species; 2) increased representation of non-pesticide industrial organic and inorganic chemicals; 3) increased representation of metals; 4) improvement in taxonomic classification schemes; and 5) model expansion and modification integrating novel (example: genomic/proteomic) endpoints.

ORD research is:

- Reducing uncertainties associated with the current use of (eco) toxicity data and existing empirically based interspecies extrapolation models to better estimate the toxic effects of chemical exposures on wildlife and aquatic species. This is being done using two general approaches: (1) improving accessibility and usefulness of available toxicity information through the ECOTOX database, a comprehensive Web-based system maintained by ORD; and (2) refining empirical models that use available information to predict toxicity across species (i.e., ICE) and across endpoints (ACE). 2.1.1
- Furthering the development of mechanistically based models to extrapolate toxicity information among chemicals, species and lifestages. This is being done using two general approaches: development and testing of a physiologically based toxicokinetic (PBTK) model for a fish species and an improved experimental method to parameterize such models; and, development and testing of a MOA model to predict inter-species differences from *in vitro* data. 2.1.2
- Developing techniques for extrapolation of toxicological effects across endpoints, species and chemicals by utilizing three small fish species, the Japanese medaka, zebrafish and fathead minnow and a systems-based approach to define toxicity pathways for model chemicals with well-defined MOA within the HPG axis. The studies employ a combination of state-of-the-art molecular biology,

bioinformatic and modeling approaches, in conjunction with whole animal testing protocols. 2.1.3 (linked to second grant described in 1.2.4)

APG - Provide methods for characterizing population-level risks of toxic chemicals to aquatic life and wildlife – FY 2015

OPP is redesigning their ecological risk assessment tools to make their risk assessments more ecologically relevant. In support of this need, OPP and ORD scientists are working to implement the approaches identified in the Wildlife Research Strategy. This specific effort is aimed at incorporating population models into the OPP risk assessment process and, thereby, taking a significant step forward in terms of making their risk assessment process more ecologically relevant.

ORD research is:

- Refining population models that have been developed for OPP and integrating these models with the exposure and effects models currently used by OPP. Some of the factors for consideration include the software code, and how to make a seamless connection between our population models and OPP's existing risk assessment models; translating OPP effects endpoints into stressor response models that can be used in the population models; data quality for population model parameter estimates; model interpretation, including decisions on which population model endpoints will are most appropriate for assessing risks; and some level of training and continuing technical support. This effort moves from conceptual models of addressing population response to actual implementation of population level analyses. Furthermore, this research advances our understanding of the usefulness of population models for accurately characterizing risks in ecological risk assessments, and determining if the quality of the population model is sufficient to answer the risk management question. 2.2.1
- Providing methods to support probabilistic ecological risk assessments and more explicitly addressing higher tier ecological risk assessment needs to take into account greater realism and complexity in projecting population responses to stressors. This is being done using a combination of theoretically and empirically based approaches and field-collected, laboratory-derived, and simulation-based information. Selected species will include those used frequently in toxicity testing, which historically have provided important information to regulatory process, including the registration of pesticides. Research is addressing four specific areas: (1) Probabilistic models, (2) density dependence, (3) genetic, and (4) spatial effects. This research will provide approaches and guidance on the need for and uses of more complex population modeling approaches as tools for integrating and projecting more realistic effects of stressors on wildlife populations. 2.2.2

APG - Provide approaches for evaluating the relative risks from chemical and nonchemical stressors on spatially structured wildlife populations across large areas or regions, and provide methods for characterizing population-level risks of toxic chemical to aquatic life and wildlife – FY 2009

This research area introduces issues associated with the spatial and temporal heterogeneity of populations and stressors, and extends the analyses under the previous areas to applications in real landscapes. Because different stressors tend to be distributed differently in the landscape, this approach will address the interactive effects of contaminants, habitat alteration, and introduced species on wildlife populations. Models and analyses are being designed both to

assess risks from multiple stressors and to evaluate the relative effectiveness of alternative management strategies. The basic GIS and population modeling platform is in development under the Ecosystem Protection Research Program so as to serve the needs of all EPA Program and Regional Offices. Under the SP2 research program, efforts are focused on the relative risk assessments for bird populations exposed to toxic chemicals and landscape disturbances within the natural range of the species. Birds are the class of vertebrate wildlife of principal concern due to their higher susceptibility as compared to mammals to past and current generations of pesticide chemicals, their high visibility, and high public concern. Programs such as the Breeding Bird Survey conducted by the USGS and the multisectorial program Partners in Flight provide a high level of public input and scrutiny to monitoring bird populations and biodiversity. ORD is also developing spatially explicit fish population models to assess large-scale pesticide exposure and risk in coastal areas, significantly enhancing the understanding of OPP staff about how coastal fish are impacted by pesticides and other stressors.

ORD research is:

- Enhancing the PATCH model (Program to Assist in Tracking Critical Habitat), is a spatially explicit, individual-based life history simulator that incorporates GIS representations of real or hypothetical landscapes, and tying it specifically to pesticide issues. The PATCH model is being used to simulate wildlife population responses to pesticide application within agricultural landscapes and ORD is exploring the incorporation of multiple landscape configurations, wildlife life histories, and stressor regimes. 2.3.1
- Designing and implementing a probabilistic exposure analysis system, undergirded by a suite of sophisticated process-based models of pesticide environmental chemistry and biology, for direct assistance to EPA's regulatory programs in their mandated pesticide risk assessments. One objective is to provide OPP with improved tools for assessing offsite drift of pesticides, and expanding the capabilities of AgDRIFT and AGDISP to assess near-field pesticide drift from aerial applications by including source term algorithms for ground sprayers, orchard airblast sprayers, and revolatilization. 2.3.2

APG - Provide improved methods to assess direct and indirect risks to non-target plant species and plant communities from pesticide use - FY 2012

There is evidence for a wide variation in response of various plant species to chemical herbicides (about three orders of magnitude), making it difficult to extrapolate potential risk to non-target, uncultivated plants from the current suite of 10 plant test species (all agronomic species). Given the increasing use of herbicides, the FIFRA Science Advisory Panel recommended restructuring the Tier II risk assessment process to expand the number of species tested and the endpoints evaluated. Current testing is limited to a very small portion of a plant's life cycle that does not include reproduction and lacks information relating to field tests, especially in terms of determining survival of threatened and/or endangered plant species, preservation of native plant communities, and for maintenance of productive habitat for wildlife.

The objective of the research is to develop methods to determine the short and long term effects of pesticides on non-target plants and plant communities, including impacts on wildlife habitat. This effort includes: 1) Development of spatial analysis tools, i.e., a web-page based, geographic

information system (GIS) platform, to identify geographic areas and types of plants with the greatest risk for non-target herbicide effects. This spatially explicit framework also will provide access to databases to support OPP's ecological risk assessments. 2) Determination of reproductive/developmental responses of plants to chemical herbicides for proposed life-cycle tests. 3) Development of methodology for greenhouse and field-plot studies to determine herbicide effects on native plants and plant communities. Both constructed communities with mixtures of plant species seeded/planted in various relative densities and proportions, and *in situ* native plant communities will be studied in the field. Endpoints will include % cover, biomass, reproduction to provide information on changes in species composition and system dynamics. This information will be used to develop guidelines for revised field tests and for assessments of impacts of pesticides on quality of plant communities for wildlife. 4) Development of molecular indicators (gene expression and subsequent protein production) of whether a plant has been affected by specific herbicides. This information will be used to predict the potential susceptibility of different plant species to herbicides (especially native plants and threatened and endangered plant species).

ORD research is:

- Determining effects from off-target movement of chemical herbicides on plants and plant communities through: 1) providing a spatially explicit, geographically based framework to access databases to select plant species for testing, and for ecological risk assessments; 2) improving phytotoxicity testing guidelines, focusing on terrestrial plant effects, with an emphasis on reproductive effects and native plants; 3) improving ecological testing guidelines to determine plant community responses; and 4) evaluating molecular indicators can be used to assess whether a plant has been affected by specific herbicides, or whether a species may be susceptible to a specific herbicide.

Long Term Goal 3: OPPTS and/or other organizations use the results of ORD's biotechnology research as the scientific foundation for decisionmaking related to products of biotechnology.

Ultimate Outcomes: *OPPTS will use the results from this research program to update its requirements of registrants of products of biotechnology and to help evaluate data submitted to them.*

Synopsis: Intramural and extramural research is:

- improving the evaluation of potential ecological effects of biotechnology products, specifically plant incorporated protectants (PIPs), on non-target species; the impact resulting from the escape of altered plants to the natural environment and the likelihood and effects of gene transfer; the development of pesticide resistance in the target insect species; the development of risk management approaches; and development of methods to assess for the potential allergenicity of genetically engineered plants.

As noted previously, OPPTS, in carrying out its Congressional mandates, 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. OPP regulates the use of all pesticides in the US and establishes maximum levels for pesticide residues, including genetically engineered pesticides. 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. 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 and that any potential unreasonable risks can be managed.

In order to carry out its mandates, OPPTS needs the scientific information to assess and manage the potential human health and ecological risks of the various products of biotechnology. Because of limited resources in the area of biotechnology research, the SP2 program is currently focused on OPPTS' highest priority of providing the tools and scientific knowledge needed to understand the nature and magnitude of potential risks and benefits resulting from the use of genetically engineered (GE) pesticide products in commerce and the means to prevent or control any such risks.

The use of biotechnology has led to new pesticide products that control a variety of pests. These biologically produced pesticides, which use the inherent pest-fighting abilities of many existing plants and microbes, have properties that distinguish them from those of conventional chemical pesticides. When these products have unique biological properties they may also pose unique regulatory challenges. To address these challenges, the EPA, USDA, and FDA have shared responsibility for regulating agricultural biotechnology in the US (US regulatory agencies unified biotechnology website - http://usbiotechreg.nbii.gov). In particular, EPA regulates pesticides created through biotechnology as a part of its regulatory jurisdiction over all pesticides marketed and used in the US. As such, EPA has tailored its basic regulatory framework to fit the distinctive characteristics of these GE biological pesticides.

In assessing safety, the basic framework for pesticide regulation provides guidance as to the nature of any new risks. Many of the traditional approaches used to assess chemical pesticides are applicable to assessing risks from genetically engineered plants which produce their own pesticides, also known as plant-incorporated protectants (PIPs). PIPs are created when through the use of biotechnology, specific genetic material from a bacterium are transferred to a plant to create plants that produce pesticidal proteins that the plant could not previously produce. Under FIFRA, EPA has the authority to regulate the new protein and its genetic material when it is pesticidal in nature. Before making a regulatory decision about a pesticide, EPA requires data on a range of subjects to ensure that the product meets federal safety standards. For all pesticide products, including GE pesticides, EPA requires testing of product composition and chemical properties, human health effects, environmental effects on nontarget pests, and the fate of the

pesticides in the environment (www.epa.gov/pesticides/biopesticides/pips/index.htm). Therefore, the research conducted under other SP2 LTGs will be of value in assessing these products, to some extent, as well. However, OPP also recognizes that PIPs may pose uniquely different risks from traditional, chemical pesticides. Therefore, OPP requires additional scientific information and tools in order to adequately assess and manage potential risks. For example, while there is very low worker exposure and no chemical pesticide spray drift, there are issues regarding gene flow from PIPs to wild relatives and pollen movement spreading the new pesticides to non-altered crops. Cross-pollination of wild relatives can disrupt a local ecosystem by changing the makeup of local plants, crowding out related species and changing the local habitat. In addition, while the level of protein produced by the newly engineered plant is very small, because proteins can be allergens, special emphasis on assessing potential allergenicity is needed of these products.

From a human health perspective, a major area of uncertainty is on the potential toxicity and allergenicity associated with biotechnology derived foods. Potential adverse effects can be from intended modifications (i.e., from the pesticidal substance) or from unintended effects resulting from production of an unexpected substance from the insertion of the new genetic material into the host genome. To date, the products approved by EPA for use in human food have all been proteins that degrade rapidly, so no chronic effects would be anticipated. This approach has been accepted by the FIFRA Scientific Advisory Panel. However, some members of the public have raised issues about the potential long term exposure to eating foods containing these newly created proteins. It is well accepted that the genetic material itself will not cause an acute or chronic toxic effects and has been exempted from tolerance.

With respect to environmental risk, OPP needs effective tools and methods to evaluate and subsequently, if needed, minimize the likelihood of negative ecological effects such as the following:

Ecosystems

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

Agri-Systems

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

Both

- 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.];
- 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.

From an ecological perspective, the regulation of biotechnology products is focused on cases where there is little prior experience with the new trait and host combination; a transformed organism may persist and perhaps replicate in the environment without human intervention; genetic exchange is possible between a transformed organism and unaltered organisms; or the trait confers an advantage to the transformed organism over native species in a given environment. Additional concerns about rapid evolution of resistance in targeted pest species; potential risk to nontarget invertebrates; and alterations in terrestrial or aquatic food chains also have been raised.

The goal of the SP2 biotechnology research program is to provide the scientific information needed to assess and manage the potential human health and ecological risks of products of biotechnology. The research will provide the tools needed to generate information about biotechnology products and the knowledge needed to understand the nature and magnitude of potential risks and benefits resulting from the use of biotechnology products in commerce and the means to prevent or control such risks.

The biotechnology research that ORD is conducting intramurally or supporting through the extramural STAR program is not only addressing specific high priority needs identified by OPP but also is in concordance with needs identified by the National Research Council (2000, 2002). Furthermore, it is address several science gaps that are noted in the recent document developed by the interagency AGRA committee (not publicly released yet).

APG - Provide improved capability to assess the risks of allergenicity of genetically engineered crops - FY 2011

There is a concern 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. OPP is currently unable to adequately evaluate the potential allergenicity of proteins introduced into the food supply by gene transfer because valid animal models to test proteins for potential allergenicity following oral exposure, and other methods to readily identify proteins that may be potent allergens have not been adequately developed. Without reliable assessment methods, the mechanisms underlying the development of food allergy and the factors that contribute to individual susceptibility remain poorly understood.

Research conducted both within ORD's laboratories and through the extramural STAR program is:

- Developing an improved understanding of the basis for human sensitization to dietary allergens and, which in turn, will lead to the development of methods to assess dietary allergenicity, which once validated, could become part of the battery of assays that industry must conduct in order to register a genetically engineered pesticidal product. The projected outcome will be an improved ability to assess the potential risks to human health from genetically engineered foods in the diet and an overall improvement in the knowledge of food allergens. 3.1.1

APG - Provide improved science based risk assessment tools and data support that ensure improved capability for the comprehensive evaluation of ecological risks and long term safe use of genetically engineered crops with plant incorporated protectants (PIPs) - FY 2011

The risk of unintended and unexpected adverse impacts on non-target organisms and ecosystems is a key issue in environmental risk assessment of PIP crop plants. Research is needed to examine potential impacts of the effects of *Bacillus thuringiensis* (Bt) 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. To address these science needs, ORD's research is:

- Developing standardized and streamlined methodologies to: 1) conduct base-line assessments of agricultural and near-field ecosystems non-target species diversity and abundance to measure direct impacts and secondary trophic level effects on non-target organisms, and 2) characterize assessment endpoint(s) and the use of predictive strategies to evaluate potential ecosystem level effects. 3.2.1
- Developing field methodologies to assess and monitor the impacts of the high-dose/structured refugia integrated risk management (IRM) strategy on the long-term susceptibility of target pests to *Bt* endotoxins. The result of this research will be the development of 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. 3.2.2

APG - Provide guidelines and tools to mitigate gene-transfer and non target effects and the development of resistance in targeted pest populations to aid the management of environmental risks associated with PIP crops to help maintain the biological integrity of the environment while minimizing the use of chemical pesticides in agriculture – FY 2015

Laboratory and small scale field testing have been the basis for evaluating the likely safety of biotechnology products, but long term, extensive monitoring has not been conducted to determine whether the effects predicted in such assessments actually occur in the field. The research objectives are to:

• develop models to estimate likelihood of insect resistance development 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 nonadditive effects on resistance under selective pressures in the field
• perform monitoring studies of gene transfer, the development of resistance to PIPs and impacts on non-target species to allow field validation of conclusions regarding transgenic plants with new pesticide traits, including recommendations to prevent development of insect resistance to support or to refine assessments.
• develop 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.

The effectiveness of 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 has not been adequately evaluated. Extensive work is needed to improve 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; and to evaluate whether the genetic alterations produce new organisms that are not equivalent to currently existing ones. ORD research is:

- Developing methods to adequately address the potential for gene flow and introgression to occur and the ecological fitness changes that might result. Studies are being conducted to demonstrate: 1) methods for monitoring pollen dispersal and gene exchange between crops and co-located wild relatives and 2) the feasibility of developing effects response information on genes controlling plant reproduction, yield, through standard backcrossing and plant community competition experiments. 3.3.1
- Gathering population genetic data that will improve the current models used to delay resistance, in order to help prevent development of resistance to Bt-corn in Western Corn Rootworm (WCR). ORD is using genetic crosses and artificial selection to identify resistance genes in wild populations of WCR across North America. These approaches will be evaluated for their utility to improve current insect resistance monitoring (IRM) plans and for their utility in risk assessment of future PIP varieties. 3.3.2
- Preparing improvements to PIP crop monitoring by: 1) developing methods to identify PIP corn in the field, 2) identifying and assessing the severity of infestation to corn from insect pest populations, exploring the use of remotely sensed hyperspectral imagery and less expensive techniques of spatially and spectrally resampling existing hyperspectral imagery to simulate satellite imagery; and 3) applying results from the first two goals and developing an IRM program. 3.3.3
- Examining the strengths and limitations of the diagnostic dose and F_2 screen to develop better, standardized protocols for use across the entire Corn Belt. Standardized protocols will assist the collection of monitoring data for PIP crops that can be compared across seasons. The information quality improvement undertaken by this research is designed to assist OPP to meet the requirements of the Data Quality Act. 3.3.4
- Determining to what extent simulation models can be used to reliably predict the onset of pest resistance to PIP crop controls. This research has been designed to provide substantive information about the operation and capabilities of different resistance management models to assist the regulatory expert in its proper use and interpretation of results. 3.3.5
- Starting in 2007, ORD will begin to scope out conducting a cross-laboratory/center effort to develop a cost-effective agro-ecosystem monitoring program designed to assess changes in pesticide exposure and effect accompanying transgenic crop adoptions. It should be applicable to a variety of crops, transgenic constructs, and spatial/geographical orientations. ORD's initial research goal will be to develop indicators that can be selectively chosen to efficiently establish causality relationships between transgenic cropping systems, off-farm exposures and ecological responses, thereby providing OPP and the industry with an ecological accountability tool. 3.3.6

VII. Relationship to Other Multi-Year Plans

As noted earlier, there are a number of high priority science needs in OPPTS programs which represent such fundamental and complex scientific challenges that ORD has committed core research efforts to the problems as follows:

- Research in support of pollution prevention issues can be found in the Sustainability Research Program and its MYP, and therefore, are not addressed through the SP2 Research Program other than the work that is done related to products of biotechnology.

- The scientific gaps in our capability to assess cumulative and aggregate risks, susceptible sub-populations amongst human or other vulnerable species, and stochastic exposure scenarios as well as to screen chemicals for their potential to disrupt the endocrine systems all comprise additional core ORD research efforts under the Human Health and Endocrine Disruptors MYPs, both of which are providing underlying science and tools to OPPTS to meet its mandates, especially those under FQPA.
- The need for prioritization tools is also being addressed by ORD's core Comp Tox Research Program, and to a lesser extent the Human Health Research Program.
- Risk assessment frameworks and methodologies are developed through the Human Health, Human Health Risk Assessment and Ecological Protection MYPs.

Similarly, some of OPPTS' science needs are being addressed by other problem-driven research programs as follows:

- The scientific gaps pertaining to the toxic effects of respirable dusts are being addressed by a comprehensive Particulate Matter (www.epa.gov/osp/myp/pm.pdf) MYP in support of the Office of Air and Radiation.
- Most recently, the initiative to understand the implications of nanotechnology on human health and the environment, as well as to explore their applications on improving the environment are leading to the development of a new research strategy on the subject.

In addition, the SP2 research program while specifically developed to support OPPTS needs may also provide indirect benefits to other Agency Goals and ORD Research Programs. Examples of this research include the development of probabilistic spatially-explicit ecological assessment framework and characterizing the occurrence of pesticides following drinking water treatment. Additional examples are included in the table below.

Examples of SP2 Research - Goal 4.4	**Goals and MYPs that benefit indirectly**
Prioritization and screening tools	Goal 4; Human Health MYP, Comp Tox Framework
Enhanced interpretation of data	Goals 1-4; Ecosystem Protection, Human Health, Human Health Risk Assessment, Endocrine Disruptors MYPs, Comp Tox Framework
Occurrence of pesticides in drinking water	Goal 2; Drinking Water MYP
Releasability of asbestos	Goals 1 and 3; Air, Land MYPs
Effects, exposure, degradation data on perfluorinated chemicals	Goals 1-4; Air, Drinking Water, Water Quality, Land, Human Health, Endocrine Disruptors MYPs

Ecological probabilistic risk assessment tools	Goals 2 and 4; Water Quality, Ecosystem Protection MYPs

It should be recognized that, as other related ongoing research has been noted previously, that there is a need to coordinate the SP2 research with that conducted through other ORD research programs, in other federal agencies, and other non-governmental science organizations, and with our international counterparts. The mechanisms for collaboration with outside-ORD organizations are highlighted in Section III. In order to improve coordination across the MYPs within ORD, the NPD for the Pesticides and Toxics Research Program meets periodically with the NPDs for each of the relevant MYPs as well as the leaders for other programmatic areas (e.g., computational toxicology, nanotechnology, homeland security) who oversee research that is ongoing in support of OPPTS. These discussions are important not only to ensure that are programs are not conducting duplicative efforts but also so that we ensure that the products of the research are disseminated to those who may find them of indirect benefit.

VIII. Relationship to the Research & Development (R&D) Investment Criteria

As part of the President's Management Agenda (http://www.whitehouse.gov/omb/budintegration/pma_index.html), explicit criteria were developed for managers to use for assessing R&D programs. The R&D Investment Criteria consist of three categories, including:

- Relevance- R&D programs must have clear plans and demonstrate relevance to national priorities, agency missions, and "customer" needs
- Quality-programs should maximize the quality of the research through the use of clearly stated, defensible methods for awarding a majority of their funding
- Performance- programs should maintain a set of high priority, multi-year R&D objectives with annual performance outputs and milestones that show how one or more outcomes will be reached

The R&D Investment Criteria will be used by two separate groups to evaluate the SP2 research program in FY07. On February 7-9, 2007 a subcommittee of ORD's Board of Scientific Counselors will meet to address a number of charge questions, including being asked comment specifically on how the program meets the R&D investment Criteria. OMB will also use the Criteria in the evaluation of the SP2 research program using the Program Assessment Rating Tool (PART) during FY07.

IX. Communication

Because of the breadth of the research program on SP2, effective implementation of this MYP requires extensive coordination and communication at multiple levels and multiple stages. Dedicated coordination and communication is crucial to effective research planning, allocation of resources, and implementing a cohesive intramural and extramural research program that is targeted to achieve both the breadth and depth of balance needed to address the multiple environmental problems that are addressed by the SP2 program.

During planning of the research:

- Coordinate identifying the highest priorities for research through a research planning committee that includes ORD representatives from the Laboratories/Centers/Offices and OPPTS and Regional scientists; ORD, OPPTS, and Regions are partners in planning the program.
- Communicate priorities to other ORD, OPPTS, and Regional senior managers.

During conduct of research:

- Coordinate and communicate across branches and divisions within a particular ORD Laboratory/Center where research is addressing a common issue.
- Coordinate and communicate across ORD National Laboratories/Centers where research is addressing a common issue; this includes working with STAR grantees, where appropriate.
- Keep the client offices and stakeholders aware of the progress of the research through meetings and seminars. Seminars will be scheduled through the ORD-OPPTS seminar series that has been ongoing since 2000.
- Hold periodic progress reviews or workshops where the intramural and extramural researchers will meet with Agency scientists and managers and other clients and stakeholders to share study results and build collaborations.

Upon completion of research:

- When researchers complete a body of work, e.g., resulting in a publication, meeting a milestone or APM, they are responsible for informing the relevant ORD managers and transferring the information in an appropriate format to the appropriate stakeholders.
- When an APG is completed, consideration will be given to preparing a synthesis document, where appropriate, that integrates findings of all of the research to demonstrate how the multiple studies have contributed to meeting the APG.

To facilitate communication within the Agency and with the public, an SP2 Research Program website will be developed and maintained.

X. References

National Research Council (NRC). *Building a Foundation for Sound Environmental Decisions*. Committee on Research Opportunities and Priorities for EPA. National Academy of Sciences, Washington, DC, 1997.

National Research Council (NRC). *Environmental Effects of Transgenic Plants: The Scope and Adequacy of Regulation.* National Academy of Sciences, Washington, DC, 2002.

National Research Council (NRC). *Genetically Modified Pest-Protected Plants: Science and Regulation.* National Academy of Sciences, Washington, DC, 2000.

APPENDIX I
POTENTIAL ADDITIONAL RESEARCH IF RESOURCES INCREASED 10-20 PERCENT

Should additional resources become available for the SP2 Research Program, the planners will work collaboratively to identify current high priority scientific uncertainties to address. In FY06, OMB introduced a pilot program within the Agency, whereby an additional $4.5 M for research was recommended to address specific Program Office needs for the Air, Water, Solid Waste, and Pesticides and Toxics offices. In the area of SP2, teams of managers and scientists from across ORD's laboratories and centers, OPP, OPPT, OSCP, and the lead Region for pesticides and toxics held a series of meetings to determine how these additional resources should be used. Within a short period of time, the multiple parties reached a consensus on identifying the research needed and allocating the resources accordingly. The planners used the previous SP2 MYP as the overall framework to guide their decisions. In a number of instances, the additional resources went to accelerate projects already planned. In other cases, new research that was complementary to ongoing efforts was identified. Many of these efforts are described within this MYP. Others that are more closely related to the Endocrine Disruptors Research Program will be described in the updated version of that MYP. This approach and partnership resulted in a portfolio of research that is already having an impact on Agency decisions even just less than a year after implementation. Therefore, the same approach will be used should additional resources become available in the future.

For example, there have been initial discussions between ORD and OPPT on using additional resources to address a new emerging biotechnology issue on the assessment of the safety of fluorescent proteins to human health and the environment. A draft background paper has been developed by OPPT that highlights the scientific questions that need to be addressed and how research would impact their decisionmaking process. A brief excerpt from that document follows as an example of new research needs that could be addressed.

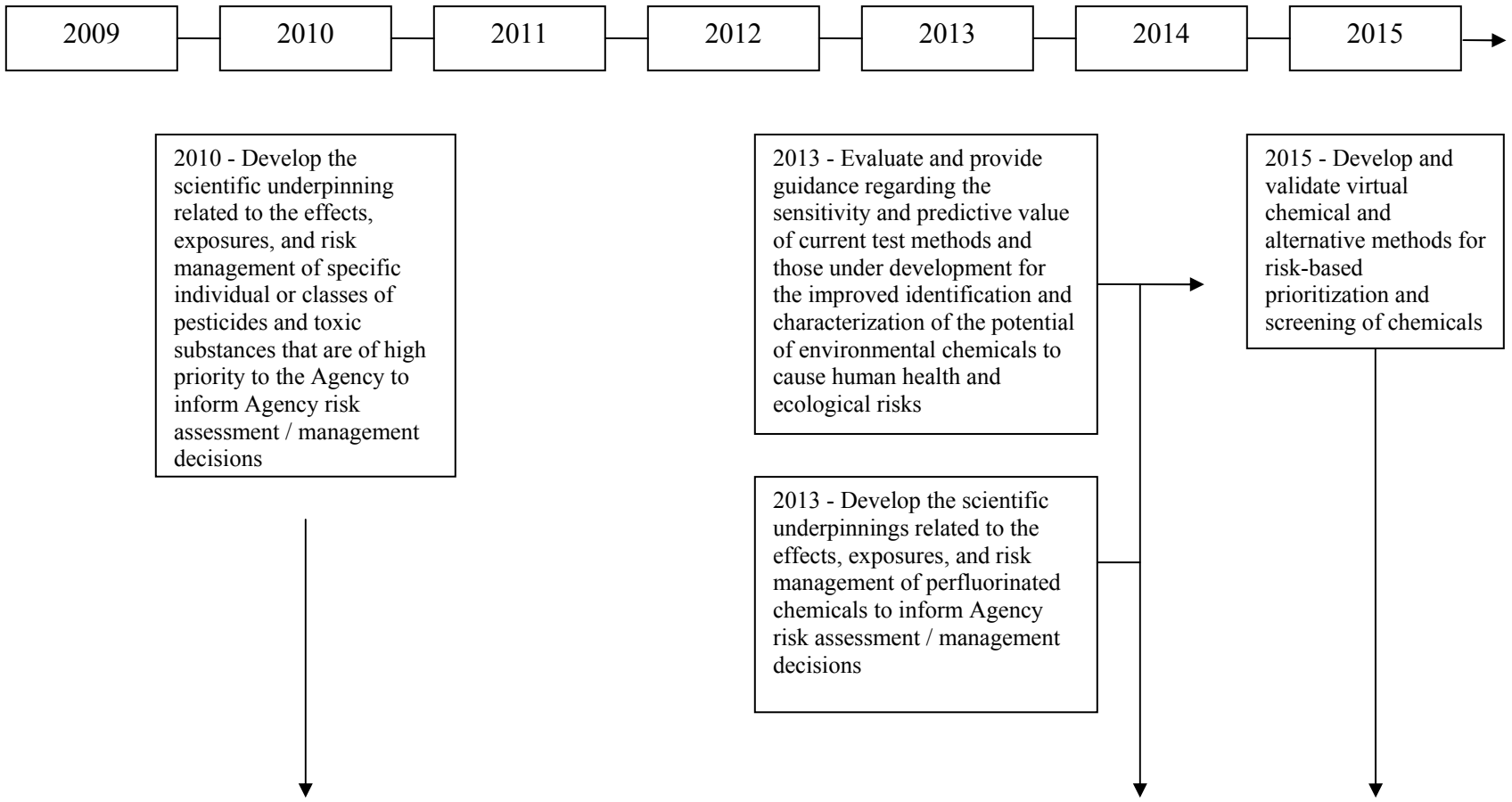
Problem Statement: OPPT has received submissions over the past several years using microorganisms marked with fluorescent proteins, both green fluorescent proteins (gfp) and a red fluorescent protein, DsRed originally isolated from the jellyfish *Aqueorea victoria* and from a reef coral, *Discosoma* sp., respectively. Given the potential widespread dissemination of fluorescent proteins in OPPT- and OPP-oriented microbial and plant applications, especially with the DsRed where there is the potential for it to enter the food supply (an application is in house which uses a genetically marked bacterium carried by an insect vector that feeds on numerous crop species), there is concern regarding unknown effects of fluorescent proteins to human health and the environment. **Approach:** OPPT proposes that ORD initially evaluate the potential human health effects of a variety of these fluorescent proteins if they were to enter the food supply in terms of their stability in the human digestive system and their potential toxicity, including allergenicity. It would also be desirable to evaluate potential ecological effects of these fluorescent proteins, including toxicity to honey bees and/or other nontarget species and the effects of protein acquisition in photosynthetic microorganisms.

Other areas where OPPTS and ORD have had discussions regarding conducting research to address unmet science needs include: 1) detection, analytical, and risk management approaches for dealing with prions and 2) nanotechnology. For the latter, there is a separate research strategy under development by a team of ORD and cross-Agency scientists. OPPTS will be a major client of that research program and it is quite conceivable that in the future that some, if not most, aspects of that research may become incorporated into the SP2 research program. Additional resources in SP2 could then be used to expand the efforts beyond what they currently are. Regional Offices have expressed the continued need for research regarding clean-up methods for common household items contaminated from pesticide applications

APPENDIX II

FLOW DIAGRAMS

Figure 3 - Linkage and Timeline for APGs to Meet SP2 Long-Term Goal 1



Long Term Goal 1. OPPTS and/or other organizations use the results of ORD's research on methods, models, and data as the scientific foundation for: 1) prioritization of testing requirements, 2) enhanced interpretation of data to improve their human health and ecological risk assessments, and 3) decisionmaking regarding specific individual or classes of pesticides and toxic substances that are of high priority.

Figure 4 - Linkage and Timeline for APGs to Meet SP2 Long-Term Goal 2

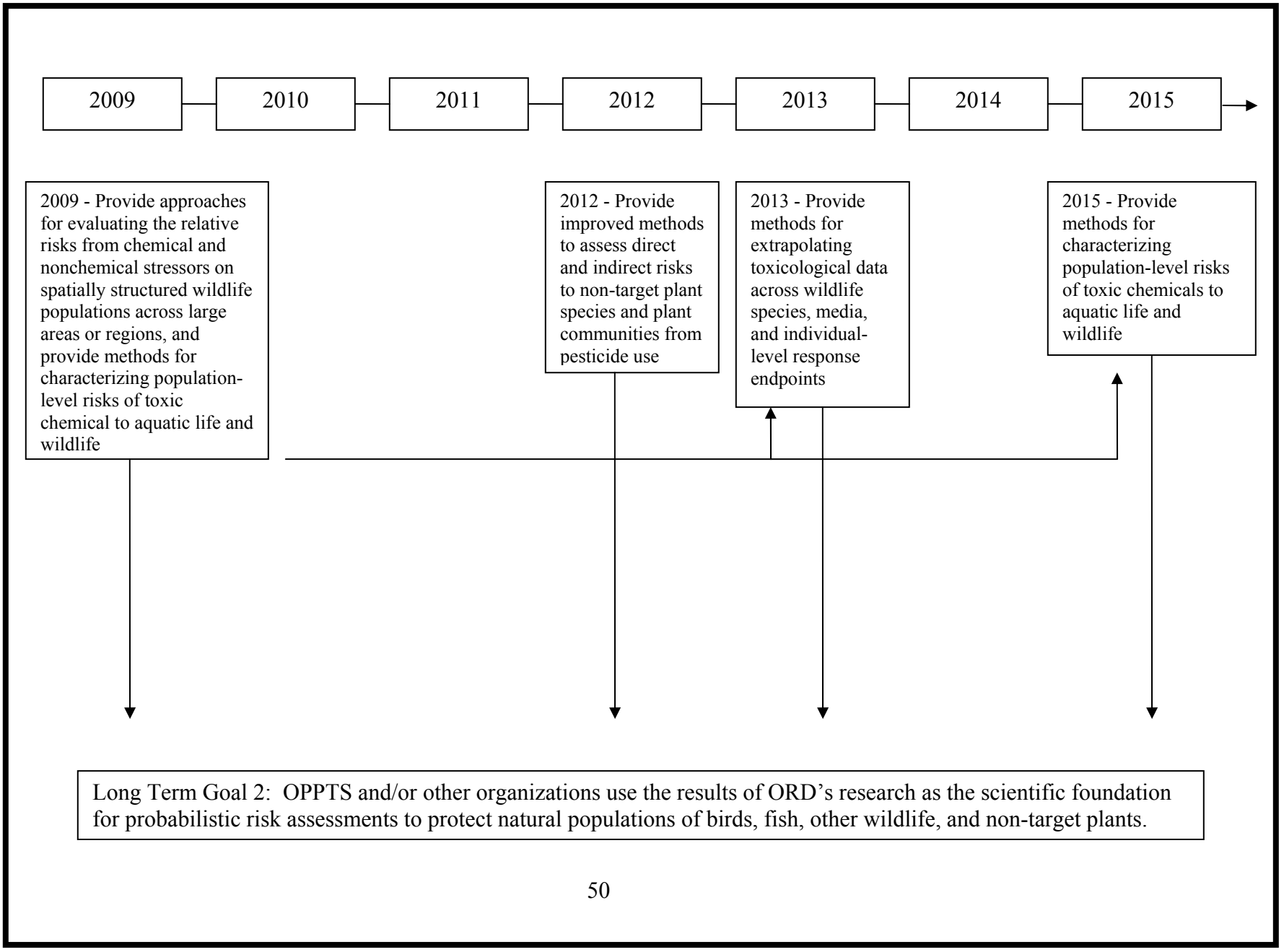
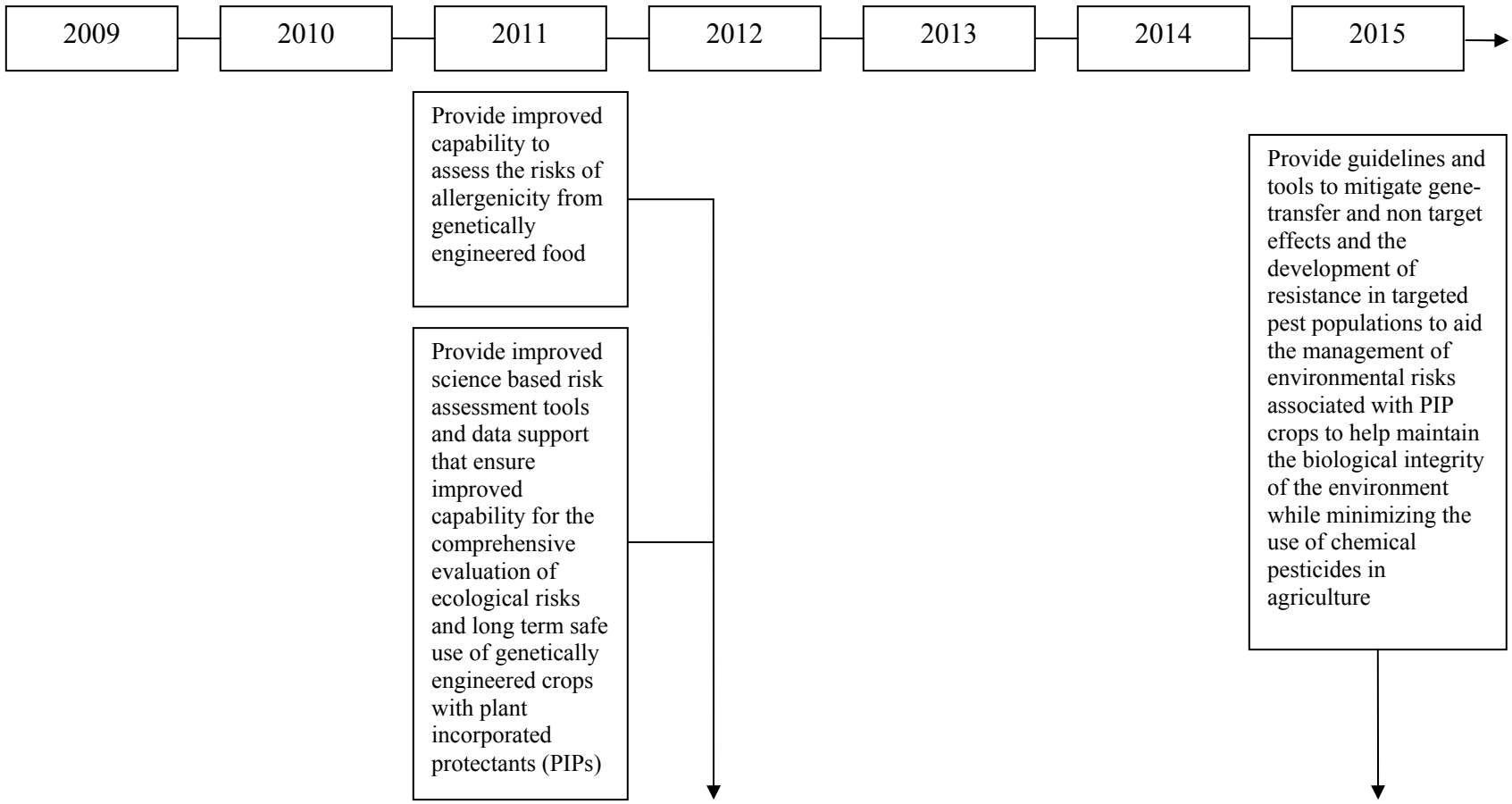


Figure 5 - Linkage and Timeline for APGs to Meet SP2 Long-Term Goal 3



Long Term Goal 3: OPPTS and/or other organizations use the results of ORD's biotechnology research as the scientific foundation for decisionmaking related to products of biotechnology.

APPENDIX III

Annual Performance Goals/ Annual Performance Measures TABLE 1.

Long Term Goal 1. OPPTS and/or other organizations use the results of ORD's research on methods, models, and data as the scientific foundation for: 1) prioritization of testing requirements, 2) enhanced interpretation of data to improve their human health and ecological risk assessments, and 3) decisionmaking regarding specific individual or classes of pesticides and toxic substances that are of high priority.

	Annual Performance Goals and Measures	Year	Lab/Center
APG	**Develop and validate virtual chemical and alternative methods for risk-based prioritization and screening of chemicals**	**2015**	
APM	Report on the development of *in vitro* models for determining the effects of chemicals on T4 synthesis	2007	NHEERL
APM	Release the ASTER system via the EPA intranet	2007	NHEERL
APM	Report on approach to evaluate and enhance metabolism simulator performance through incorporation of metabolic maps for reaction types and chemicals on EPA priority lists	2007	NHEERL
APM	Report on inventory ranking for OPP lists	2008	NHEERL
APM	Release the ASTER ranking module	2008	NHEERL
APM	Report on cross-species protein expression recognition models in fish in which are predictive of chemical mode of action affecting reproductive processes	2008	NHEERL
APM	Provide ORD and the Program Offices with evaluated techniques for characterizing temporal and compensatory aspects of exposure using NMR-based metabolomics	2008	NERL
APM	Report on using a sensitive Japanese medaka (*Oryzias latipes*) fish model for endocrine disruptor screening	2008	NCER
APM	Report on a high throughput zebrafish embryo gene expression system for screening endocrine disrupting chemicals	2008	NCER
APM	Report on development and application of a bioluminescent yeast-reporter systems for screening chemicals for estrogenic and androgenic effects	2008	NCER
APM	Report on Sertoli cell based screening assay	2009	NHEERL
APM	Report on mechanistic approach to screening chemicals and mixtures for endocrine activity using an invertebrate model	2009	NCER
APM	Report on prioritization approaches for multiple inventories and endpoints	2010	NHEERL
APM	Update ASTER toxicity pathway identification software with substructures associated with new pesticide active ingredients	2010	NHEERL

APM	Report on quantitative, high-throughput assays of neurodevelopmental processes for cell-based and non-mammalian models	2010	NHEERL
APM	Report on Biomarker identification, characterization and potential applicability to high throughput reproductive toxicity testing	2010	NHEERL
APM	Provide ORD and OPPTS with evaluated techniques for screening large chemical lists using NMR-based metabolomics	2010	NERL
APM	Report on the development of a systems model of the thyroid axis of *Xenopus*	2011	NHEERL
APM	Identify genomic, proteomic and or other throughput approaches that improve the efficiency and effectiveness of immunotoxicity testing	2012	NHEERL
APM	Report on linking proteomic biomarkers to traditional reproductive endpoints used in chronic fish bioassays	2012	NHEERL
APM	Report on enhancement of metabolic simulations and use in hypothesis formulation and testing	2012	NHEERL
APM	Provide OPPTS with documented markers of chemical exposure for selected high-priority classes of pesticides and toxic chemicals	2012	NERL
APM	Report on predictive ability of a test battery for developmental neurotoxicity	2014	NHEERL
APG	**Evaluate and provide guidance regarding the sensitivity and predictive value of current test methods and those under development for the improved identification and characterization of the potential of environmental chemicals to cause human health and ecological risks**	**2013**	
APM	Deliver prototype software, input templates, and demonstration database for capture of pesticide metabolism pathway data.	2007	NHEERL
APM	Report on an evaluation of the use and relevance of the individual components of DNT, with recommendations for revisions	2008	NHEERL
APM	Report on progress toward building metabolism and degradate data systems data.	2009	NHEERL
APM	Report on the application of genomics and proteomics for characterizing the mode of action for conazole-induced mouse liver tumors and their relevance for human health risk	2009	NHEERL
APM	Report on estrogen elicited gene expression network elucidation in the rat uterus	2009	NCER
APM	Report on systems biology modeling of fathead minnow response to endocrine disruptors	2009	NCER
APM	Report on chemical induced changes in gene expression patterns along the HPG-axis at different organizational levels using a small animal model (Japanese medaka)	2009	NCER
APM	Report on proteomic approaches using small fish models to predict chemical mode of action in chemical mixtures	2010	NHEERL
APM	Report on assay fidelity in predicting reproductive toxicity	2012	NHEERL
APM	Report on New Jersey Research Center for Environmental Bioinformatics and Computational Toxicology	2012	NCER

APM	Report on Carolina Environmental Bioinformatics Research Center	2012	NCER
APG	**Develop the scientific underpinnings related to the effects, exposures, and risk management of perfluorinated chemicals to inform Agency risk assessment/management decisions**	**2013**	
APM	Develop a database for the PFAA content in new AOC by combining the data from this project and those published in peer-reviewed journals	2007	NRMRL
APM	Deliver to OPPT a summary of the concentrations of perfluorinated compounds (PFCs) in soil samples collected from around the United States and globally to be used in a risk assessment regarding PFCs in the environment	2008	NERL
APM	Provide data to OPPT on the major PFAA sources in the indoor environment and the PFAA emission rates from AOC based on accelerated aging tests and other migration tests	2008	NRMRL
APM	Comparative profiles for developmental toxicity of various PFAA pertinent to risk assessment	2009	NHEERL
APM	Define the PFAA doses required for different adverse effect endpoints for determination of BMD	2009	NHEERL
APM	Relative sensitivity of developing liver to PFAA	2009	NHEERL
APM	Reporting on the effects of PFAA on developmental expression and activation of PPAR-signaling pathways	2009	NHEERL
APM-R	Deliver to OPPT a series of methods for describing the distribution of PFCs in key environmental and biological media and for characterizing potential human exposures to these compounds	2009	NERL
APM	Experimental Methods to Evaluate the Stability of Fluorotelomer-based Polymer Products	2009	NRMRL
APM	Evaluation of fluorochemical degradation during aerobic wastewater treatment (OECD 303a)	2009	NRMRL
APM	Characterize the immunotoxic potentials of PFAA and the role of PPAR signaling pathway in the PFAA-induced immunotoxicity	2010	NHEERL
APM	Pharmacokinetic models for selected PFAA for interspecies extrapolation	2010	NHEERL
APM	Deliver to OPPT a summary evaluating degradation of fluorotelomer-based polymer products (FBPPs) in soil materials to be used in a risk assessment regarding FBPPs	2010	NERL
APM	Provide data to OPPT on PFAA precursors (e.g., fluorotelomer alcohols) in the indoor environment and their implications to PFAA exposure	2010	NRMRL
APM	Comparative pharmacokinetic profiles for selected PFAA pertinent to risk assessment	2011	NHEERL
APM	Description of hepatotoxicity of selected PFAA and the biological pathways involved	2011	NHEERL
APM	Determining the effects of PFAA on PPAR-signaling functions that impact prenatal and postnatal growth regulation	2011	NHEERL
APM	MOAs for tissue dysplasia/tumor formation following early life exposure to PFAA	2011	NHEERL

APM	Extend the evaluation of developmental toxicity of other PFAA deemed relevant by OPPT	2011	NHEERL
APM	Wastewater screening study to evaluate FC loadings to the environment	2011	NRMRL
APM	Profiles of hormonal disruption induced by PFAA and their implication for human health hazards	2012	NHEERL
APM	Evaluation of fluorochemical degradation during anaerobic wastewater treatment (OECD 311)	2012	NRMRL
APM	Common modes of action for PFAA developmental toxicity	2013	NHEERL
APG	**Develop the scientific underpinning related to the effects, exposures, and risk management of specific individual or classes of pesticides and toxic substances that are of high priority to the Agency to inform Agency risk assessment/management decisions**	**2010**	**APG**
APM	Provide the Program Office and Regional Asbestos Risk Assessors with enhanced exposure tools for assessing human exposures to asbestos in soils and air.	2007	NERL
APM	Provide OPPT with a report incorporating the science needs outlined in the issue paper and workshop discussions on the development of Pb test kits	2007	NERL
APM	Asbestos releasability data and model development completed for use by Regional Offices and others for greater understanding of expected asbestos airborne concentrations levels from various sources under certain environmental and typical disturbance/activity conditions	2007	NRMRL
APM	Provide OPP with information that will inform guidance to the public regarding the ability of coating products to reduce dislodgeable CCA residues on the surfaces of CCA-treated wood	2007	NRMRL
APM	Provide OPP with data that may inform the CCA risk assessment	2007	NRMRL
APM	Evaluated protocols submitted to OPP	2007	NRMRL
APM	Treatment study results submitted to peer journals and to OPP on acetochlor, molinate and terbufos, on carbamate pesticides, and on triazole degradates	2007	NRMRL
APM	Provide OPP with data summarizing the enantioselectivity, exposure, transformation and effects of conazoles and other modern chiral pesticides for use in risk assessment and regulatory activities	2008	NERL
APM	Provide OPPT with a summary report on the laboratory research conducted to modify the performance of Pb test kits with the recommendation(s) for a modified Pb paint test kits(s) that can be readily commercialized to support the Pb Renovation, Remodeling, and Painting rule	2008	NERL
APM	Provide OPPTS with evaluated tools for forecasting the fate of pesticides and toxic chemicals in drinking water treatment systems	2008	NERL
APM	Treatment study results submitted to peer journals and to OPP on acetochlor, molinate and terbufos, on carbamate pesticides, and on triazole degradates	2008	NRMRL

APM	Provide OPPTS with a report highlighting the fate of perfluorinated chemicals under simulated drinking water treatment conditions	2009	NERL
APM	Provide a compendium of AHS Pesticide Exposure Study results to OPPTS, NCI, and NIEHS for use in assessing and refining exposure classification in an important agricultural epidemiological cohort and to provide information about exposures and related factors.	2009	NERL

TABLE 2.

Long Term Goal 2: OPPTS and/or other organizations use the results of ORD's research as the scientific foundation for probabilistic risk assessments to protect natural populations of birds, fish, other wildlife, and non-target plants.

	Annual Performance Goals and Measures	Year	Lab/Center
APG	**Provide methods for extrapolating toxicological data across wildlife species, media, and individual-level response endpoints**	**2013**	
APM	Report on quantitative model to estimate in vivo metabolic rates for fish from microdialysis sampling data	2007	NHEERL
APM	Guidance document for performing histopathology on gonadal tissues from small fish exposed to endocrine disruptors	2007	NHEERL
APM	Report on protocol for assessing transgenerational effects of reproductive toxicants on the HPG axis in medaka	2007	NHEERL
APM	Demonstrate a systems-based approach to utilizing toxicogenomic data to extrapolate across species and biological levels of organization	2007	NHEERL
APM	Report on and deliver web-ICE and validated ACE to OPPTS to support pesticide risk assessments.	2008	NHEERL
APM	Evaluate species, endpoint, and associated hazard for ECOTOX data used in the Pesticide Re-registration process	2008	NHEERL
APM	Demonstration of an approach for predicting population-level impacts in fish based on molecular responses to chemicals with different toxic mechanisms of action	2008	NHEERL
APM	Report on the utility of in vitro metabolic assays to predict in vivo metabolism in fish	2009	NHEERL
APM	Genomic characterization of toxicity pathways in fish as a basis for extrapolation across species	2009	NHEERL
APM	Report on and deliver updated web-ICE and ACE to OPPTS to support pesticide risk assessments.	2010	NHEERL
APM	Evaluate species, endpoint, and hazard for ECOTOX data associated with listed USFWS threatened and endangered species	2010	NHEERL
APM	Systematic, global analysis of biological networks to facilitate extrapolation across species and chemical exposures in support of population-level risk assessments for fish	2011	NHEERL
APM	Assessment of the utility of short-term reproductive and developmental assays with fish for predicting adverse population-level effects	2012	NHEERL
APG	**Provide methods for characterizing population-level risks of toxic chemicals to aquatic life and wildlife**	**2015**	
APM	Methods for assessing the quality of published demographic parameters for use in population-level risk assessments	2007	NHEERL
APM	Review of published methods and models to incorporate genetics into population viability models	2007	NHEERL

APM	Methods for translating existing avian toxicity data into estimates of change in demographic parameters for use in population models	2008	NHEERL
APM	Provide guidance to program office on the development, application, and interpretation of simple modeling approaches in a regulatory context for risk assessment.	2009	NHEERL
APM	Development of stochastic population modeling approaches for risk assessments of aquatic and avian populations	2010	NHEERL
APM	Report on and provide spatial population models for coastal fish to OPPTS to support pesticide risk assessments.	2010	NHEERL
APM	Evaluate methods to incorporate complex ecological processes into population models	2012	NHEERL
APM	Report on and provide improved spatial aquatic population models, including expanding to additional species, stressors, locations, and fish communities	2014	NHEERL
APM	Provide guidance on the development, application, and interpretation of population models to tiered assessment process	2014	NHEERL
APG	**Provide approaches for evaluating the relative risks from chemical and nonchemical stressors on spatially structured wildlife populations across large areas or regions, and provide methods for characterizing population-level risks of toxic chemical to aquatic life and wildlife**	**2009**	
APM	Web-accessible, GIS with information on cropping practices, pesticide use, and avian demographics	2007	NHEERL
APM	PATCH II framework version (Windows) with a generalized life history module, a general stressor module, and GIS accessibility	2008	NHEERL
APG	**Provide improved methods to assess direct and indirect risks to non-target plant species and plant communities from pesticide use**	**2012**	
APM	Input for protocol for measuring reproductive and developmental endpoints with annual species	2008	NHEERL
APM	Development of molecular methods for tracking exposure and assessing effects of plants exposed to low-dose, high-potency herbicides	2008	NHEERL
APM	Refined regional assessment tools for probabilistic assessments of risks to plants from herbicides based on GIS framework	2009	NHEERL
APM	Methodologies to determine how chemical stressors and natural factors control native plant populations	2011	NHEERL

TABLE 3.

Long Term Goal 3: OPPTS and/or other organizations use the results of ORD's biotechnology research as the scientific foundation for decisionmaking related to products of biotechnology.

	Annual Performance Goals and Measures	Year	Lab/Center
APG	**Provide improved capability to assess the risks of allergenicity from genetically engineered food**	**2013**	
APM	Develop and use animal models to assess potential risks of allergenicity associated with plant incorporated pesticides (PIPs)	2010	NHEERL
APM	Report on improved animal model for assessment of allergenic potential of foods through selective deletion of T cells and global gene expression analysis	2012	NCER
APM	Report on risk assessment of food allergenicity by a data base approach	2012	NCER
APM	Report on delineation of appropriate specific and targeted IgE serum testing to assess the potential allergenicity of proteins introduced by genetic engineering	2012	NCER
APM	Report on safety assessment of dietary proteins for allergenicity using an adjuvant-free mouse model	2012	NCER
APG	**Provide improved science based risk assessment tools and data support that ensure improved capability for the comprehensive evaluation of ecological risks and long term safe use of genetically engineered crops with plant incorporated protectants (PIPs)**	**2011**	
APM	A scoping meeting regarding the integrated project with appropriate EPA and outside experts	2007	**NCEA,** NERL, NHEERL, NRMRL
APM	External Review Draft report outline appropriate tools for monitoring resistance development to GM crops in the field and the sue of target pest ecology to refine insect resistance management strategies to support OPPTS ecology risk assessments of risk management techniques	2007	NCEA
APM	External review draft of report on a conceptual framework for assessing the ecosystem scale impacts of genetically modified crops to support OPPTS risk assessments	2008	NCEA
APG	**Provide guidelines and tools to mitigate gene-transfer and non target effects and the development of resistance in targeted pest populations to aid the management of environmental risks associated with PIP crops to help maintain the biological integrity of the environment while minimizing the use of**	**2015**	

	chemical pesticides in agriculture		
APM	Assessment of pest genetic architecture (population structure and genetic trait analysis) in order to inform optimized resistance management plans	2006	NERL
APM	Acquire hyperspectral imagery over production fields in Illinois, Iowa, Nebraska and Minnesota	2006	NRMRL
APM	Generate prototype PIP status and infestation extent maps and distribute to field personnel for assessment	2006	NRMRL
APM	Detailed field data collection on PIP status, insect infestation levels and general agronomic conditions of close to a hundred fields	2006	NRMRL
APM	Map assessment for accuracy and refinement based on 2006 results	2006	NRMRL
APM	Acquisition over production fields repeated over a larger area using more automated map generation methods	2006	NRMRL
APM	SOP for microarray methods for plant samples	2007	NHEERL
APM	Provide report to OPPTS on the development and evaluation of a gene expression assay of plant incorporated protectant exposure to non-target insects	2007	NERL
APM	Provide report to OPPTS on the genetic characterization of WCR resistance to Cry3Bb based on artificial selection and quantitative molecular genetic approaches	2007	NERL
APM	Methods to measure ecological effects of gene flow on plant communities: herbicide resistant model crops	2008	NHEERL
APM	Provide expert panel recommendations to ORD for designing a genetic monitoring program to assess long-term effects of PIPs on non-target organisms	2008	NERL
APM	Office of Pesticide Programs (OPP) project involvement	2008	NRMRL
APM	Generate maps over large cross sections of the corn producing areas of the US and distributed to OPP personnel for their use and assessment	2008	NRMRL
APM	Molecular indicators of PIP (insect resistance and fungal disease resistance) gene expression, introgression and population level markers in GM crop and compatible non-target plants.	2009	NHEERL
APM	Nearly automated production of map development and distribution for use by OPP, seed registrants and growers for a precise assessment of PIP status and insect infestations	2009	NRMRL
APM	Incorporate the variation in crop phenology as observed from the aerial detection including phenological differences between and within fields.	2009	NRMRL
APM	Methods to measure ecological effects of gene flow on plant communities: insect resistant model crop	2010	NHEERL
APM	Provide a report to OPPTS on the results of artificial selection for resistance in WCR- implications for resistance management programs	2010	NERL
APM	Development and assessment of a multi-species molecular assay for use by Agency scientists to assess exposures to *Bt* toxins by target and non-target beetles	2010	NERL
APM	Examine landscape configurations involving within field refuge areas and compare results to whole field evaluations reported	2010	NRMRL

	earlier		
APM	Each model will be transparently documented for an audience ranging from professional to public citizenry	2010	NRMRL
APM	Provide OPPTS with an assessment of the results from an ORD monitoring program to evaluate ecosystem-scale changes associated with transition to biotechnology-based agriculture	2012	TBD
APM	Modeling ecological effects of gene flow from herbicide resistant and insect resistant GM crops on plant communities.	2012	NHEERL
APM	Methods to measure ecological effects of gene flow from GM fungal disease resistant crops on plant communities.	2013	NHEERL
APM	Apply the developed technology for resistance monitoring of pests in cotton production	2013	NRMRL
APM	Methods to measure the ecological effects of GM plants with stacked PIPs in the presence/absence of selective pressures and environmental stressors	2014	NHEERL
APM	Refine the methodology that combines analytical and simulation techniques using initial allele frequencies reported from field experiments	TBD	NRMRL

APPENDIX IV
DETAILS ON RESEARCH THEMES

Long Term Goal 1:
OPPTS and/or other organizations use the results of ORD's research on methods, models, and data as the scientific foundation for: 1) prioritization of testing requirements, 2) enhanced interpretation of data to improve their human health and ecological risk assessments, and 3) decisionmaking regarding specific individual or classes of pesticides and toxic substances that are of high priority

APG - Develop and validate virtual chemical and alternative methods for risk-based prioritization and screening of chemicals - FY 2015

1.1.1 Screening and Prioritization Models

Research Goals and Approaches: Research is: 1) Developing toxicity pathway-based QSARs for prioritization within large chemical lists, 2) Providing access to peer-reviewed literature and MOA-based QSAR models (ASsessment Tools for Evaluation of Risk), and 3) Simulating metabolism to enhance effects modeling.

1) The QSAR pilot project will use a multifaceted approach to determine the chemical structural requirements for initiation of distinct toxicity pathways. It incorporates QSAR-based hypothesis generation, strategic chemical selection for hypothesis testing, in vitro assay optimization and targeted testing, and QSAR evaluation and improvement for mechanistic classifications for OPP pesticidal inerts and antimicrobials, chemicals for which data are lacking and predictions welcomed. The approach is grounded in seeking mechanistic understanding of underlying chemical-biological interactions and defining chemical similarity in terms of biological activity. The objective is to present a process applicable to recurring issues surrounding determining structural attributes associated with toxicity and leading to adverse biological consequence. Determinations must be made with enough specificity to result in reliable predictions but have broad applicability to numerous diverse chemicals. The process used strives for mechanistic interpretability and transparency, allowing evaluations of coverage within inventories to which models are applied.

2) ORD is collaborating with OPP on the release of ASTER, with its existing battery of MOA-based QSARs, to EPA's intranet. Models within ASTER are upgraded (e.g., ecotoxicity, environmental partitioning, environmental persistence, chemical bioconcentration in tissues) and the system capabilities is expanded (e.g., ability to search the ECOTOX database for structural analogs). This effort puts a revised version of a tool currently used by the program office directly on their desktops, providing the program office the capability to rank large lists of chemicals based on measured data (in ECOTOX) and predicted values where measured data is absent.

3) An existing metabolism simulator will be refined to focus on metabolic transformations most likely to increase toxic potential for an endpoint under study in a related project (Developing toxicity pathway-based QSARs for prioritization within large chemical lists). Metabolic transformation types shown to enhance estrogenicity, but that currently are underrepresented in an existing simulator, will be studied using chemicals selected from priority OPP lists. For selected chemicals, metabolic pathways will be determined using rat (or fish) *in vitro* metabolism systems. Analytical methods will be developed and used to verify bioactivated metabolites formed in each system under study. Chemicals additionally will be tested in metabolically competent liver slices from male fish. Chemical binding to fish ER also will be verified for chemicals and their putative bioactivated metabolites (when available) in an associated project. Newly generated metabolic maps will be used to retrain the metabolism simulator and, thus, increase reliability and predictivity. The ultimate goal is to demonstrate an approach for predicting chemical potential for metabolic activation. As building of metabolism and degradate databases progresses (see ***1.2.1*** – metabolism/degradate databases) they will provide a rich source of metabolic map information specific to OPP chemicals thus serving as the foundation for developing a metabolism simulator relevant to reaction types and chemicals of OPP concern.

Impact and Outcomes: 1) The toxicity pathway-based QSAR research will develop and apply *in vitro* and *in silico* techniques for prioritization and ranking within a regulatory context for a defined toxicity pathway. Model development and applicability is provided with guidance on use to enhance data interpretation. 2) The updating and improvements to the ASTER system will facilitate the identification of structural analogs and associated toxicity information to estimate potential hazard of untested chemicals or chemicals with limited toxic effects information. 3) The metabolism simulator research helps in better understanding toxicity pathways from initiating events to response for metabolically activated chemicals. A computational tool is provided that allows prediction and prioritization of chemicals for which measured data are lacking.

1.1.2 Nuclear Magnetic Resonance (NMR)-Based Metabolomics

Research Goals and Approaches. ORD will be using NRM-based metabolomics to: 1) understand and link the exposure and effects of EDCs within the HPG axis of small fish models. This includes linking responses from genomics, proteomics and metabolomics. Information will be integrated in a systems and population modeling context.; 2) define markers of exposure, and to better understand the cumulative risk from triazole pesticides. Research will differentiate responses from triazole pesticides that exhibit different MOA, assess the impact of metabolism of the parent triazole pesticide on the observed outcome and determine the extent of conservation of triazole metabolism behavior and metabolomic profiles across various species (e.g., fish and rats); 3) identify markers of exposure for important PFCs such as PFOA and PFOS to gain a better understanding of toxic MOA and investigate the occurrence of markers of exposure from fish collected in contaminated field sites; 4) develop a HTP metabolomics approach that involves exposing cell cultures to potentially toxic chemicals in order to screen large inventories of chemicals, such as the HPV chemicals, rapidly for potential adverse outcomes. Chemicals found to greatly alter the metabolite profile of cell cultures (relative to the control case) would be candidates for more extensive testing; 5) investigate the feasibility of metabolomics to describe the impacts of exposure of small fish models to nano-materials. Changes in endogenous metabolites would be used as an indicator and descriptor of detrimental exposure; and 6) identify endogenous metabolite-based markers of exposure to toxicants in human epidemiology studies in collaboration with the National Cancer Institute (NCI). This includes unique sample sets that NCI has collected on occupational benzene exposure in China and benzidine exposure in India. (Note: Part of this project is linked with the second grant under **1.2.4** and **2.1.3**)

Impacts and Outcomes: The research will result in: 1) validated markers of biologically relevant chemical exposure to important classes of pesticides and toxic chemicals; 2) information on the temporal and compensatory aspects of chemicals exposures; 3) information on similarities and differences xenobiotic metabolism and the impact of exposures across key species (small fish, rats, etc.); 4) information on the linkage of exposure events to whole organism adverse outcomes. These new methods of prioritization will be used by the Program Office to: 1) winnow down the list of chemicals to those most likely to be of concern to humans and ecosystems; 2) extrapolate impacts across species where direct measurements are not available. Furthermore, the markers of exposure that ORD will provide will be used by Program Offices to determine when effective exposure has occurred and to aid in decisions about regulating chemicals according to a common MOA.

1.1.3 Screening and Prioritization Methods

Research Goals and Approaches: ORD research is developing: 1) Alternative methods for screening and prioritization of developmental neurotoxicants, 2) cell culture and biomarker-based screening methods for nonendocrine reproductive toxicology, and 3) toxicity-pathway-specific protein expression models for chemical screening and prioritization

1) ORD will develop *in vitro* cell culture models of the key events in brain development and develop methods to measure behavioral, morphological and neurochemical outcomes in a limited number of non-mammalian species. Assays of developmental endpoints will be optimized for quantitative analysis using HTP technologies. The predictive capability of the *in vitro* and non-mammalian test batteries will be assessed using a training set of known developmental neurotoxicants, from different chemical classes. Data will be gathered from the literature and OPP data sets and will be incorporated into a searchable DNT database.

2) ORD will utilize Sertoli cell cultures challenged with a panel of known reproductive toxicants to identify insult induced effects *in vitro*. Sertoli cell markers will be examined and their utility as endpoints for HTP screening determined. Cell cultures will also be evaluated using genomic and proteomic methodology to identify novel biomarkers suitable for inclusion in the screening model. This approach subsequently will be extended to additional reproductive cell lines (i.e., epididymal cells), seminiferous tubules, and intact animals to identify additional biomarkers. Emphasis will be on identifying markers with the potential to be used in current one generation tier testing.

3) Surface Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (SELDI-TOF MS) is used to examine protein expression profiling as a means to screen chemicals for their MOA. *In vitro* exposure of fish hepatocytes and short-term *in vivo* minnow exposures are used to link diagnostic expression profiles between tissue level and whole organism assays, and across multiple fish species. Culture media (*in vitro*) or plasma (*in vivo*) samples from control and exposed treatments are applied to Protein Chip arrays to produce protein expression profiles unique to each treatment. A binary classification model is constructed from control and treatment protein profiles to identify differentially expressed proteins predictive of the MOA of interest. As proof of concept, research efforts are initially focused on developing diagnostic models with established estrogenic and androgenic chemicals.

Impact and Outcomes: 1) A screening approach using cell-based models and alternative species addresses the need to delineate a strong linkage between responses observed at lower and higher levels of biological organization. This research addresses several questions associated with developing a first tier

approach for screening for developmental toxicity including: 1) are there predictive biomarkers of key events in the developing nervous system that can be assessed *in vitro*; 2) how do we apply technological advances in HTP testing, genomics, and/or proteomics to develop rapid in vitro screening assays; 3) is there a homologous, non-mammalian model of neurodevelopment that can be used as a rapid screen for developmental neurotoxicity. 2) Screening for reproductive effects using biomarkers and *in vitro* cell cultures will aid in prioritizing the testing of chemicals under review and characterization of new biomarkers will enhance interpretation of existing information on the effects of reproductive toxicants. This research will specifically approach the needs to utilize emerging technology to develop rapid screening and prioritization tools and to identify new markers of effect that would increase our understanding of current information on reproductive toxicity. 3) Protein expression profiling addresses the need for targeted *in vivo* tests based on MOA and targeted omics-based *in vitro* screens. Profiling the differential expression of proteins associated with established toxicological pathways, and linking these profiles across multiple levels of biological organization provides OPPTS with a powerful predictive tool for screening and prioritization of chemicals. This research addresses OPPTS needs for increased efficiency and effectiveness of testing programs while providing information necessary to estimate the toxicological potential of a chemical or chemical class to elicit an adverse outcome

1.1.4 STAR: Development of HTP Screens

Research Goals and Approaches: In 2002 an RFA was released through the Computational Toxicology STAR program to solicit research proposals that would lead to the development of HTP screening systems for identifying chemicals with estrogen, androgen, or thyroid hormone activities. The goal was to develop an extramural portfolio of research on the development of HTP screening systems to assist in prioritization of chemicals for further screening and testing of their potential as endocrine disruptors that would complement ORD's internal efforts. Four STAR awards were made in 2003:

Development and Application of a Bioluminescent Yeast-Reporter System for Screening Chemicals for Estrogenic and Androgenic Effects

Objective: Researchers have re-engineered the Saccharomyces cerevisiae YES colorimetric estrogen reporter system to produce bioluminescence in response to estrogen or environmental estrogens (S. cerevisiae BLYES). Bioluminescence is a reagentless system eliminating the need for expensive chromophores. Light-detection is more sensitive than absorbance detection thus shortening the development time of the assay. The colorimetric-based YES has been widely used and is a very useful tool for assessing estrogenicity of a compound or environmental sample. Development of the BLYES system has the potential to enhance its utility. This research is: 1) validating the BLYES system and developing a standard operating procedure for routine chemical analysis; and 2) developing an androgen bioluminescent reporter system analogous to the BLYES system.

Mechanistic Approach to Screening Chemicals and Mixtures for Endocrine Activity Using an Invertebrate Model

Objective: Research is developing a mechanism-based, high-throughput screening assay for evaluating estrogen, androgen, and thyroid (EAT)-like activities in an invertebrate species that also can be used to evaluate interactive effects of endocrine-active compounds through receptor cross-talk. The research is addressing two deficiencies in the Agency's current Tier 1 screening battery for detecting endocrine activity of chemicals: 1) no invertebrate screen is included despite the proposed use of a Tier 2

multigenerational test with a crustacean; and 2) the battery is not equipped to assess combined effects of diverse endocrine toxicants.

A High Throughput Zebrafish Embryo Gene Expression System for Screening Endocrine Disrupting Chemicals

Objective: Researchers are investigating the proposition that perturbations in the normal amount or timing of a hormone-regulated gene product can be taken as evidence of chemical exposure and used as an end-point in a screening assay to detect potential endocrine disrupting activity using the zebrafish.

Using a Sensitive Japanese Medaka (Oryzias latipes) Fish Model for Endocrine Disruptors Screening

Objective: Research is developing a rapid, sensitive, biologically-integrated screening assay to identify EDCs using a sensitive medaka (*Oryzias latipes*) fish model. Specifically, the research is focusing on two identified areas of interest: identification and evaluation of EDCs, and their categorization into (anti)estrogenic, (anti)androgenic, and (anti)thyroidogenic activity.

Impact and Outcomes: The research is expected to contribute to the development of HTP screening systems to assist in prioritization of chemicals for further screening and testing of their potential as endocrine disruptors. The methods generated from these studies will provide for multiple platforms for the HTP screening of potential endocrine disrupting chemicals, in some cases even allowing for the remote, near real-time monitoring of potential endocrine disrupting chemicals in the environment.

APG - Evaluate and provide guidance regarding the sensitivity and predictive value of current test methods and those under development for the improved identification and characterization of the potential of environmental chemicals to cause human health and ecological risks - FY 2013

1.2.1 Databases for Hypothesis Formulation and Testing

Research Goals and Approaches: In collaboration with OPP**,** ORD is developing databases for structural depiction of metabolic maps and degradation products. 1) *Metabolism*. ORD researchers are developing software which will allow easy access to currently poorly accessible and unsearchable metabolic map information that exists in OPP files. Software for the rapid and efficient depiction of metabolic maps is enhanced to: allow depiction of hierarchical connection sequences of parent chemical and all listed metabolites; track radiolabel within a pathway; combine (or separate) maps from associated radiolabel studies; identify all maps that contain a specific metabolite (or substructure) of toxicological concern; compare complete maps across chemicals to find commonalities/differences; compare maps across species to detect commonalities, differences, etc. Associated chemical identifiers, as well as bioassay and analytical chemistry data, and OPP tracking codes are included. Early outputs of the project demonstrate capabilities of the system by coding metabolic maps for a sample set of pesticides selected by OPP. Progress is also being made toward population of the database with OPP data. This is perhaps the largest collection of metabolic map data collected under the same guidelines, and as such serves as a valuable resource to ORD for hypothesis generation and testing to better understand and predict chemical potential for metabolic activation (see ***1.1.1***, part 3 – Metabolism Simulator). 2) *Degradates*. ORD researchers are optimizing the metabolic map software to capture chemical degradate data. This will facilitate the identification of potentially toxic degradates for the same chemicals assessed for metabolic activation by OPP, and will enhance the efficiency of these comparisons across the OPP divisions doing these assessments. As for metabolism data, the system would allow ready access to degradate structures

associated with parent chemicals, and, where sufficient data permits, to identify pathways of degradation associated with certain reaction types. Based on name or structure (sub-structure), degradates of particular concern can be identified; other documented occurrences of the same degradate (or the substructure of interest) can be determined; and, the association between degradate and parent chemical structures can be assessed. This also allows a better understanding of enzymatic reaction types. Initial outputs of the project will demonstrate capabilities of the system by coding degradate maps for a sample set of guideline study types selected in partnership with OPP. Because degradates are observed in various study types the challenge is to determine where sufficient depth of data (i.e., number of chemicals) is available within a given type of guideline study. This is required to better understanding and eventually predicting reaction types leading to degradate formation which can be generalized from those which may be assay specific. Due to these factors, success with metabolism database efforts is prerequisite to determining the detailed approach and resource commitment needed to develop a system capable of predicting degradation pathways for OPP chemicals (see ***1.1.1***, part 3 – Metabolism Simulator).

Impact and Outcomes: 1) Access to metabolism/degradate data in a searchable format is key to: understanding the role of metabolic activation in toxicity pathways; to generating targeted hypotheses that will address the highest priority uncertainties; and to doing so for the types of chemicals of most concern to the Program Offices. 2) Electronically stored structures of metabolites/degradates that include pathway connectivity are necessary first steps to developing and applying genetic algorithms and related artificial intelligence systems to predict environmental metabolism and degradation pathways. The development of searchable databases is key to efficient use of existing information and moving toward a new paradigm based on hypothesis-driven testing and prioritization.

1.2.2 Enhanced Interpretation of Existing Guideline Data

Research Goals and Approaches: Research is being conducted to evaluate and provide guidance on major issues regarding the methods used in the current DNT guidelines. Study reports will be reviewed and summarized in a manner that will allow evaluation of the power and limitations of the data collected under current guidelines. Parameters such as variability, reliability, and sensitivity will be evaluated across studies and laboratories conducting the tests. There are collaborative efforts currently underway among multiple organizations in ORD and OPPTS to address these issues.

Impact and Outcomes: This project will assist the Agency by enhancing interpretation of data from DNT studies. The outputs from the project will assist the Agency to revise the DNT guidelines to provide better and more accurate data and increase the ability to interpret data. Evaluation of current data also will identify areas where hazard identification or data interpretation is problematic and, therefore, assist in prioritization of research to develop new predictive diagnostic markers of DNT.

1.2.3 Identifying Predictive Functional and Molecular Endpoints

Research Goals and Approaches: ORD is exploring the development of screening assays in the areas of immunotoxicity and thyroid toxicity and the incorporation of multiple endpoint assessments into a single integrated testing protocol. 1) Research will be conducted to identify sensitive *in vivo* (using rodents) and *in vitro* approaches for recognizing and screening for alterations in immune function, including immune suppression and chemical allergy. The feasibility of applying genomics as a screen for alterations of immune function will be pursued. The potential increased susceptibility of the developing immune system, as well as the reproductive and central nervous systems, to environmental chemical exposure is a major concern given that these "sensitive" populations would be at greater risk. An

integrated testing approach for developmental neurotoxicity, reproductive toxicity and immunotoxicity will be pursued. 2) Research will use in vitro and in vivo exposure systems coupled with genomic analysis to identify key events in cancer pathways. These new technologies, in combination with traditional toxicology experimental systems, will provide a common accepted set of principles for informing extrapolation across test systems, species, and dose. These will enable the design of targeted and relevant studies to more rapidly predict the potential for induction of cancer. 3) Research will use *in vitro* and *in vivo* approaches to understand and discriminate the compensatory and toxicological responses of the highly regulated HPT system. Development of an initial systems model will be based on the current understanding of the amphibian HPT axis and the compensatory processes involved in thyroid hormone homeostasis. Experiments will be conducted to better understand the relationships of the critical sub-components of the system. Particular emphasis will be placed on understanding the relative importance of gene expression in the pituitary, thyroid, and peripheral tissues under normal conditions and following exposure to chemicals known to interfere with TH synthesis. These molecular changes will be linked with functional measurements of key hormones and enzymes that are part of the HPT pathway, all of which will be interpreted in the context of organismal-level effects.

Impact and Outcomes: The employment of *in vivo* and *in vitro* functional assays, along with the application of genomics, should provide a significant link to the identification of potentially undesirable environmental chemicals that impact the immune, reproductive and central nervous systems, can induce cancer, and disrupt the endocrine system. A comparison of the sensitivity of *in vivo* and *in vitro* function results versus genomics should lead to the identification of the most sensitive metrics for immuno-, repro-, neurotoxicity, or tumor development testing. Exploration of the conditions under which increased susceptibility of the developing immune system will be an important goal. In addition, results of this research will develop a sufficient understanding of the HPT so that predictive models can be developed, testing protocols can be abbreviated, and efforts in inter-species extrapolation can be improved. One of the most likely uses for a HPT systems model is to aid in the understanding and discrimination of different MOA. As such, this work further enables the development of QSARs by providing a basis for sorting chemicals by mode of action, a necessary step prior to quantifying features of chemical structure associated with a particular type of toxicity. If these relationships can ultimately be established, then predictive models can be developed to prioritize/rank chemicals for future *in vivo* testing.

1.2.4 STAR: Systems Biology

Research Goals and Approaches: In 2003 an RFA was released on Computational Toxicology and Endocrine Disruptors: Use of Systems Biology in Hazard Identification and Risk Assessment. The research goals are: 1) Development of integrative, quantitative models of the function of the HPG or HPT axes with emphasis on the descriptions of the normal physiological processes and mechanisms of perturbation following exposure to xenobiotics (e.g., EDCs), in rats or a commonly used small fish toxicology model (e.g., fathead minnow, medaka, zebrafish); 2) Cross-species extrapolation of integrative, quantitative models of the perturbed HPG or HPT axes following exposure to xenobiotics from rats to humans or a commonly used small fish toxicology model (e.g., fathead minnow, medaka, zebrafish) to other vertebrates (i.e., within the same class or across classes). Three STAR awards were made in 2004:

Chemical induced Changes in Gene Expression Patterns Along the HPG-axis at Different Organizational Levels Using a Small Animal Model (Japanese medaka)

Objective: Researchers are developing a screening method to use molecular techniques such as *in situ* hybridization, *in situ* RT-PCR and immuno-histochemical staining to screen for effects of chemicals on the HPG axis with a special emphasis on steroidogenic pathways and hormonal control mechanisms along the HPG-axis in the Japanese medaka. The methods will allow for screening of multiple effects in multiple tissues, even at points in development when the tissues are too small to be accurately dissected for use in more traditional molecular techniques. The methods will be applied to a set of "model" and "test" compounds for a set of target genes. Once the methods have been developed and validated, they can be adapted for use with other genes and/or species of interest and used to efficiently and completely screen for endocrine disruptor effects beyond simple receptor binding.

Systems Biology Modeling of Fathead Minnow Response to Endocrine Disruptors

Objective: This study is developing a computational model to evaluate molecular and protein biomarkers in relation to reproductive dysfunction in fathead minnows exposed to environmental estrogens. The model is incorporating a number of biochemical endpoints along the entire HPG axis, direct evaluation of physiological changes and reproductive endpoints and the pharmacodynamics and kinetic distribution of the contaminants. The hypothesis being tested is that genomic and proteomics biomarkers will be diagnostic of the estrogenic effects of environmental estrogens and that they will provide a global understanding of mechanisms of action that will relate specifically to reproductive endpoints in fathead minnow that are adversely affected by exposure to estrogenic compounds. This research is being done through a cooperative agreement with ORD researchers (see research project **2.1.3**).

Impact and Outcomes:
This research is expected to result in the development of integrative, quantitative models of the function of the HPG or HPT axes with emphasis on normal physiological processes and mechanisms of perturbation following exposure to EDCs and improve the ability to extrapolate results across species.

Estrogen Elicited Gene Expression Network Elucidation in the Rat Uterus

Objective: Systems biology involves the iterative development of strategies that integrate disparate physiological and biochemical data into computational models that are capable of predicting the biology of a cell or organism. In order to facilitate hazard identification and risk assessment, a comprehensive quantitative understanding of the molecular, cellular, physiological, and toxicological effects that are elicited following acute and chronic exposure to synthetic and natural chemicals is required within the context of the whole organism. This research is developing a computational model that will identify and predict critical estrogenic endocrine disruptor elicited changes in gene expression which play a central role in the observed physiological/toxic effects based on systematic and quantitative data obtained from comparative *in silico*, genomic, molecular and histopathological approaches using a rat uterus model.

1.2.5 STAR: Environmental Bioinformatics Research Centers

Research Goals and Approaches: In 2004 an RFA was released to establish an Environmental Bioinformatics Research Center. Applicants were asked to focus their proposals in research that is consistent with the first and at least one other strategic objective of the computational toxicology program: 1) Improve understanding of the linkages in the continuum between the source of a chemical in the environment and adverse health and ecological outcomes; 2) Provide predictive models for screening and testing; and 3) Improve quantitative risk assessment. Two STAR awards were made in 2005. Both

of these projects are building bioinformatics capabilities and are working collaboratively under cooperative agreements with scientists from ORD and EPA program offices.

The Carolina Environmental Bioinformatics Research Center

Objective: The Carolina Environmental Bioinformatics Research Center is bringing together multiple investigators and disciplines, combining expertise in biostatistics, computational biology, chem-informatics and computer science to advance the field of Computational Toxicology. The Center is developing novel analytic and computational methods, creating efficient user-friendly tools to disseminate the methods to the wider community, and is applying the computational methods to data from molecular toxicology and other studies.

New Jersey Research Center for Environmental Bioinformatics and Computational Toxicology

Objective: The Research Center is bringing together a team of computational scientists, with diverse backgrounds in bioinformatics, cheminformatics and enviroinformatics, from the University of Medicine and Dentistry of New Jersey, Rutgers, and Princeton Universities, and the FDA's Center for Toxicoinformatics. This team is addressing, in a systematic and integrative manner, multiple elements of the toxicant Source-to-Outcome sequence (Investigational Area 1, as identified in the RFA) as well as developing cheminformatics tools for toxicant characterization (Investigational Area 2, Predictive Models for Hazard Identification). The computational tools will be extensively evaluated and refined through collaborative applications involving Center scientists as well as colleagues from the three universities and EPA; particular emphasis will be on methods that enhance current quantitative risk assessment practices and reduce uncertainties.

Impact and Outcomes: The research is expected to contribute to development and application of dose-response information analysis and enhance current quantitative risk assessment practices and reduce uncertainties. The computational methods developed will link to data from molecular toxicology and other studies in order to move risk assessment from a hypothesis-driven science toward a predictive science.

1.2.6 STAR: Applying Bioinformatics Data to In Silico Predictive Environmental and Biomedical Models and Simulations

Research Goals and Approaches: The goal of the research is to develop predictive environmental and biomedical computer-based simulations and models that address data gaps in environmental and human health risk assessment and will strengthen the ability of predictive scientific data to guide sound future scientific policy and decisions. An RFA is being released in Fall 2006 and 3 awards are expected to be made with research beginning in 2007. The RFA is being developed in concert with intramural research efforts and it is expected that some of the awards may be made as cooperative agreements in order to take advantage of the experience of the intramural investigators.

Through this RFA ORD will seek to fund research that will synthesize mathematical and computational models of biological systems that capture knowledge through the explicit representation of dynamic biochemical and biophysical processes and the application of these models to understand complex biological system functions in response to environmental exposures. Research grant applications driven by computational and mathematical principle, design, or validation will be given highest priority.

Impact and Outcomes: The envisaged long term end product will be the creation of simulations and models for predicting toxicity pathways and health impacts as a result to environmental exposures and their use in human health and environmental health risk management.

APG - Develop the scientific underpinnings related to the effects, exposures, and risk management of perfluorinated chemicals to inform Agency risk assessment/management decisions – FY 2013

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1.3.1 Perfluoroalkyl Acids—Developmental Toxicity Characterization

Research Goals and Approaches: Because PFOS and PFOA are the most predominant PFCs detected in humans as well as in the environment, adverse developmental outcomes from *in utero* and lactational exposure to these two chemicals will be examined in laboratory rodents. As PFCs are persistent in the environment and have long half-lives in humans and laboratory animals, the adverse effects of PFOS and PFOA will be tracked at various life stages, from perinatal periods to adolescence (puberty), and well into adulthood. One of the goals of this research project is to identify an alternative animal model that lacks the gender difference in chemical clearance that is seen in the rat with some of the PFCs (e.g., PFOA). The serum and tissue levels of PFCs will be determined in the appropriate animal model and these internal dose metrics will be used for dose response assessment. Once adverse effects are identified from the PFOS and PFOA exposure, ORD's investigation will be extended to include other PFCs (such as 8:2 Telomer Alcohol, C-6, C-9, and C-10) that are pertinent to the risk assessment effort at OPPTS.

Impact and Outcomes: This research addresses OPPTS's immediate need to characterize the hazards of these chemicals for human populations. Descriptive findings will help form the basis for complementary research projects that focus on MOAs for PFAA toxicity.

1.3.2 Perfluoroalkyl Acids—Pharmacokinetic Modeling

Research Goals and Approaches: The goals of this research are three-fold: 1) to provide a descriptive pharmacokinetic profile of PFOA in the mouse (as an alternative model for rat); 2) to discern whether the pharmacokinetic parameters of PFOS and PFOA in immature rodents are different from those of adults, thereby accounting for the unusually high levels of PFOS and PFOA noted in children; and 3) to evaluate the patterns of PFOS and PFOA accumulation in various tissue compartments in rodents, and to explore the bases for bio-persistence of these chemicals in the body. This research is being conducted collaboratively across multiple ORD organizations. Depending on additional needs from OPPTS, similar pharmacokinetic studies (particularly those in immature animals) can be extended to other PFAA such as Telomer alcohols, C-6, C-9, and C-10 compounds.

Impact and Outcomes: These studies will fill an important informational gap to provide a basis for the margin of exposure (MOE) paradigm of risk assessment of PFOA. Findings will also provide insights to the bio-persistence (long half-lives) of these chemicals in both animals and humans, as well as the unusually high levels of these chemicals detected in children. Studies will be conducted in concert with a comprehensive investigation coordinated by NTP, OPPTS and ORD to examine the pharmacokinetic profiles of 18 PFAA identified by OPPT.

1.3.3 Perfluoroalkyl Acids— Characterization of Immunotoxicity, Hepatotoxicity and Hormonal Imbalance

Research Goals and Approaches: ORD research aims to confirm the reported immunotoxic potential of PFOA in the mouse, establish NOAEL and LOAEL values, as well as correlate changes in immune function with serum PFOA concentrations. The investigation will be extended to include PFOS (and potentially other PFCs), and to the rat model. In addition, developmental immunotoxicity studies will determine the relative sensitivity of the developing and mature immune systems to PFAAs and persistence of effects following developmental or adult exposure.

A few histological features of hepatotoxicity have been described in adult rats after exposure to PFOS and PFOA. ORD research is designed to extend these findings to include biochemical evaluation of PFCs-induced hepatotoxicity, including the use of genomic and proteomic techniques to pinpoint to cellular insults in the liver, in addition to histological lesions. Hepatotoxic potentials of PFOS and PFOA will be evaluated in developing rodent models for comparison with the adult, to determine if the adverse effects are more sensitive during developmental stages, and if these effects are reversible later in life.

Based on preliminary findings from our laboratory, exposure to PFOS abruptly reduced thyroxine and triiodothyronine in circulation, without activating the classical HPT feedback pathway to produce an elevation of thyroid-stimulating hormone. ORD's research aims to explore the underlying mechanisms of these hormonal changes. More importantly, studies will be conducted to determine if these unconventional thyroid hormone changes are translated into physiological dysfunction, and if these changes are relevant to human health hazards. Additional work will investigate if thyroid hormone imbalance represents a hallmark response to PFAA exposure. Furthermore, limited data have suggested that PFOA might produce estrogenic effects in the rat. These findings will be confirmed and extended to elaborate the endocrine disruptive potentials of PFOA and PFOS (and other PFCs, if warranted).

Impact and Outcomes: This work will identify the hazard potentials of PFCs regarding immunotoxicity, hepatotoxicity and hormonal interruption and provide OPPT with needed data on additional endpoints for their risk assessment of these chemicals.

1.3.4 Perfluoroalkyl Acids—Characterization of MOA

Research Goals and Approaches: To evaluate the role of PPAR in PFAA toxicity, ORD will construct transfected cell lines with a reporter gene to screen for the specific PPAR activities. PFCs with a high PPAR affinity will be investigated for their ability to alter lipid homeostasis, fatty acid transport and metabolism, placental function, and hematopoiesis. Gene expression of the PPAR isoforms, co-factors and co-activators will be investigated in various embryonic, fetal, and placental tissues, and patterns of changes in gene expression will be correlated with cellular endpoints indicating alterations in growth. Positive results will guide the third phase of our approach, which will utilize a PPAR gene knock-out mouse model to confirm the PPAR-mediated effects. In addition, ORD will determine the specific involvement of the PPARα pathway in PFOA-induced developmental toxicity, hepatotoxicity and immunotoxicity by the use of PPARα-knocked out mouse model. If warranted, this transgenic mouse model can be extended to investigation of other PFCs. MOA studies will also address the role of the hypothalamic pituitary adrenal (HPA) axis in PFOA-induced immunotoxicity.

Induction of mammary gland tumors, Leydig cell tumors and ovarian hyperplasia by PFOA has been reported in the rat. To determine if these tumor incidences are extended to the mouse model, testis, ovary and mammary glands will be examined in mice exposed to PFOA during gestational and lactational periods. Circulating steroid hormone levels and P450 enzyme activity in these tissues will be monitored to ascertain whether tumor induction is mediated by disruption of steroid hormone metabolism.

Based on the previous observation from ORD laboratories, pulmonary insufficiency is a likely cause for the neonatal mortality associated with *in utero* exposure to PFOS or PFOA. In follow-up research, ORD will attempt to determine the critical window of susceptibility to PFOS-induced neonatal mortality and to assess the effects of PFOS on prenatal lung development. Lungs will be collected during the perinatal period and assessed for their degree of maturity by histological, morphometric, biochemical, and molecular techniques. ORD also will measure surfactant proteins in lung lavage fluid from neonates to assess release of surfactant into the terminal air sacs. Additionally, gene expression analysis of a variety of markers of lung maturation will be examined for clues into the possible MOA of PFOS.

Impact and Outcomes: Results will provide information regarding the affinity of PFAA compounds for PPAR isoforms, and comparisons of the affinities of the compounds in rodent versus human receptors. This information will be shared with our NTP collaborators for prioritizing the 18 PFCs chemicals in their investigation. The PPAR-KO studies will provide valuable insights into the MOA for developmental toxicity, neonatal growth and survival, immunotoxicity and hepatotoxicity. The work described here will also better define the target organs (e.g. developing lung) and the physiological functions (steroid hormone metabolism) responsible for the developmental toxicity of PFCs.

1.3.5 Perfluorinated Compounds Methods Development and Validation — Environmental and Biological Matrices

Research Goals and Approaches: A wide range of matrix specific collection and analytical methods need to be developed and validated to begin evaluating environmental distributions, potential human exposures, and corresponding risks of PFCs. This research is specifically designed to provide sampling and analytical methods that will be used to: characterize environmental distributions of the PFCs; help determine how humans are exposed to PFCs; and help evaluate the toxicity, metabolism, and systemic disposition of PFCs for the risk assessment process. Emphasis will be placed on developing and applying methods for water and soil, the two environmental matrices that may influence human and ecological exposures. Priority will also be placed on methods for biological media (e.g., serum, tissue homogenates) that will measure body burden, disposition, and/or metabolism in ecological and risk assessment studies. This research will help provide the methods needed to determine the most important exposure pathways and risks for the highest priority PFCs.

Impact and Outcomes: This research will meet OPPTS's need to develop and validate tools (methods and protocols) for characterizing the environmental distributions of the PFCs and to assess which pathways are important for global transport and human exposure.

1.3.6 Perfluoroalkyl Acids—Stability of Fluorotelomer-Based Polymers and Distribution of Perfluorinated Compounds in Soils

Research Goals and Approaches: The stability of the selected FBPPs will be investigated through a series of laboratory experiments in which FBPPs are exposed to selected natural and amended soils and sediment materials. Successful completion of this research will include quantitative analysis of perfluorinated acids and alcohols in complex soil matrices at lower detection limits than have been reported thus far. Achieving these detection limits entails analyses of these compounds in a wide variety of soil matrices collected from around the world to demonstrate the validity of the methods being developed.

Impact and Outcomes: The findings from this research will help OPPT develop a more accurate assessment of the potential risks posed by PFOA and similar compounds.

1.3.7 Determining the Fate of Fluorotelomer Alcohol-Based Polymer Products During Wastewater Treatment

Research Goals and Approaches: The specific goals of the research are to: 1) develop analytical and experimental methods to characterize fluorinated surfactants in various environmental matrices, 2) describe the composition of FTOH polymer formulations released to the environment via down-the-drain disposal, 3) determine the environmental loadings of FTOHs and PFCs from WW treatment, and 4) determine the potential for FTOH polymer products to transform or degrade during WW treatment.

Research on other aspects of FTOH polymer product fate are planned to address gaps in the risk management of these formulations in wastewater. Screening studies of effluents and biosolids will be completed to understand fluorinated surfactant loading to the environment. The fate of these fluorinated compounds during anaerobic WW treatment will also be explored using simulated anaerobic WW conditions. These studies, along with the OPPT focused work, will provide a more thorough understanding of fate of FTOH-based polymers during WW treatment.

Impact and Outcomes: This research addresses OPPTS's request and immediate need to characterize the environmental stability of FTOH polymer products. Descriptive findings will advise OPPTS in their negotiations with industry [Supplemental Environmental Projects (SEP) and Enforceable Consent Agreement (ECA)]. The research will also clarify if and how FTOH polymer products contribute to FTOHs and PFAAs observed in WW effluents and other environmental matrices.

1.3.8 Perfluoroalkyl Acids — Article Testing and Chamber Studies

Research Goals and Approaches: The main goals are to characterize the source, transport, and fate of PFCs in the indoor environment and the factors that may affect PFCs release from AOC. This study will be carried out in three phases: Phase 1: conduct AOC screening tests to determine how much PFC is available in new AOC, and which AOC are potentially the most important PFC sources in the indoor environment; Phase 2: conduct accelerated aging tests to determine whether AOC can release PFCs to the indoor environments and, if so, at what relative rates; Phase 3: conduct aging and migration tests under realistic use and exposure conditions to determine what role AOC may play in human exposure to PFCs, and the major exposure routes.

Impact and Outcomes: This research was initiated by ORD in response to OPPT's immediate need for better understanding the sources of PFCs to which the general population is exposed and the major exposure routes in the indoor environment. The findings will close a data gap in PFCs risk assessment and suggest potential risk management solutions.

APG - Develop the scientific underpinning related to the effects, exposures, and risk management of specific individual or classes of pesticides and toxic substances that are of high priority to the Agency to inform Agency risk assessment/management decisions – FY 2010

1.4.1 Control of Pesticides in Drinking Water

Research Goals and Approaches: This research is designed to produce tools (models, methods, protocols) to address OPP's drinking water treatment research needs. Specifically, OPP has requested that ORD: 1) develop and evaluate a protocol for selected pesticides identified by OPP, and 2) perform drinking water treatment studies for other selected pesticides wherein OPP must conduct risk assessments. For both the protocol and ORD's studies, treatment processes will include those commonly used by drinking water treatment plants and those less commonly used but known or anticipated to be effective. These processes include: coagulation and clarification, softening, adsorption onto powdered activated carbon (PAC) or granular activated carbon (GAC), and oxidation by chlorine, chloramine, chlorine dioxide, permanganate and ozone. Parent pesticides will be studied and, to the extent possible, transformed pesticides created by hydrolysis or oxidation will also be studied.

Originally evaluated using three different types of pesticides, the protocol will also be used as a basis for providing treatment data for two classes of pesticides of interest to OPP – carbamates and fungicide triazole degradates. Additional classes will be targeted in the future.

ORD also is conducting research to: 1) provide chemical-specific information on the effects of water treatment on pesticide transformation pathways, 2) provide physicochemical parameters for transformation products, and 3) develop predictive models for forecasting treatment effects that cross chemical class and treatment conditions. The treatment processes currently being investigated are lime softening (hydrolysis) and chemical disinfection (oxidation) as processes with transformation potential, and which have been shown to transform pesticides to either less or more toxic forms.

Impact and Outcomes: This research will: 1) allow OPP to provide an easy-to-use/implement protocol to pesticide manufacturers for their use in submitting data to OPP in a consistent manner. It will also provide data over a class of pesticide compounds that challenges the protocol's methods; 2) provide drinking water treatment data and its interpretation to OPP for inclusion in their risk assessment, as under FQPA the contribution of pesticides in drinking water must be considered in the overall risk assessment.; 3) provide OPP with a drinking water degradation model that can be used to predict transformation pathways across chemical class and treatment conditions; and 4) provide OW, OPP, and OPPT with treatment data on contaminants that may be used in their risk assessments. This is a demonstration of a multi-program opportunity for cooperation in research and implementation.

1.4.2 Reducing Risk Due to Contact with CCA-Treated Wood – Impact of Coatings on Dislodgeable Chromium, Copper, and Arsenic Residues

Research Goals and Approaches: The goals of this work are to characterize the impact of selected coating on dislodgeable CCA residues on the surfaces of CCA-treated wood and, through demonstration of the approach, provide industry with a methodology that can be used to further develop and demonstrate the performance of new and improved products. Twelve coating products available to consumers were applied to mini-decks constructed from decking materials reclaimed from in-service decks. Dislodgeable CCA residues were monitored on the surfaces of coated and uncoated control decks over a two-year period of outdoor weathering using a wipe methodology developed and demonstrated by the Consumer Product Safety Commission (CPSC). In concert with EPA's study, the staff of the CPSC conducted a similar two-year study by applying eight of the same products to mini-decks constructed from new CCA-treated wood. EPA and CPSC collaborated on development of the protocol.

Impact and Outcomes: This research was initiated by ORD in response to OPPT's and CPSC's need for coatings performance data. The findings will close a data gap in the CCA risk assessment and provide a demonstrated method that industry can use to further develop and demonstrate products.

1.4.3 Agricultural Health Study - Pesticide Exposure Study

Research Goals and Approaches: The noted limitations of previous agricultural studies are being addressed through the AHS, a prospective epidemiological study to quantify the cancer and non-cancer risks in the agricultural community and to study the relationship between agricultural exposures and disease. The study uses questionnaires to provide information regarding pesticide use, work practices, and other agricultural exposures, as well as information on other activities that may affect either exposure or risk for a large (more than 89,000) cohort of licensed agricultural pesticide applicators and their spouses in Iowa and North Carolina. Information derived from study questionnaires is used to develop exposure-classification procedures for subsequent investigation of associations between pesticide exposure and specific diseases. Exposure measurement data are being developed and analyzed to assess these questionnaire-based exposure-classification procedures. The AHS Pesticide Exposure Study, an exposure-measurement field study for a subset of agricultural pesticide applicators and participating family members in the larger AHS cohort, was led by EPA to provide information to assess and refine the exposure-classification procedures developed from the AHS questionnaire data and to better understand factors affecting exposures to 2,4-D and chlorpyrifos for agricultural pesticide applicators and their families.

Impact and Outcomes: The AHS Pesticide Exposure Study will improve exposure and health risk assessments made by NCI and NIEHS in the AHS epidemiological study. This will increase the value of epidemiological study results for OPP use in pesticide exposure and health assessments. Identifying and understanding key exposure factors can also guide development of improved exposure reduction strategies and guidance developed by OPP and other organizations. The results may provide information for pesticide safety educators on how pesticides can be handled more safely to reduce the exposures to pesticide handlers and their families.

1.4.4 Asbestos Exposure Research—Air, Soil and Bulk Material Scenarios

Research Goals and Approaches: ORD is conducting research to determine which sampling and analytical approaches best support asbestos analyses. To address the major gaps in exposure as identified by the EPA Asbestos Coordination Team, two studies were initiated. The first study reviewed the state of the science to identify analytical procedures and counting rules needed for asbestos detection and quantitation in bulk samples, air, settled dust, and soil for field monitoring and risk analysis in various exposure scenarios. Five areas are specifically addressed in this investigation: 1) Identifying analytical procedures and counting rules needed for asbestos detection, 2) Comparing the efficiency of 0.45 μm pore size filters versus 0.8 μm pore size filter media, 3) Rating the efficiency of polycarbonate versus mixed cellulose ester filters, 4) Recommendations for bench-level testing of filters to provide information on the performance of these filter media, and5) Testing on soil/asbestos mixtures. Soils will be of differing particle-size distributions and organic matter contents.

The second study addresses the uncertainty in measuring true asbestos fiber concentrations in soil and bulk materials and estimating possible exposures (e.g. including friability and releasability from these materials). In particular, research is needed to determine how to separate the asbestos from the soil while maintaining the integrity of the asbestos fibers (i.e., without fiber length reduction). An initial method for

non-destructive separation of asbestos was identified for testing from the peer reviewed literature. Spiked soil samples of known asbestos content and asbestos fiber distribution will be prepared and analyzed by this method. If the method fails to adequately and efficiently separate the asbestos from the soils tested, a modified method will be developed.

Impact and Outcomes: This research addresses OPPTS's immediate need to characterize the hazards of asbestos for human populations, especially susceptible populations (e.g. school children) and highly exposed subpopulations (e.g. Libby, Montana workers and families and users of nonstochastically distributed vermiculite products from the Libby mine, as well as areas with elevated concentrations of asbestos in natural rock formations). The results of the filter data analyses will be used to reduce current uncertainties resulting from the use of different filter media and/or filter pore size. A new method for sampling asbestos in soils will be developed and provided for use by regional asbestos risk managers. The findings will decrease uncertainties in sampling and analysis approaches used in asbestos exposure and risk assessments.

1.4.5 *Asbestos Releasability Research*

Research Goals and Approaches: The goals of this research are to: 1) develop a framework for modeling asbestos breathing zone concentrations generated by activities of varying intensity on outdoor and indoor surfaces and 2) obtain asbestos fiber releasability data from soil and carpet for calculation of emission factors. These data will be obtained from existing activity-based sampling (ABS) data, generation of ABS data, and from instrument tests; i.e., vertical elutriator and/or releasable asbestos field sampler (RAFS).

Impact and Outcomes: These data and model(s) will allow EPA Regional Offices and others to make rapid decisions about whether a soil or other bulk material is contaminated with asbestos.

1.4.6 Lead Paint Test Kits Research to Support the EPA "Lead; Renovation, Repair, and Painting Program; Proposed Rule"

Research Goals and Approaches: To meet the new rule requirements to reduce exposure to lead hazards created by renovation, repair, and painting activities that disturb lead-based paint, it is necessary that new efficient, cost effective technologies meeting the desired sensitivity within a specified range of false positive and false negatives rates be developed and made available commercially. This research will initially focus on the investigation of available state-of-the-art analytical technology for its potential to be modified to meet OPPT's needs for the Lead Renovation and Repair (Pb R&R) rule. The development of these tests will be guided by the parameters of cost effectiveness, short analysis time, and field use by OPPT's primary audience (remodeler/ building contractor complying with the ruling). The kit configuration should allow for a prompt commercialization effort. To meet the short timeframe (12 months), two concurrent tracks are being followed: Technical Outreach Support and In-house Laboratory Research.

An issue paper is being developed describing currently available and emerging Pb test kits, performance of test kits for Pb in paint (not limited to spot test kits), availability and types of reference/known Pb-based paint materials, and the performance evaluation protocol options. Cost factors of required materials and protocols are being addressed in the issue paper. A Workshop is being planned for Fall 2006. The workshop objectives include: to obtain additional information on the accuracy, precision and cost of Pb test kits to determine Pb in paint at the action level; to obtain additional information on the specifications,

availability, and costs of testing/reference materials to evaluate the performance of test kits for Pb in paint at the action level; to obtain additional information on the specifications and availability of protocols to evaluate the performance of test kits for Pb in paint at the action level; and to determine the cost to perform these protocols.

Concurrently, in the laboratory, modifications to adjust the sensitivity and performance requirements of the currently available test kits will be investigated. Four areas that impact the kit's sensitivity and reproducibility have been identified. These areas can be grouped into chemistry-based or technique/application–based. The four areas that will be experimentally investigated to determine if they can be optimized or adjusted, as appropriate, to help the performance of the paint test kit approach the specifications given in the Pb R&R rule are: 1) Paint sampling; 2) Pb extraction from paint; 3) Response to Pb; 4) Detection of Pb response. The objectives for each area are: 1) Reproducible paint sampling of a known area or weight; 2) Quantitative/reproducible extraction for all Pb-containing paint types; 3) and 4) Response to Pb and/or detection of the response to Pb adjusted to the action level and reproducible.

Impact and Outcomes: The research findings will be used to help develop a low cost lead paint test kit that can be rapidly commercialized and made available to renovators and remodelers following requirements of the EPA Pb R&R Rule. It is expected that the developed kit will be commercially available within the next three (3) years. The kit, as well as the proposed rule, supports the attainment of the Federal government's goal of eliminating childhood lead poisoning by 2010.

1.4.7 Chiral Pesticides

Research Goals and Approaches: The primary research goal is to determine the environmental occurrences, fate and effects of enantiomers of selected chiral pesticides, PCBs and other chiral pollutants with an emphasis on currently-used modern pesticides expected to have short to intermediate environmental half-lives. The approach to this research involves several steps: 1) development of analytical techniques to separate enantiomers; 2) analysis of environmental and human exposure samples expected to contain chiral pollutants to determine bioaccumulation and enantiomeric ratios; 3) measurement of enantiomer degradation in selected environmental matrices to determine selectivity and rates of enantiomer degradation; 4) preparative separation/collection of the enantiomers of important pesticides and other pollutants for effects studies; and 5) measurement of the bioaccumulation and effects of the separate enantiomers using various species and toxicity endpoints, ranging from conventional acute exposure endpoints (e.g., LC50), to biochemical indicators obtained through application of modern "omics" tools such as metabolomics and proteomics.

Impact and Outcomes: This research provides data for OPP to use to better inform risk managers on the certainty/uncertainty and need for additional information for pesticides that are either single, enriched or racemic compounds. The ultimate outcome of this research is to encourage the development, production, and use of single- or enriched-enantiomer pesticides as a green chemistry activity. Such pesticide products would relieve the environment of thousands of tons of unnecessary chemicals that may have adverse impacts on non-target species, including humans.

Long Term Goal 2:
OPPTS and/or other organizations use the results of ORD's research as the scientific foundation for probabilistic risk assessments to protect natural populations of birds, fish, other wildlife, and non-target plants

APG - Provide methods for extrapolating toxicological data across wildlife species, media, and individual-level response endpoints - FY 2013

2.1.1 Ecotoxicological Data and Toxicity Estimation Models

Research Goals and Approaches: Research will reduce uncertainties associated with the current use of ecotoxicity data and existing empirically based interspecies extrapolation models to better estimate the toxic effects of chemical exposures on wildlife and aquatic species by: 1) improving accessibility and usefulness of available toxicity information through the ECOTOX database, a comprehensive Web-based system maintained by ORD; and 2) refining empirical models that use available information to predict toxicity across species (i.e., ICE) and across endpoints (ACE).

Literature acquisition and encoding for the ECOTOX database will focus on OPP priorities (e.g., studies on pesticides undergoing reregistration meeting data quality requirements for use in final risk assessments). ECOTOX improvements will include: 1) providing chronic effects data for organic chemicals for use in the ICE/ACE models, 2) an analysis of toxic effects data available for pesticides that have undergone re-registration within OPP, 3) an analysis of toxic effects data for threatened and endangered species, and 4) a plotting feature for easy comparisons and ranking of hazard across species, organism life stages, and observed effect endpoints. Key improvements to the ICE and ACE toxicity estimation programs will include: 1) identification and expansion of toxicity databases, 2) incorporation of chemical classes and chemical MOA categories, 3) rigorous model validation, 4) improved tool functionality and user guidance, and 5) development of web-based versions of ICE and ACE. Additional research will evaluate sources of uncertainty in ICE and ACE outputs, including: 1) ACE uncertainty and protectiveness for chronic mortality versus other endpoints, such as growth and reproduction; 2) incorporation of MOA categories into ICE correlation models; and 3) approaches for reducing variability in toxicity predictions for wildlife and aquatic species.

Existing databases used for extrapolating underrepresented species, chemicals, endpoints, and lifestages will be expanded by including additional wildlife and aquatic species toxicity data. Both ICE and ACE will be validated by comparing model predictions to measured values. ICE acute toxicity estimates will be compared to measured LC50 values for multiple species, chemical classes, and MOA. ACE chronic mortality estimates will be compared to measured values for no effect and low effects on chronic survival, growth, and reproduction endpoints determined in available chronic toxicity tests. The results of the validation and software refinement will be incorporated into new user guidance to allow determination of the reliability of both ICE and ACE estimates.

Impact and Outcomes: ECOTOX is used by researchers in the development of high quality models needed to estimate population effects of toxic chemicals to wildlife species. The ECOTOX system: 1) facilitates LTG 2 efforts through quarterly releases of newly encoded and quality assured data to an openly accessible database with a robust, user interface for querying and outputting of data, 2) will be used to identify data gaps and trends in existing data sets to assist in furthering research efforts in both chemical and species extrapolation, 3) will facilitate the identification of structural analogs and associated

toxicity information to estimate potential hazard of untested chemicals or chemicals with limited toxic effects information. The ICE/ACE modeling approaches contribute to the ability to predict the effects of chemicals on aquatic and wildlife species, an essential component of the conceptual model for ecological risk assessment. Specifically, this approach facilitates the estimation of potential hazard for untested chemicals or chemicals with limited toxic effects information. Web-based versions of ICE and ACE will provide immediate action to updates and improvements, and allow greater user flexibility in searching and correlation analysis.

2.1.2 Mechanistically Based Approaches to Predict Differences in Species Sensitivity

Research Goals and Approaches: Research will further the development of mechanistically based models to extrapolate toxicity information among chemicals, species and lifestages by: 1) development and testing of a PBTK model for a fish species, and an improved experimental method to parameterize such models; and, 2) development and testing of an MOA model to predict inter-species differences from *in vitro* data.

Three interrelated efforts will advance the development and application of PBTK models for compounds that undergo metabolic biotransformation in aquatic species: 1) available information on *in vitro* fish metabolism will be synthesized and scaled up this for incorporation into PBTK models; 2) experimental work will be conducted to test the accuracy of model predictions based on *in vitro* data by making *in vivo* metabolism measurements for selected compounds; and 3) a new experimental system for HTP collection of metabolism information will be developed along with a mathematical model that translates this information into *in vivo* metabolic rate and affinity estimates.

To examine potential toxicodynamic differences among species, a pilot project will be initiated to determine the relative differences in insecticide activity at known sites of action in the nervous system. This will provide a test of concept that *in vitro* measurements of effects via a known MOA can predict species differences in toxicity. Initially, voltage-sensitive sodium channels will be selected as an endpoint, and the pyrethroids will be utilized as a class of compounds for comparisons. Both the insecticidal and acute toxicological effects of pyrethroids are mediated via voltage-sensitive sodium channels, and these channels have been cloned from insects, rodents and humans. These will serve as starting points for cross species comparisons by obtaining these clones, expressing them in *Xenopus* oocytes, and measuring pyrethroid effects on their function using electrophysiological techniques. The ability to predict toxicity will be determined by comparison of potency on ion channel function to LD/LC50 values among the different species.

Impact and Outcomes: This research will improve the accessibility for higher tiered risk assessment of mechanistically based methods, which provide a scientifically-sound basis for extrapolating toxic effects across species, and identifying sensitive species. Specifically, the PBTK modeling research addresses several questions that limit current efforts to extrapolate toxicity data among species, including 1) how well does in vitro data predict *in vivo* rates of metabolism; 2) how variable are metabolism rates within and among species, 3) under what circumstances does metabolism impact chemical bioaccumulation, and 4) what type of metabolism data has the most utility for incorporation into PBTK models. MOA research also may provide useful methodologies for predicting and identifying sensitive populations, and reduce the need to test all chemicals. In addition, these *in vitro* methods provide important alternatives to *in vivo* and whole animal methods. This research is integrated with ongoing research in the Human Health MYP as well as related to cross-species work (animal to human) that has been conducted under the air toxics research plan.

2.1.3 Extrapolation Methods for Under-Represented Taxa, Lifestages, Chemicals, and Endpoints

Research Goals and Approaches: The overall goal of this research is to utilize three small fish species, the Japanese medaka, zebrafish and fathead minnow, as a basis for the development of techniques for extrapolation of toxicological effects across endpoints, species and chemicals. To achieve this, a systems-based approach is being used to define toxicity pathways for model chemicals with well-defined MOA within the HPG axis. These pathways serve as a basis for understanding responses of the fish across biological levels of organization, ranging from molecular alterations to adverse effects in individuals to, ultimately, changes in population status. The studies employ a combination of state-of-the-art molecular biology, bioinformatic and modeling approaches, in conjunction with whole animal testing protocols.

One part of this project involves development of a harmonized medaka multigeneration exposure protocol that can be used to evaluate population-relevant endpoints (i.e., fecundity, fertility reproductive behavior, phenotypic and genotypic sex of each generation). This testing also will include a number of molecular and histological endpoints diagnostic of MOA and, hence, be useful for extrapolation across life-stages and taxa. An important component of this work will be to ascertain the degree to which short-term (partial life-cycle) assays are predictive of effects in full life-cycle tests.

The other major aspect of this project involves testing with zebrafish, a useful model from the standpoint genomic analyses, in conjunction with the fathead minnow, the small fish model most commonly used by the Agency for both laboratory testing and field monitoring. A subset of chemicals representative of different HPG MOA will be characterized extensively using a short-term reproduction assay with the fathead minnow. These results will provide input for population modeling and provide crucial information in terms of understanding the consequences of changes in gene and protein expression and metabolite profiles with respect to apical responses. Again, data will be collected at multiple biological levels of organization thereby supporting quantitative endpoint extrapolation. This phase of the research is being conducted through collaboration with an interdisciplinary network of EPA (NHEERL, Duluth; NERL, Cincinnati, Athens; NCCT, RTP), EPA grantee (University of Florida), and non-EPA partners (e.g., Joint Genome Institute of the Department of Energy; see second grant description under **1.2.4**).

Impact and Outcomes: This research will provide information concerning linkages across biological levels of organization from molecular to population responses. Further, the systems-based approach used to define toxicity pathways in these studies will serve as a basis for extrapolation across species, including taxa not directly amenable to testing. Ultimately, the approaches emanating from this work will support both diagnostic and predictive risk assessments.

APG - Provide methods for characterizing population-level risks of toxic chemicals to aquatic life and wildlife – FY 2015

2.2.1 Simple Screening Tools to Project Population Responses

Research Goals and Approaches: This effort will explicitly incorporate relatively simple screening matrix population models into the OPP risk assessment process. Population models that have been developed for OPP will be refined and integrated with the exposure and effects models currently used by OPP. Some of the factors for consideration include: 1) the software code, and how to make a seamless connection between ORD's population models and OPP's existing risk assessment models; 2) translating OPP effects endpoints into stressor response models that can be used in the population models; 3) data

quality for population model parameter estimates; 4) model interpretation, including decisions on which population model endpoints will are most appropriate for assessing risks; and 5) some level of training and continuing technical support.

Of particular emphasis will be the development of a methodology for evaluating the reliability of predictions made using population projection models that rely on published demographic parameters. ORD is using computer simulations that mimic the population-level risk assessment process at three important phases -1) data collection, 2) parameter estimation, and 3) population projection - to attempt to characterize the risk-assessment scenarios that result in the most serious errors and to provide a set of diagnostic criteria that may be used to identify when problems in the quality of population projections may be present.

Impact and Outcomes: This effort begins the important process of moving from conceptual models of addressing population response to actual implementation of population level analyses. Furthermore, this research advances our understanding of the usefulness of population models for accurately characterizing risks in ecological risk assessments, and determining if the quality of the population model is sufficient to answer the risk management question.

2.2.2 More Realistic Projections of Population Responses to Stressors

Research Goals and Approaches: This effort will provide methods to support probabilistic ecological risk assessments and will more explicitly address higher tier ecological risk assessment needs to take into account greater realism and complexity in projecting population responses to stressors. This will be done using a combination of theoretically and empirically based approaches and field-collected, laboratory-derived, and simulation-based information. Selected species will include those used frequently in toxicity testing, which historically have provided important information to regulatory process, including the registration of pesticides. Research will address four specific areas.

(1) Probabilistic models - Under this research effort, a variety of methods will be explored to incorporate stochasticity into population modeling. Data from laboratory and field studies will be used to explore useful representations of variation in population projections. Simulated data also will be used to test and compare modeling approaches. (*2) Density dependence, and (3) genetics* - The implications to population projections of the incorporation of compensatory population responses (i.e., density dependence and changes in population genetic structure) also will be explored using simulation and experimental approaches. For example, a number of data scenarios will be generated using stochastic simulation models that are parameterized to represent the range of scenarios encountered in risk assessment. Those density dependence formulations with the greatest flexibility and adaptability to assessment scenarios will be incorporated into EPA's existing suite of population modeling tools along with guidelines for their selection and use. Also, selected experimental and simulation models will be adapted and implemented to refine and test hypotheses associated with chemical risks to population persistence and genetic perturbations. Additional research will use natural populations and a quantitative genetic approach to identify genetic loci important to population persistence during chronic chemical stress. *(4) Spatial effects* - Focal species in ecological risk assessment, as well as their habitats and the stressors to which they are exposed, are often spatially heterogeneous and only partially overlapping. As a proof of the practical utility of models for assessing large-scale pesticide exposure and risks in coastal areas, spatial fish population models will be developed, evaluated and compared using an estuarine fish species. Published pesticide exposure data will be used initially in the development of demographic population models, which can then be expanded into spatial models using literature-based information on natural history, native habitat, distribution, range, and occurrence of the species within the Gulf of

Mexico. Secondary spatiotemporal data, such as EPA's Environmental Monitoring and Assessment Program (EMAP) data, will be incorporated into population-level models with coastal fish species. Resulting models will be used to project population-level effects in spatial terms and develop an approach that is intended to have broader applicability to other species, stressors, and coastal areas.

Impact and Outcomes: The application and interpretation of stochastic population modeling approaches provided through this research supports an important need by OPP for probabilistic, higher tier ecological risk assessments. This research will provide approaches and guidance on the need for and uses of more complex population modeling approaches as tools for integrating and projecting more realistic effects of stressors on wildlife populations.

APG - Provide approaches for evaluating the relative risks from chemical and nonchemical stressors on spatially structured wildlife populations across large areas or regions, and provide methods for characterizing population-level risks of toxic chemical to aquatic life and wildlife – FY 2009

2.3.1 Spatially Explicit Population Models for Avian Wildlife

Research Goals and Approaches: The research being conducted here will enhance the PATCH model (Program to Assist in Tracking Critical Habitat) and tie it specifically to pesticide issues. PATCH is a spatially explicit, individual-based life history simulator that incorporates GIS representations of real or hypothetical landscapes. The PATCH model will be used to simulate wildlife population responses to pesticide application within agricultural landscapes. Multiple landscape configurations, wildlife life histories, and stressor regimes will be explored.

Impact and Outcomes: The development of a spatially explicit wildlife population model provides a tool for scaling from individuals up to populations in a manner that addresses the complexities of real landscapes, and that evaluates the cumulative effects of pesticide use and other factors such as habitat alteration and environmental variability. The model and associated, datasets, documentation, and example analyses being developed are designed for use by OPP scientists and managers but are sufficiently generalizable to be applicable to the needs of other Program and Regional Offices.

2.3.2 Probabilistic Ecological Exposure Assessment

Research Goals and Approaches: ORD is designing and implementing a probabilistic exposure analysis system, undergirded by a suite of sophisticated process-based models of pesticide environmental chemistry and biology, for direct assistance to EPA's regulatory programs in their mandated pesticide risk assessments. Research activities extend from analysis of the reliability and uncertainty of predictive modeling tools, through production of user interfaces designed to aid regulatory personnel in their daily activities, to production of high-quality standardized databases with direct linkages to OPP modeling software and systematic technological and scientific modernization of terrestrial and aquatic fate and transport models. One important objective of this research is to provide OPP with improved tools for assessing offsite drift of pesticides, and expanding the capabilities of AgDRIFT and AGDISP to assess near-field pesticide drift from aerial applications by including source term algorithms for ground sprayers, orchard airblast sprayers, and revolatilization. These new source term models will be embedded in a transport framework to assess multiple applications and multiple sources for measuring exposures to a community or small watershed during a crop production season.

Impact and Outcomes: ORD is developing a scientifically sound approach to characterizing the exposure element of risk (directly to aquatic resources, and indirectly to humans through the fisheries) posed by pesticide contamination of aquatic, estuarine, and marine ecosystems, with credible, state-of-the-science models that include heretofore neglected areas such as benthic ecosystems, transport across the benthic boundary layer, and effects of sorption kinetics on ecosystem contamination. Research under this program generates state-of-the-art improvements in ORD products for use by OPP in implementation of probabilistic risk assessments.

APG - Provide scientific basis for assessments of direct and indirect risks to non-target plant species and plant communities from pesticide use - FY 2012

2.4.1 Effects of Herbicides on Non-Target Plants and Plant Communities

Research Goals and Approaches: Four inter-related efforts will be conducted. 1) Spatial information will be compiled in a GIS platform which will be made available, at least initially, to Agency staff through a Web page, and if successful and feasible, to the general public. Research will result in a data system appropriate for use in risk assessments of effects of pesticides on non-target plants and animals. Data will be obtained through collaborative efforts with Federal, State, and local agencies or purchased as necessary. That data will include (as feasible, but likely not be limited to) political boundaries (states and counties), human population census data, major geomorphic attributes, crop locations (both conventional and genetically engineered), resident plants (i.e., noncrop species), bird distributions (from Breeding Bird Survey data), pesticide use (location and type of registered pesticides), and wind speed. Additional layers can be added as requested by OPP or Regional Offices, and, if feasible, outputs will be made available for use in wildlife population models. 2) Research will be conducted to provide the scientific basis for phytotoxicity (Tier II) testing guidelines with the focus on terrestrial plant effects, with pilot research on aquatic plants, if possible. Candidate noncrop test species for plant tests will be based on spatial analysis, prior use in phytotoxicity testing, ecological significance, and cultural characteristics. These may include both annual and perennial and native plants. Species will be evaluated to improve traditional seedling tests and proposed life-cycle tests. 3) Ecological information initially will be obtained under controlled greenhouse conditions to determine the relative herbicide susceptibility of different native species based on exposure response studies with individual plants growing in pots. Large field-plot studies using these species will be conducted to provide a scientific basis for determining herbicide effects at the plant community level. Both constructed communities with planted species and *in situ* native plant communities would be studied. 4) ORD will evaluate whether gene arrays can be used as molecular indicators as to whether a plant has been affected by specific herbicides, what the possible effects of the herbicides may be based on the gene activity, and whether this molecular information can, in the future, be used to predict the potential susceptibility of different plant species to an herbicide (especially native plants and threatened and endangered plant species).

Impact and Outcomes: Results of these inter-related efforts will: 1) Improve models and databases to improve ecological risk assessments of chemical herbicides to plants, plant communities and possibly wildlife in a spatially explicit landscape. 2) Produce scientific information for comprehensive and efficient *in vivo* assays to evaluate adverse effects of chemical herbicides on individual plant species, especially reproductive effects. 3) Produce scientific basis to determine ecological risks of chemical herbicides on native plant communities, and how they may change over ecologically relevant time periods as a result of predicted effects of pesticides. 4) Produce comprehensive and efficient genomic and proteomic analysis to identify indicator genes and their metabolic products (fingerprints) in response to specific herbicides, and, as resources permit, other pesticides toxic chemicals and stressors. These

analyses can be used to as a scientific basis for chemical/molecular approaches to characterize risks to species beyond those initially tested, and for herbicides and other chemicals with similar MOA.

Long Term Goal 3:
OPPTS and/or other organizations use the results of ORD's biotechnology research as the scientific foundation for decisionmaking related to products of biotechnology

APG - Provide improved capability to assess the risks of allergenicity of genetically engineered crops - FY 2011

3.1.1 Develop Methods to Assess Potential Dietary Allergenicity of Novel Proteins in Genetically Engineered Food

Research Goals and Approaches:
ORD is addressing the need to develop methods to assess dietary allergenicity and improve understanding of the basis for human sensitization to dietary allergens through a combination of intramural and extramural research projects:

Project 1. Development of an animal model to assess the potential allergenicity of genetically engineered food (conducted intramurally)

- A dietary allergy model in a laboratory rodent is being developed using a modification of the respiratory allergy protocols. Suckling, weanling, and adult rodents (BALB/c or C3H/HeJ mice, or Brown Norway rats) are exposed by gavage or injection multiple times with various doses of a known food allergen to establish the ability to induce food-allergy responses. Allergic responsiveness is judged based on the induction of antigen specific IgE, IgG1 and possibly IgA, in addition to gut mucosal eosinophil influx and respiratory responses.
- Once the model is developed, rodents will be fed or gavaged multiple times with various doses of a prototype transgenic pesticide protein. Allergic responsiveness will be assessed based on results obtained from the above studies. Appropriate positive and negative controls will be incorporated into the model. The responses to the transgenic pesticide protein allergen in both a purified form and in a food matrix will be assessed. The relative potency of transgenic pesticide proteins, when compared with known food allergens will be assessed.
- The model will be: 1) validated in several laboratories, 2) used to assess effects of respiratory exposure following oral sensitization and oral exposure following respiratory sensitization, 3) used to assess the vulnerability of neonatal and weanling animals relative to adults, 4) used to assess the potential influence of toxic contaminants (e.g. aflatoxin) on the development of food allergy, 5) extended to assess the potential allergenicity of other proteins introduced into the food supply, such as fluorescent biomarkers, and 6) used to understand mechanisms and identify potential *in vitro* test strategies.

Project 2. Solicitations through the STAR extramural program to develop *in vitro, in vivo*, and *in silico* methods to assess the potential allergenicity of genetically engineered food

The goal of the extramural STAR research project is to engage the external research community in developing methods to assess dietary allergenicity and improve understanding of the basis for human sensitization to dietary allergenicity. One RFA has already been issued calling primarily for specific research on safety assessment including: 1) Development and evaluation of animal models for safety assessment; 2) Development of targeted or specific serological assays, and 3) Determination of structure-activity relationships of allergen proteins. A secondary area of interest is research on basic issues underlying sensitization to food allergens. The ability to accurately predict the risk of allergenicity posed

by the introduction of a novel protein into the food supply is currently impeded by a lack of understanding of the basic mechanisms underlying the development of food allergy and factors that lead to susceptibility. These basic issues are not limited to allergies developing from GE food, but have broader implications for the diagnosis, treatment, and prevention of, the sometimes life threatening allergic reactions to food. Four grants have been awarded with the first solicitation. Future solicitations will re-evaluate where the data gaps still exist related to this topic.

Impact and Outcomes:
The research will directly impact the ability of OPPTS to assess the risk to human health from novel pesticidal proteins that is required for regulatory activities related to agricultural biotechnology. The research will contribute to EPA's mission by helping to ensure the safety of the food supply. The principal output of this research will be the development of methods to assess potential allergenicity of novel pesticidal proteins in food. The projected outcome will be an improved ability to assess the potential risks to human health from genetically engineered foods in the diet and an overall improvement in the knowledge of food allergens. Development and evaluation of animal models suitable for assessing potential allergenicity relative to other food proteins is a high priority for EPA and other federal regulatory agencies. By developing an appropriate animal model, hypotheses regarding conditions (e.g., age, genetics) that contribute to susceptibility can be tested. Research on i*n vitro* screening methods will improve the ability to detect and predict the allergenicity of both known and novel proteins in the human diet. Development of a structure-activity database will reduce the reliance on animal testing and improve the predictability of allergenicity in humans.

APG - Provide improved science based risk assessment tools and data support that ensure improved capability for the comprehensive evaluation of ecological risks and long term safe use of genetically engineered crops with plant incorporated protectants (PIPs) - FY 2011

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3.2.1 Non-Target and Ecosystem Impacts From Genetically Modified Crops Containing PIPs

Research Goals and Approaches: The goal is to develop methodologies to measure direct impacts and secondary trophic level effects non-target organisms, and to characterize assessment endpoint(s) and the use of predictive strategies to evaluate potential ecosystem level effects. The risk of unintended and unexpected adverse impacts 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 impacts (or lack of impacts) 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.

ORD will develop standardized and streamlined methodologies to conduct base-line assessments of agricultural and near-field ecosystems non-target species diversity and abundance. In addition to broad field censuses bio-indicators 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. ORD will identify a suite of ecologically significant indicator species at different trophic levels in, for example, *Bt* corn and cotton agro-ecosystems.

Potential impacts of PIP crop plants will also be examined in terms of ecosystem functions, such as nutrient cycling, predator-prey interactions and the provision of non-target wildlife habitat. ORD will develop methodologies 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.

Impact and Outcomes: The research results will inform regulatory decision-making by OPP and will provide critical tools to Regional Offices involved in field test and post commercialization monitoring. The experimental data and critical evaluations will reduce uncertainty and strengthen risk assessments of PIP crops submitted to the EPA for registration under FIFRA. Data from this work has been used to support the drafting of proposed regulations for the Agency.

3.2.2 Field Assessment of IRM for PIPs

Research Goals and Approaches: The goal is to develop field methodologies to assess and monitor the impacts of the high-dose/structured refugia IRM strategy on the long-term susceptibility of target pests to *Bt* endotoxins. The development of target pest resistance to the *Bt* transgene[s] used as PIPs is a serious risk both to the sustainability of *Bt* crops and to the wider utility of environmentally 'soft' microbial *Bt* pesticides. Therefore, the EPA requires growers of *Bt* crops to follow the high-dose/structured refugia strategy to delay or prevent resistance development. 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.

The research 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 lepidopteron cotton and corn pests, and beetle pests. ORD proposes to address these ecological data gaps in a series of field and regional-scale studies.

Impact and 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 insect resistance monitoring (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. Results from spatial/temporal analyses of insect sensitivities to Bt toxin will be used by OPP staff in evaluating results from current monitoring efforts and future management planning.

APG - Provide guidelines and tools to mitigate gene-transfer and non target effects and the development of resistance in targeted pest populations to aid the management of environmental risks associated with PIP crops to help maintain the biological integrity of the environment while minimizing the use of chemical pesticides in agriculture – FY 2015

3.3.1 Ecological Effects of Gene Flow

Research Goals and Approaches: Although industry has not yet requested registration of a PIP in a plant that has relatives in the US with which it can hybridize, such requests are certain to be made in the very near future. As with any risk assessment, methods are needed that adequately address the potential for gene flow and introgression to occur (e.g., pollen dispersal distances; sexual compatibility) and the ecological fitness changes that might result (e.g., increased weediness; changes in reproductive vigor). Studies were conducted that demonstrated methods for monitoring pollen dispersal and gene exchange

between crops and co-located wild relatives. Other studies underway in the greenhouse and mesocosms are demonstrating the feasibility of developing effects response information on: 1) genes controlling plant reproduction; 2) yield, through standard backcrossing experiments; 3) plant community competition. Because of significant differences in plant types, two model crops are used: creeping bentgrass, which is a monocot, perennial, wind-pollinated, cool season turfgrass and canola, an annual dicot that is primarily pollinated by insects. ORD used commercially available GE genotypes of each of these crops engineered to be resistant to the herbicide glyphosate (Roundup®) through expression of the selectable marker *CP4EPSPS*. More recently, via academic collaborators, ORD has obtained GE canola resistant to insects and creeping bentgrass that has disease tolerance.

Impact and Outcomes: Research from this project has provided the Agency with useful tools for generally assessing nontarget risks from PIPs and has helped inform Agency decisions on the environmental safety of the products of agricultural biotechnology, particularly with regard to potential long term effects on plant communities. Studies on pollen dispersal and gene flow have changed the paradigm of understanding the distances and probabilities of gene transmission. Protocols for conducting plant microarrays will allow the Agency to critically review registrant submissions. Lessons learned have been applied to discussions of rule making for exemption of viral coat protein PIPs. The project also has contributed to the general scientific knowledge of potential ecological consequences of genetic engineering through publication of journal articles and presentations at national and international conferences.

3.3.2 Long-Term Responses of Targeted Pests and Non-Target Organisms to PIPs

Research Goals and Approaches: In order to help prevent development of resistance to B*t*-corn in WCR, ORD will be gathering population genetics data that will improve current models used to delay resistance. In addition, ORD is using genetic crosses and artificial selection to identify resistance genes in wild populations of WCR across North America. Molecular genetic approaches are used to identify candidate resistance genes that can then be further characterized at molecular and physiological levels. Resistance genes are used to develop PCR-based assays to monitor populations based on allele frequencies at these candidate genes. These approaches will be evaluated for their utility to improve current IRM plans and for their utility in risk assessment of future PIP varieties.

The long-term ecological outcomes associated with *Bt*-corn adoption remains a concern to EPA. To aid in the assessment of long-term ecological risk, a second research goal is to evaluate an approach to long term monitoring for potential consequences of GE crops for non-target organisms. Specifically, ORD is investigating the use of genetic data to 1) develop a molecular assay for detection of biologically relevant exposure to Cry3Bb protein in non-target organisms, based on change in gene expression; 2) estimate changes in numbers of effective breeders and gene flow in non-target populations; 3) provide a quantitative and objective measure of biodiversity. The initial approach is to focus on ground beetles and rove beetles and then beginning in FY09, to expand to other valued ecological endpoints, particularly beetle predators and competitors, in order to better assess broader ecological consequences.

Impact and Outcomes: ORD's research has direct relevance to OPP's policy and regulatory decision-making and addresses public uncertainty about ecological risks of GE crops. Resistance biology research provides a sound scientific basis to defend and improve EPA-mandated resistance management plans. Molecular methods ORD developed for genetic analysis of WCR are already being used by academia and USDA to monitor WCR populations and evaluate existing population models. By improving the biological realism of models used to decide how to delay/prevent insect resistance to *Bt*-corn, ORD's

work will improve the longevity of *Bt* -corn products and, hence, their environmental benefits. Methods to evaluate long-term, ecosystem-scale outcomes of Bt-corn will allow OPP to make informed decisions about the eventual consequences of greater GE crop adoption. ORD's non-target monitoring protocols are expected to be a key component of future ecological assessments designed to evaluate the long-term ecological costs and benefits associated with GE agriculture adoption.

3.3.3 Improvements to PIP Crop Monitoring through the use of Remote Sensing

Research Goals and Approaches: The project has three primary goals: 1) develop methods to identify PIP corn in the field; 2) identify and assess the severity of infestation to corn from insect pest populations. The project partners are exploring use of remotely sensed hyperspectral imagery to determine if these goals can be met, but will also assess whether less expensive, more accessible satellite imagery can be used by spatially and spectrally resampling existing hyperspectral imagery to simulate satellite imagery; and 3) take results from the first two goals and develop an IRM. The IRM program will use coarser resolution satellite imagery to evaluate regional corn growing areas for insect pest infestation potential and then use the greater resolution provided by hyperspectral imagery to investigate whether resistance is developing in specific PIP corn locations. If it appears probable infestations are occurring in PIP corn, field crews will be deployed to these locations to sample for PIP resistant insect pests.

Impact and Outcomes: Completion of this research will lead to a number of beneficial outcomes for the EPA, seed companies and growers, including the development of an improved management plan for Bt stewardship that minimizes resistance development and maximizes effectiveness of Bt toxins. Additionally, growers will receive feedback on effectiveness of Bt in their fields and information on insect pest damage to their crop if it develops.

3.3.4 Standardization of PIP Crop Resistance Assay Procedures

Research Goals and Approaches The goal of resistance monitoring is to aid researchers in evaluating resistance management strategies by determining baseline susceptibility levels to transgenic insecticides, detecting changes in the resistance allele frequencies, documenting changes in the level of resistance in the field, and documenting control failures due to resistant insects. Several methods for monitoring resistance to Bt proteins have been proposed for the European corn borer. Two of these methods have produced the primary data EPA has been using for regulatory decisions regarding Bt corn by discriminating concentration assays and the F_2 screen. Researchers and regulatory agencies must have sound confidence in interpretation of resistance monitoring results and understand limitations of the various methodologies to make economically and environmentally sound decisions. To effectively monitor frequency of resistance alleles in wild populations of insects, researchers must balance concerns of statistical precision at low allelic frequencies, costs of sampling, and the organization and labor required to intensively sample many individuals. The purpose of the research is to examine the strengths and limitations of the diagnostic dose and F_2 screen to develop better, standardized protocols for use across the entire Corn Belt.

Impact and Outcomes: ORD is working with USDA to extend the standardization effort to cotton insects. Recent field results have underscored the importance of standardized analytical methodology and care of field collected insects. Standardized protocols will assist the collection of monitoring data for PIP crops that can be compared across seasons. The information quality improvement undertaken by this research is designed to assist the Office of pesticide programs to meet the requirements of the Data Quality Act.

3.3.5 Evaluation and Testing Simulation Model for the Evaluation of Resistance Development in Pest Populations

Research Goals and Approaches: Methodology has been developed for combining analytical and simulation modeling techniques to take advantage of the best features of both methods of evaluating resistance management plans. By combining the two techniques, the simulation models can be used to generate input parameters for the simpler analytical models. Using this methodology, a wider range of resistance management strategies can be evaluated at realistic allele frequencies. Preliminary results suggest that there are several situations where resistance to transgenic crops may not evolve in the pest population.

Impact and Outcomes: Verification that an IRM model conforms to evolutionary theory using the procedures developed for this project should become a standard for future IRM models. Sampling from complex simulation models to generate the input parameters for use in the analytical models should significantly reduce the time required to evaluate resistance management plans. This methodology will identify the range of landscape conditions where evolution of resistance is unlikely and provide guidelines for the landscape conditions that pose the greatest risk of resistance evolution.

3.3.6 Systematic Development of an Agro-Ecosystem Monitoring Program to Assess Environmental Risks and Benefits of Pesticidal and Herbicide-Tolerant Transgenic Crops

Note: This project represents a cross laboratory cooperative effort that extends from the work done at the individual labs and center since FY2003. Because this is a transitional project, a detailed research plan will be developed in FY2007 and actual implementation of the research begun in FY2008.

Annual Performance Goal: To provide science based risk assessment tools and data support that ensure the comprehensive evaluation and long term safe use of genetically modified crops with plant incorporated protectants (PIPs).

Problem Statement: Despite widespread adoption of transgenic crops and clear indications of economic and environmental benefits from recent research, there are sufficient contrary results which, along with continuing public concern and the promise of new crop varieties, warrant new approaches to EPA's research. A systematic approach to monitoring PIP crop usage, conventional pesticide exposure, and associated ecological effects directly has been done only on a limited basis and without a view to development of broadly applicable schemes. Existing data are not sufficient to clearly establish the degree to which transgenic crop adoption, separate from other environmental and agricultural trends, has resulted in improved human health and decreased detrimental effects on non-target organisms at national or regional scales. The development of a cost-effective monitoring program that clearly links transgenic crop usage to conventional pesticide exposure and ecological effects will fill this data gap.

Research Theme: This project is a cross-laboratory effort to develop a cost-effective agro-ecosystem monitoring program designed to assess changes in pesticide exposure and effect accompanying transgenic crop adoptions. It should be applicable to a variety of crops, transgenic constructs, and spatial/ geographical orientations. ORD's initial research goal will be to develop indicators that can be selectively chosen to efficiently establish causality relationships between transgenic cropping systems, off-farm exposures and ecological responses. The 7-year objective will be to provide OPP and the industry with an ecological accountability tool. The monitoring program will be designed to address such questions as:

- To what degree does transgenic agriculture contribute to reductions in broad spectrum chemical pesticide applications within the Corn Belt?
- Is GE agriculture contributing to an overall reduction in risk to native biodiversity via reduced pesticide exposure or altered agricultural practices? Does the proportionate risk to different species change with shifts in these agricultural practices?
- Does GE agriculture adoption result in a decrease in human pesticide exposure for agricultural communities in the Corn Belt?

The research challenge for ORD is to develop a rigorous monitoring program that provides statistical power at a manageable cost. Development of the program will require input and analysis from a wide range of disciplines. Therefore, building upon the resident ORD experience, the first year will be dedicated to development and organization of a detailed plan by OPP and ORD personnel and then followed by outside peer review. Although the ultimate design is contingent on the outcomes of this detailed planning, ORD believes the basic framework for ecological and human monitoring will incorporate longitudinal studies at fixed monitoring sites to aid in causality analysis, combined with probabilistic sampling of additional sites for purposes of spatial extrapolation. Ideally, the ecological assessment will demonstrate whether expected changes in weed, lepidopteron and coleopteran communities propagate into effects on sensitive vertebrate communities, particularly birds and amphibians.

Because the design goal is to provide trends information on exposures and effects at minimal cost, successful development and application of powerful new indicators and approaches (e.g., hyperspectral imaging analysis, genomic technologies, and molecular population genetic analyses that estimate multigenerational responses and model long term population outcomes at regional scales) are needed. Partnering with other Federal agencies will also be pursued. A scoping meeting with appropriate EPA and outside experts will be scheduled in FY2007.

APPENDIX V
Key ORD Investigators

Research Theme	Key ORD Investigators
1.1.1	P. Schmieder, C. Russom
1.1.2	T. Collette, D. Ekman, J. Kenneke, C. Mazur, Q. Teng, T. Whitehead
1.1.3	W. Mundy, J. Welch, M. Hemmer
1.1.4	D. Mustra, NCER Project Officer; Grantees: G. Sayler, G. LeBlanc, G. Callard, S. Teh
1.2.1	P. Schmieder
1.2.2	K. Crofton
1.2.3	R. Smialowicz, R. Luebke, M Selgrade, S. Degitz
1.2.4	D. Mustra, Project Officer; Grantees: J. Giesy, N. Denslow, T. Zacharewski
1.2.5	D. Mustra, Project Officer; Grantees: F. Wright, W. Welsch
1.2.6	D. Mustra, Project Officer
1.3.1	C. Lau, J. Rogers, B. Abbott, S. Fenton, G. Klinefelter, A. Lindstrom, M. Strynar
1.3.2	C. Lau, A.Lindstrom. M. Strynar, H. Barton
1.3.3	R. Luebke, D. Wolf, C. Lau, M. Rosen, M. Gilbert, C. Gordon
1.3.4	J. Rogers, B. Abbott, S. Fenton, G. Klinefelter, M. Rosen, C. Lau
1.3.5	A. Lindstrom, M. Strynar, C. Lau , J. Washington, M. Mills, ORISE Fellows: Shoji Nakayama, XiBiao Ye
1.3.6	J. Washington, J. Ellington
1.3.7	B. Boulanger, C. Acheson, T. Holdsworth, M. Mills
1.3.8	X. Guo, M. Mason, K. Krebs, X. Liu
1.4.1	R. Miltner, S. Duirk
1.4.2	M. Mason
1.4.3	L. Sheldon, C. Croghan, P. Jones
1.4.4	D. Vallero, B. Schumacher
1.4.5	G. M. Shaul
1.4.6	S. Harper, J. Van Emon, K. Rogers
1.4.7	W. Garrison, E. Ulrich, M. Tapper
2.1.1	C. Russom, M. Barron, S. Raimondo
2.1.2	J. Nichols, T. Shafer
2.1.3	G. Ankley, R. Johnson, D. Bencic, J. Lazorchak, T. Collette, R. Conolly, Grantee: N. Denslow
2.2.1	J. Grear, G. Thursby, T. Gleason, R. Bennett, M. Etterson, S. Raimondo
2.2.2	J. Grear, D. Nacci, S. Raimondo, M. Barron
2.3.1	N. Schumaker, R. Bennett, M. Etterson
2.3.2	L. A. Burns, S. A. Bird
2.4.1	D. Olszyk, T. Pfleeger, E.H. Lee
3.1.1	M. Selgrade, C. Bowman, S. Laessig
3.2.1	B. Frederick

3.2.2	B. Frederick
3.3.1	L. S. Watrud , J. R. Reichman, E. H. Lee, C. Burdick, T. Shiroyama, B. M. Smith, R. Waschmann
3.3.2	U. Stolz, S. Franson, B.Daniel, M. Bagley
3.3.3	J. Glaser, M. Carroll
3.3.4	J. Glaser
3.3.5	J. Glaser
3.3.6	B. Frederick, M. Bagley, L. Watrud, A. Fairbrother, J. Glaser

APPENDIX VI
ACCOMPLISHMENTS

Long Term Goal 1

Safe Communities – Research program in LTG 1 prior to 2003.

- Sensitivity of the Young to Pesticides: ORD research developed data that: 1) identified pesticides to which the young are uniquely sensitive; and 2) directly influenced regulatory actions and risk assessment decisions for these pesticides (NHEERL).
    - OPP cancelled or reduced household and agricultural uses of selected cholinesterase-inhibiting pesticides to decrease potential for exposure in the young.
    - OPP issued a Data Call-In (DCI) for all registered organophosphates (~30) to collect data on comparative sensitivity of the young. ORD data were instrumental in developing the testing paradigm required by OPP of pesticide registrants for this evaluation. This DCI has provided information used by the Agency to evaluate the risk to infants and children
    - ORD research contributed to the development of guidance for selecting appropriate age groups for assessing childhood exposures to pesticides.
- Mixtures: ORD modeled organophosphorous pesticide (OP) mixtures in adult and developing rats in conjunction with OPP, as part of the OP Cumulative Assessment (NHEERL).
    - Results demonstrated greater-than-additive responses to a mixture of 5 OPs, with effects exacerbated in the young. The experimental design and statistical analysis used to determine the effects of mixtures has also been successfully applied to carbamates, pyrethroid insecticides, and thyroid disrupting chemicals.
- Research on chemicals that modify the regulation of luteinizing hormone: This work has demonstrated that a toxicant-induced alteration in luteinizing hormone secretion is a common mode of action underlying altered reproductive function and other critical reproductive outcomes (NHEERL).
    - ORD research has been used in the Cancer Risk Assessment of Atrazine and the Chlorotriazine Cumulative Risk Assessment. It also identifies the hypothalamic-pituitary-gonadal axis as an area of concern for OPPTS' endocrine disruptors screening and testing program, and eliminates some forms of cancer as outcomes of concern with respect to animal to human extrapolation.
- Models and data were critical for risk assessments and regulatory decisions on the developmental effects of specific pesticides (e.g., chlorpyrifos) http://www.epa.gov/oppsrrd1/REDs/chlorpyrifos_ired.pdf (NHEERL).
- ORD researchers have provided extensive expertise and review of numerous IRIS (Integrate Risk Information System) and OPPTS Risk Assessments (e.g., acrylamide, perchlorate, thiourea, perfluorooctane sulfonate, perfluorooctanoic acid, perchlorate, 2,3,7,8-Tetrachlorodibenzo-P-Dioxin (TCDD) And Related Compounds) http://www.epa.gov/iris/ (NHEERL).
- In collaboration with OPPTS, ORD developed harmonized guidelines (for cross-agency use) for immunotoxicity testing, developmental immunotoxicity testing, and the Risk Assessment Guidelines for Immunotoxicity (NHEERL).

- ORD researchers provided the expertise needed for the review of the Draft OECD 426 Developmental Neurotoxicity Guidelines (NHEERL).

Safe Pesticides/Safe Products – Research program in LTG 1 since 2003:

- Transition of staff to a new program focused on development of high-throughput *in vitro* and alternative species methods and peer review of program goals (NHEERL).
- Developed and supported the first Center for Alternatives to Animal Testing (CAAT) TestSmart Developmental Neurotoxicity (DNT) Workshop on alternative methods for developmental neurotoxicity testing http://caat.jhsph.edu/programs/workshops/testsmart/dnt/index.htm (NHEERL).
- Established an in house fish colony and published a medium throughput test method for exposure of fish embryos http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=16620995&query_hl=7&itool=pubmed_docsum (NHEERL).
- Developed *in vitro* assays and strategic chemical selection in a systematic approach for prioritization of large chemical inventories within a defined toxicity pathway (NHEERL).
- Delivered test version of software for rapid and efficient depiction of structure searchable metabolism pathways and associated bioassay data (NHEERL).
- Provided extensive technical support and a wide range of research activities in support of the OPPT perfluorooctanoic acid (PFOA) Risk Assessment and Enforceable Consent Agreement (ECA) activities (http://www.epa.gov/oppt/pfoa/). Extensive research on the toxicity and pharmacokinetics of PFOA and perfluorooctyl sulfonate (PFOS) was critical for these activities and is still under way. Sampling and analytical methods have been developed and validated for characterizing PFOA and other PFAAs in numerous environmental and biological matrices. Protocols have been developed and are being used to evaluate the potential degradation of selected fluoropolymer-based polymers in soil and sediments. The biodegradability of the same polymer products will be evaluated using both long term high microbial population exposures (OPPTS SCAS testing) and simulated aerobic wastewater treatment conditions (OECD Simulations testing). Test protocols for both thermal generation of PFOA by fluoropolymers and accelerated aged article testing have been developed which have been adopted by the fluoropolymer industry (in principle). Quantitative method(s) to analyze PFAAs using LC Q-TOF MS which will be used to assess PFAA concentrations for all experimental conditions and matrices were developed. ORD's research is producing state-of-the-science results that inform OPPT regarding the potential risks associated with environmental exposures to PFAAs as they continue to negotiate with industry through the ECA process.. (NHEERL, NRMRL, NERL).
- Completed a 24-month study assessing the effectiveness of deck sealants to reduce exposure to arsenic in CCA-treated wood. This research is part of a multi-year effort that supported OPP's risk assessment and risk management activities on chromated copper arsenate (CCA). First year preliminary results were released in early May 2005. A report has been completed and is expected to be finalized in the Fall 2006 (NRMRL).
- Developed and/or evaluated test protocols for detecting pesticides regulated by the Safe Drinking Water Act (SDWA) or for pesticides on the SWDA's Contaminant Candidate List (CCL) under consideration for regulation. A model has been developed, and validated

through laboratory experiments, for characterizing the fate of OP pesticides and their degradation products as they travel from natural source waters through conventional drinking water treatment plants (protocol website). Data generated from the protocols was used in OPP's draft cumulative risk assessment document on n-methyl carbamates in 2005 (NRMRL and NERL).

- Collaborating with NCI, NIEHS, and NIOSH on the Agricultural Health Study (AHS), a prospective epidemiological study to quantify the cancer and non-cancer risks in the agricultural community and to study the relationships between agricultural exposures and disease. ORD conducted the AHS Pesticide Exposure Study (AHS PES) at a small subset of the AHS farms in Iowa and North Carolina (~120 out of ~89000) to assess farm applicator exposures to 2,4-D or chlorpyrifos during a single agricultural pesticide application. The study results are being used by NCI, NIEHS, NIOSH, and EPA to assess/refine exposure classification procedures for the AHS epidemiological study and to better understand the key factors influencing agricultural pesticide exposures to farm applicators and their families. The AHS PES results have been provided to NCI and NIEHS for updating the AHS algorithms and future questionnaires. EPA is reviewing the study results to address questions regarding the 2,4-D reregistration eligibility decision (NERL).
- Established an ORD NMR-Metabolomics Research Center with supporting data bases, for identifying toxicity pathways and prioritizing testing. This center is supporting various research programs being implemented within and outside ORD including the conazoles assessment, PFOA and other PFAAs (LTG 1), and the small fish study (LTG 2). Delivered test version of software for rapid and efficient depiction of structure searchable metabolism pathways and associated bioassay data. (NERL, NHEERL).
- Completed research in support of the Agency's Asbestos Research Plan (http://www.epa.gov/opptintr/asbestos/ and http://www.epa.gov/asbestos/pubs/vaiframework.pdf.). Exposure research was initiated to evaluate filter collection efficiencies for airborne asbestos for various filter media and filter pore size. A method for characterizing asbestos in soils has been developed and provided to the regional asbestos site coordinators. The Releasable Asbestos Field Sampler (RAFS) has been developed to obtain asbestos fiber releasability data from soil for calculation of emission factors. The RAFS unit was first used in cooperation with EPA Regions 9 and 10 where Regional scientists were on site conducting activity tests while ORD worked alongside them collecting data with the RAFS unit. Preliminary data, study results and draft final report are expected by December 31, 2006**.** Developed and published dose-response relationships using a fiber dose database compiled from past ORD characterizations of exposures associated with toxicity studies conducted by ORD and other research groups. This supports the on going IRIS reassessment of cancer risks associated with asbestos,. (NERL, NRMRL, NNHEERL)
- Demonstrated that several chiral pesticides and other pollutants are enantioselectively transformed by microbes in various environmental matrices, with some that selectively accumulate in fish being toxic. A definitive feature article, "Probing the Enantioselectivity of Chiral Pesticides" (*Environmental Science and Technology,* Jan 06) summarizes the research and highlights the importance of considering chirality in product registration (NERL).

- Issued three Requests for Applications for proposals from non-profit institutions in the area of Computational Toxicology. Awarded a total of six grants and three co-operative agreements, two of which established research centers on environmental bioinformatics (NCER).

Long Term Goal 2

- Made available ECOTOX Release 4.0 August 2006. The ECOTOX database provides single chemical toxicity information for aquatic and terrestrial life. Enhancements include expanded taxonomic and chemical searching, and output directly to MS Excel format http://cfpub.epa.gov/ecotox/ (NHEERL).
- Expanded ICE AND ACE datasets with the fathead minnow database of acute and chronic toxicity of industrial organic chemicals, and wildlife toxicity. Beta version of web-ICE developed for wildlife http://bagel.epa.csc.com/ice/index.htm (NHEERL).
- Developed a conceptual systems model as a basis for predicting effects of hypothalamic-pituitary-gonadal (HPG)-active toxicants with differing mechanisms of action (MOA) in small fish species http://www.epa.gov/med/ (NHEERL).
- Established a consortium of ORD and STAR-awarded scientists to conduct integrated studies linking genomic responses in small fish to outcomes at individual and population levels http://www.epa.gov/med/ (NHEERL, NERL).
- Developed preliminary guidance on the development, application, and interpretation of population models for ecological risk assessment of pesticides http://www.epa.gov/aed/ (NHEERL).
- Developed PATCH, a spatially explicit, individual-based, life history simulator designed to project populations of territorial terrestrial vertebrate species through time http://www.epa.gov/wed/pages/models/patch/patchmain.htm (NHEERL).
- Developed databases, a framework for spatial analysis, and test protocols to determine effects of herbicides on non-target crop and native plant species for terrestrial plant risk assessments http://www.epa.gov/wed/pages/projects/PesticideResearchFlyer.pdf
- Developed and provided to OPP a series of ecological exposure models (PRZM/EXAMS/AgDrift) and accompanying databases in a probabilistic analysis framework (Express) for use in generating data required by FQPA (http://www.epa.gov/oppefed1/models/water/index.htm) and for the registration and/or reregistration of pesticides (http://www.epa.gov/ceampubl/swater/express/index.htm) (NERL).

Long Term Goal 3

- Developed methods for monitoring gene dispersal via pollen or seeds and probability of establishment of viable genetically modified (GM) plants or hybrids in natural areas http://www.epa.gov/wed (NHEERL).
- Developed methods for genomic response (e.g., plant microarrays) and changes in fitness characteristics of plants with GM material as inputs for ecological risk assessments http://www.epa.gov/wed (NHEERL).

- Conducted in-depth review of scientific literature on genetic methods for long-term ecosystem monitoring, implemented a pilot genetic monitoring program for ground beetle communities, and coordinated a joint ORD/OPP workshop to explore ways to incorporate these methods into agroecosystem assessment and monitoring (NERL).
- Delivered a report on the genetic architecture of western corn rootworm, a target pest for new varieties of Bt-corn. Successfully selected for Bt resistant populations, assessed gene flow pattern across the USA, described the molecular structure of a cadherin-like protein that is a candidate resistance gene, and developed a PCR assay to precisely monitor and detect localized changes in the frequency of alleles at this gene (NERL).
- Issued one Request for Applications for proposals from non-profit institutions in the area of developing models for allergenicity in support of the biotechnology research program. Awarded a total of four grants (NCER).

APPENDIX VII
ACRONYMS

ACC	American Chemistry Council
ACE	Acute to Chronic Estimations
AGRA	Agricultural Biotechnology Risk Analysis
AHS	Agricultural Health Study
ALSase	Acetolactate Synthase
AOC	Articles of Commerce
APG	Annual Performance Goal
APM	Annual Performance Measure
ARS	Agriculture Research Service
ASTER	Assessment Tools for the Evaluation of Risk
BMD	Bench Mark Dose
BOSC	Board of Scientific Counselors
BRWG	Biotechnology Research Working Group
Bt	*Bacillus thuringiensis*
CCA	Chromated Copper Arsenate
CEBRC	Carolina Environmental Bioinformatics Research Center
CENR	Committee on Environment and Natural Resources
CLA	CropLife America
CPRI	Crop Protection Research Institute
Comp Tox	Computational Toxicology
CPSC	Consumer Product Safety Commission
CTRP	Computational Toxicology Research Program
DNT	Developmental Neurotoxicity
DOD	Department of Defense
DQA	Data Quality Act
DW	Drinking Water
EAT	Estrogen, Androgen, and Thyroid
ECA	Enforceable Consent Agreement
EDCs	Endocrine Disrupting Chemicals
EDRP	Endocrine Disruptors Research Program
EFED	Ecological Fate and Effects Division
EPA	Environmental Protection Agency
EPRP	Ecological Protection Research Program
ERAs	Ecological Risk Assessments
EU	European Union
FBPP	Fluorotelomer-Based Polymer Products
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FQPA	Food Quality Protection Act
FTE	Full-Time Employment Equivalent

FTOH	Fluorotelomer Alcohol
FY	Fiscal Year
GE	Genetically Engineered
GIS	Geographical Information System
HED	Health Effects Division
HHRP	Human Health Research Program
HHRARP	Human Health Risk Assessment Research Program
HPA	Hypothalamic-Pituitary-Adrenal
HPG	Hypothalamic-Pituitary-Gonadal
HPV	High Production Volume
HPT	Hypothalamic-Pituitary-Thyroid
HTP	High-Throughput
ICCVAM	Interagency Coordinating Committee on Validation of Alternative Methods
ICE	Interspecies Correlation Estimations
ID	Identify
IgE, IgG1, IgA	Immunoglobulins
IOAA	Immediate Office of the Assistant Administrator
IPCS	International Programme on Chemical Substances
IRM	Insect Resistance-Monitoring
KO	Knock Out
LC	Lethal Concentration
LD	Lethal Dose
LOAEL	Lowest Observed Adverse Effect Level
LRI	Long-Range Research Initiative
LTG	Long-Term Goal
LRAT	Long Range Atmospheric Transport
MOA	Mechanism/Mode of Action
MOE	Margin of Exposure
MYP	Multi-Year Plan
NCEA	National Center for Environmental Assessment
NCER	National Center for Environmental Research
NCI	National Cancer Institute
NCT	National Center for Toxicogenomics
NERL	National Exposure Research Laboratory
NHEERL	National Health and Environmental Effects Research Laboratory
NIEHS	National Institute of Environmental Health Sciences
NJRCEBCT	New Jersey Research Center for Environmental Bioinformatics and Computational Toxicology
NMR	Nuclear Magnetic Resonance
NOAEL	No Observed Adverse Effect Level
NPD	National Program Director
NRC	National Research Council
NRMRL	National Risk Management Research Laboratory

NTP	National Toxicology Program
OAR	Office of Air and Radiation
OECD	Organization for Economic Cooperation and Development
OIA	Office of International Activities
OMB	Office of Management and Budget
OPP	Office of Pesticides Programs
OPPT	Office of Pollution Prevention and Toxics
OPPTS	Office of Prevention, Pesticides and Toxic Substances
ORD	Office of Research and Development
ORMA	Office of Resources Management and Administration
OSCP	Office of Science Coordination and Policy
OSP	Office of Science Policy
OSTP	Office of Science and Technology Policy
OW	Office of Water
PART	Program Assessment Rating Tool
PATCH	Program to Assist in Tracking Critical Habitat
Pb	Lead
PBTK	Physiologically Based Toxicokinetic
PCBs	Polychlorinated Biphenyls
PCR	Polymerase Chain Reaction
PFAA	Perfluoroalkyl Acids
PFCs	Perfluorinated Chemicals
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctyl sulfonate
PIPs	Plant Incorporated Protectants
PMN	Pre-Manufacture Notice
PPA	Pollution Prevention Act
PPAR	Peroxisome Proliferator Activated Receptor
PTRP	Pesticides and Toxics Research Program
QSAR	Quantitative Structure Activity Relationship
R&D	Research and Development
RFA	Request for Applications
RP	Research Program
R&R	Renovation & repair
SBIR	Small Business Innovation Research
SEP	Supplemental Environmental Projects
SP2	Safe Pesticides/Safe Products
SRP	Sustainability Research Program
STAR	Science to Achieve Results
S&T	Screening and Testing
TH	Thyroid Hormone
TSCA	Toxic Substances Control Act
USDA	US Department of Agriculture
USGS	US Geological Survey

WCR	Western Corn Rootworm
WHO	World Health Organization
WRS	Wildlife Research Strategy
WW	Wastewater