MULTI-YEAR PLAN FOR

ENDOCRINE DISRUPTORS
(FY2007-2013)

OFFICE OF RESEARCH AND DEVELOPMENT
US ENVIRONMENTAL PROTECTION AGENCY

DRAFT
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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 Aliving documents.@ ORD updates MYPs on a periodic basis to reflect the current state of the science, resource availability, and Agency priorities. This updated MYP took into consideration the recommendations made by the Endocrine Disruptors Subcommittee of ORD's Board of Scientific Counselors (BOSC) that reviewed the research program in December 2004. It will be reviewed through a mid-cycle assessment by the Subcommittee in September 2007. Any additional recommendations will be considered and incorporated into the version that will be reviewed for approval for finalization by ORD's Science Council later in 2007.

Endocrine Disruptors Research Program
Contributors to the Multi-Year Plan

Research Planning Team:
ORD Members:
Alva Daniels (NRMRL)
Elaine Francis (IOAA)
Ross Highsmith (NERL)
Susan Laessig (NCER)
Elizabeth Lonoff (ORMA)
Michael Loughran (IOAA)
Sue Makris (NCEA)
Jacqueline McQueen (OSP)
Marc Mills (NRMRL)
Greg Toth (NERL)
Doug Wolf (NHEERL)

Program and Regional Office Members:
Octavia Conerly (OST/OW)
Cathy Fehrenbacher (OPPT/OPPTS)
(REGION 10)
Bill Lovely (Region 1)
Kathleen Raffaele (OPP/OPPTS)
Phil Sayre (OPPT/OPPTS)
Jennifer Seed (OPPT/OPPTS)
Ingrid Sunzenauer (OPP/OPPTS)
Gary Timm (OSCP/OPPTS)
Les Touart (OSCP/OPPTS)

Key ORD Investigators:
Appendix V

Science Council Reviewers:
TBD

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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 Endocrine Disruptors describes the research program that is specifically designed to address the Agency's science needs. The purpose of the Endocrine Disruptors Research Program (EDRP) is to provide the Agency with the scientific information it needs to reduce or prevent unreasonable risks to humans and wildlife from exposures to individual pesticides and toxic chemicals and environmental mixtures of chemicals that interfere with the function of the endocrine system. It has been suggested that humans and domestic and wildlife species have suffered adverse health consequences resulting from exposure to chemicals in the environment that interact with the endocrine system. However, considerable uncertainty exists regarding the relationship(s) between adverse health outcomes and exposure to environmental contaminants. Collectively, chemicals with the potential to interfere with the function of endocrine systems are called endocrine disruptors (ED) or endocrine disrupting chemicals (EDCs). For the purposes of this document and the research that falls within the EDRP, the Agency is using the World Health Organization's definition of a potential endocrine disrupting chemical which is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function (IPCS/WHO 2002).

The EDRP is focused on: 1) reducing uncertainty regarding the effects, exposure, assessment, and management of EDCs, 2) determining the extent of the adverse impact of EDCs on humans, wildlife, and the environment; and 3) supporting the Agency's screening and testing program to identify endocrine active chemicals.

To date, adverse effects from EDCs have primarily been identified in wildlife species with relatively high exposures to specific compounds, including organochlorines such as DDT and its metabolites, PCBs and dioxins, or in domestic animals foraging on plants with high levels of phytoestrogens (Kavlock et al., 1996). Effects noted in wildlife that have a documented or presumed relationship to altered endocrine function include imposex in molluscs exposed to the alkyltins, vitellogenin induction in fish downstream from wastewater treatment plants (linked to concentrations of ethynyl estradiol, the primary estrogen in birth control pills), changes in sex steroids in fish downstream from pulp and paper mills, abnormal reproductive development in alligators in Lake Apopka following a pesticide spill, nearly complete mortality of Lake Ontario lake trout in the sac-fry stage presumably resulting from exposure to dioxin-like compounds, eggshell thinning in birds from exposure to DDT and its metabolites, birth defects in Lake Michigan cormorants exposed to poplychlorinated biphenyls (PCBs) and other Ah-receptor ligands, and masculinization of female fish found near run-offs from certain concentrated animal feeding operations (CAFOs). Also, a variety of adverse effects on reproductive development have been observed in laboratory rodents exposed to very low levels of dioxin.

There are many chemicals that have been tested in laboratory animals at higher levels than found in the environment that have been shown to cause a variety of adverse endocrine-mediated effects, depending on the dose administered, the frequency of exposure, and the life-stage of exposures. Some examples include phthalates, polybrominated diphenyl ethers, atrazine, vinclozolin, certain perfluorinated alkyls, and bisphenol A.

In humans, the consequences of prenatal exposure to diethyl stilbesterol (DES) on the reproductive tract of both females and males are well known and developmental neurological problems have been identified in children exposed to PCBs and/or PCDFs. In addition, reports of declines in the quality and quantity of sperm production in humans over the last four decades, and increases in certain cancers that may have an endocrine-related basis (breast, prostate, testicular) have led to speculation about environmental etiologies. There have been recent reports suggesting that some suspected endocrine disruptors may impact the timing of the onset of puberty and the anogenital distance in infants.

Despite the identified potential hazard, we know little about specific toxicity pathways that lead to the identified effects nor the factors influencing environmental exposures and the environmental concentrations of EDCs that would be required to induce effects at the population level. Nevertheless, it is known that the normal functions of all organ systems are regulated by endocrine factors and small disturbances in endocrine function, especially during certain stages of the life cycle such as development, pregnancy and lactation, can lead to profound and lasting effects.

Given EPA's overall mandate to protect both public-health and the environment, it is in a unique position to provide leadership in this area. Individual scientists across EPA's laboratories and centers have performed research related to endocrine disruptors for several decades. In response to increased public health concerns in the early to mid-nineties, the Office of Research and Development integrated and expanded its ongoing efforts into a consolidated endocrine disruptors research program. To initiate the program, two workshops were held in 1995 where the opinions of international experts were sought to help formulate a national research plan for endocrine disrupting chemicals (Kavlock et al., 1996; Ankley et al., 1997).

Research on endocrine disruptors was identified as one of the six high-priority topics in the ORD Strategic Plan (USEPA, 1996; USEPA, 1997, USEPA, 2000). This was based upon recognition of: *1) the potential scope of the problem, 2) the possibility of serious effects on the health of populations, 3) the persistence of some endocrine-disrupting agents in the environment, and 4) the widespread global concern about the fate and transport over national borders.* The EPA Strategic Plan (2006) values the EDRP as one of the "means and strategies for reducing risks from chemicals and pesticides" (www.epa.gov/ocfo/plan/2006/entire_report.pdf).

ORD's *Research Plan for Endocrine Disruptors* (www.epa.gov/ORD/WebPubs/final/revendocrine.pdf), published in 1998, was developed from the recommendations provided by the USEPA-sponsored workshops, the scientific judgment of the ORD Research Planning Committee, and reviews and input from the chairpersons for the risk assessment breakout groups of the Raleigh workshop, internal peer reviewers from across the Agency, the ORD Science Council, and an external peer review panel convened by the Agency's Risk Assessment Forum. The framework for ORD's EDCs research program is designed around the risk assessment/risk management paradigm. The objectives of the EDCs research program are to improve our knowledge and understanding of endocrine disruptors in the environment so that we can improve our methods of assessment and risk management. This, in

turn, will assist the Agency in: 1) identifying the chemicals and other environmental stressors that pose an unreasonable risk, 2) developing ways to prevent or reduce their release into and impact on the environment, and 3) developing means to remediate in-place EDCs that pose an unreasonable risk. Further, the research plan specifically addresses scientific questions that have arisen as a result of legislation enacted in 1996. The Food Quality Protection Act (FQPA) and the Safe Drinking Water Act Amendments (SDWAA) mandate the development of a screening and testing program to evaluate the potential of chemicals found in drinking water sources and food, respectively, to have estrogenic or other hormonal activity. Thus, EPA's research program strikes a balance between "core" and "problem-driven" research. In this way it 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 and that core research improves the basic underlying science. The EDRP includes areas that are uniquely of importance to EPA in helping the Agency meet its legislative mandates and includes research areas that serve to improve the basic understanding of EDCs, in general. Furthermore, as noted in Section II and VII the EDRP is coordinated with the "core" research in the complementary programs.

ORD's EDCs 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 EDRP is providing the Agency with the tools it needs to make decisions regarding chemicals that interact with the endocrine system.

The EDRP is one of a few ORD research programs that include diverse multi-disciplinary efforts in the areas of human health and wildlife 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., characterization of exposures, concentrated animal feeding operations), 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 EDCs, to "conduct research that contributes to the overall health of people, communities, and ecosystems."

The current ED MYP arrays ORD's research program for the period 2007-2013 and revises and updates the previous MYP (US EPA, 2003). The ED MYP provides a focused research framework and direction that reflects available ORD scientific capabilities and capacity. The research described in this MYP assumes annual resources of approximately 40.7 FTEs (full time equivalents) and $ 10.1 million, including payroll, travel and operating expenses throughout the life of the plan.

Decisions regarding the conduct of specific research efforts under the EDRP are based on ORD's strategic and annual planning processes. This is a dynamic process which depends on continuous input and regular reassessment of prioritization of research by ORD and Program and Regional Office members of research planning teams as well as other Agency (e.g., OPPTS, OW, ORD) senior managers, risk assessors, and stakeholders. ORD has partnered most closely with the Office of Science Coordination and Policy (OSCP) in OPPTS on the development and implementation of the EDRP, since that Office is responsible for overseeing the development and implementation of the Endocrine Disruptors Screening Program (EDSP), to ensure close alignment across the research and programmatic elements. The methods, models, and data developed through the EDRP are externally peer-reviewed and widely disseminated. The EDRP undergoes periodic external review by the Endocrine Disruptors Subcommittee of ORD's Board of Scientific Counselors (see Section VIII for additional details).

It should be noted that throughout the document ORD describes research that it is conducting either on EDCs, in general, or on specific chemicals or classes of chemicals. It should not be construed that just because ORD is studying specific chemicals or classes of chemicals that this means that the Agency has determined that these chemicals or classes are officially designated as "endocrine disruptors." Those determinations will be made by the Agency through the implementation of the EDSP and the traditional risk assessment processes followed by the relevant regulatory offices, the Regional Offices, and/or the National Center for Environmental Assessment.

Furthermore, it is important to point out that there are many hormonal systems in humans, laboratory animals, and wildlife. The emphasis of ORD's current EDRP is on mechanisms related to impacts on estrogen, androgen, and thyroid systems. However, in addition, there are activities on other mechanisms such as through steroidogenesis, aromatase, the hypothalamic-pituitary-gonadal and hypothalamic-pituitary-thyroid axes, and ecdysoids.

II. BACKGROUND

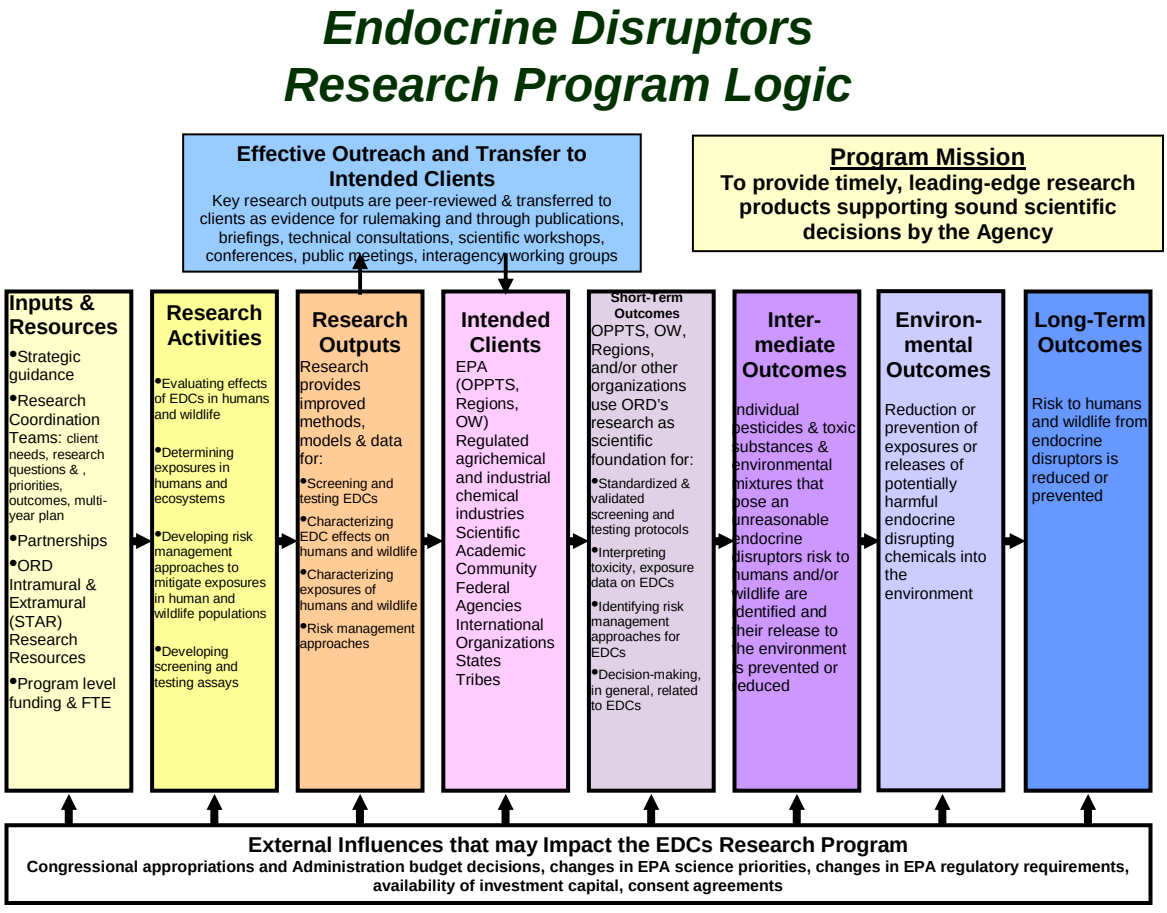
Agency's priorities and regulatory programs

The authorities and responsibilities of EPA are mandated primarily by thirteen major environmental statutes (CRS Report to Congress, 1995). These statutes direct EPA to perform a wide variety of activities with the goal of protecting human health and the environment. The authorities to evaluate the potential risks and, where appropriate, regulate chemicals, including endocrine disruptors, are amongst these mandated activities. In order to meet the needs of its mandates, EPA needs the tools to be able to: 1) identify EDCs, 2) evaluate their potential effects on human health, wildlife, and the environment, 3) characterize potential environmental exposures to EDCs, 4) discern when additional data are needed, 5) develop the appropriate protocols, should additional data be required, 6) set allowable levels of exposure or releases to the environment that are protective of human health and the environment, 7) develop technological controls to prevent/reduce releases, and 8) remediate the risks associated with in-place EDCs. The *Research Plan* and this MYP set forth the research that is needed by program and regional offices to carry out their legislative requirements.

The scope of the EDRP has been developed largely in partnership with OPPTS and to a lesser extent with the Agency's Regional Offices and Office of Water (OW). 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). OW is responsible for carrying out the mandates of other laws, including the Safe Drinking Water Act, Clean Water Act, and Drinking Water Act. Both OPPTS and OW play leading roles in regulatory risk assessment in EPA and, consequently, a number of their research needs are similar to those of other EPA Offices. Both Offices have other problem-driven research programs conducted through ORD that also support their needs – e.g., for OPPTS there is the Safe Pesticides/Safe Products Research Program (www.epa.gov/osp/myp/sp2.pdf). and for OW there are the Drinking Water (www.epa.gov/osp/myp/dw.pdf), and Water Quality Research Programs (www.epa.gov/osp/myp/wq.pdf). In addition, research conducted under ORD's core human health (www.epa.gov/osp/myp/eHH%20MYP%20Final.pdf), human health risk assessment (www.epa.gov/osp/myp/HHRA.pdf), and ecological (www.epa.gov/osp/myp/eco.pdf) research programs are providing many of the scientific methods and models needed by OPPTS, OW, and the Regions. (See Section VII for more details on relationship of EDCs research with those in other ORD programs).

Working with Program Office and Regional Office clients, ORD identified three overarching science needs for the EDRP to address. 1) Provide a better understanding of the science underlying the effects, exposure, assessment, and management of endocrine disruptors; 2) Determine the extent of the impact of endocrine disruptors on humans, wildlife and the environment; and 3) Support the Agency's screening and testing program. The three identified overarching long-term science needs have been structured as the long-term research goals for the EDRP. See Section V for a detailed description of the SP2 Long Term Goals (LTGs).

Figure 1 provides a conceptual framework for the EDRP, which links the resources to the outputs and programmatic outcomes to reduce or prevent risks to humans and wildlife from EDCs. The principal client for the EDRP is the Agency, in general, with OPPTS, OW, and the Regions the organizations with the greatest scientific needs for this research. 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, as scientific foundation for: 1) standardized and validated screening and testing protocols; 2) interpreting toxicity and exposure data on EDCs; 3) characterizing real-world EDC exposures and understanding the factors influencing these exposures; 4) identifying risk management approaches for EDCs, and 5) general decisionmaking related to EDCs. Progress is measured by the extent to which methods, models and/or data from the EDRP are actually used in peer-reviewed risk assessments and other decisionmaking. The use of EDCs research products by OPPTS, OW, and others will contribute to decisionmaking related to reduction or prevention of exposures or releases of potentially harmful EDCs into the environment.



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

Research outside of EPA

The broad nature of the EDCs issue necessitates a coordinated effort on both the national and international level. In November 1995, the Committee on Environment and Natural Resources (CENR), under the President's National Science and Technology Council (NSTC), identified endocrine disruptors as an initiative. The CENR established a working group on endocrine disruptors that was chaired by ORD/EPA with vice chairs from the Department of the Interior's US Fish and Wildlife (DOI) and the National Institute of Environmental Health Sciences (NIEHS). In addition to the three aforementioned agencies, participants include: the National Oceanic and Atmospheric Administration (NOAA), the National Science Foundation (NSF), the Food and Drug Administration (FDA), the Centers for Disease Control and Prevention (CDC), the Agency for Toxic Substances and Disease Registry (ATSDR), the National Cancer Institute (NCI), the Smithsonian Institution, the Department of Energy (DOE), the Department of Agriculture (USDA), the Department of Defense (DOD), and the Office of Science and Technology Policy (OSTP). In 2004, after a short hiatus, the interagency working group (IWG) was reconstituted, still chaired by EPA and with many of the same member Agencies with the exception of NCI and DOE and with the addition of the US Geological Survey (USGS), the Office of Management and Budget (OMB).

The earlier IWG on ED: 1) Developed a framework for federal research related to the human health and ecological effects of EDCs. The document reviews the state of the science and major uncertainties related to endocrine disrupting chemicals and established a framework for research areas that need attention. This framework categorizes major research needs into three groups; methods development, model development, and laboratory and field data acquisition. 2) Developed an internet-accessible searchable data base of on-going federally funded research on EDCs (htpp://www.epa/gov/endocrine). 3) Overlaid the framework with the inventory to identify high priority research gaps in the federal portfolio (Reiter et al., 1998). The inventory and the analysis point out what research is ongoing across the federal government that relates to the various LTGs and APGs identified in this Multi-Year Plan. The current IWG held a workshop on 'Endocrine Disruption in the Environment' in February 2007 to determine the progress the Federal agencies have made in addressing the research needs identified in the 1996 framework document, provide an overview of current research and monitoring activities, and identify collaborations on research and monitoring.

A separate IWG on Pharmaceuticals in the Environment (PiE) was convened in 2004 also under the auspices of the CENR. At first its charter took into consideration personal care products. However, it has since evolved to examining the body of knowledge for both human and veterinary pharmaceuticals, including antibiotics and the implications for antibiotic resistance. The IWG is developing research strategies for human and veterinary pharmaceuticals in the environment (December 2007) and for antibiotic resistance (December 2008). Those strategies will help identify, prioritize, and address the scientific issues associated with the potential ecological and human health risks associated with exposure to these classes of pharmaceuticals

in the environment. The IWG is co-chaired by EPA, USGS, and FDA and includes USDA, NOAA, CDC, NIEHS, OSTP and OMB. A workshop was held in August 2005 that brought together the Federal agencies, states, water utilities and industries, and academic researchers to share information, identify scientific gaps, and build collaborations. The chairs of the IWGs on ED and PiE collaborate with one another on their activities.

The aforementioned activities, along with the recognition that the key uncertainties regarding endocrine disruptors are complex, that interests across the agencies are overlapping, and that federal resources are limited, have helped ensure that there is close cooperation and collaboration on endocrine disruptors research across the federal government.

Some endocrine disrupting chemicals have been shown to persist in the environment stimulating global concern about their fate and transport over international boundaries. While evidence for a global concern is growing, the breadth of the current scientific uncertainties related to what effects are actually attributable to environmental exposures, what chemicals are responsible for the effects, and what risk management steps need to be taken to protect public health and the environment necessitate international cooperation and communication. To begin to address this issue from a global perspective, several key collaborative efforts have been undertaken. The US federal inventory of research was updated and expanded in 1998 to include research projects from Europe, Canada, and Japan and, thus, establish a Global Endocrine Disruptors Research Inventory (GEDRI) that included almost 800 projects. The inventory provided a searchable database as to what research is ongoing across continents that relates to the various LTGs and APGs identified in this Multi-Year Plan. In addition, the US chaired a steering committee under the auspices of the International Programme on Chemical Safety/World Health Organization/Organization for Economic Cooperation and Development that developed a "Global State-of-the-Science Review," (WHO, 2002). Both the inventory and the international assessment were a result of recommendations made at the 1997 G-8 Environmental Ministers' Meeting.

The European Commission has been providing funding research on EDCs since their Fourth Framework Programme (1994-1998); they are now on their Seventh Framework Programme (2007-2013) (http://ec.europa.eu/environment/endocrine/documents/studies_en.htm). They have provided over 56 million euros to support studies on the impact of EDCs on a variety of projects including: studying their impact on the aquatic environment; establishing the Cluster of Research into Endocrine Disruption in Europe (CREDO) which encompasses 63 laboratories working on four projects; and setting up a large network of excellence, CASCADE, which is focused on research, risk assessment, education, and information on chemicals, including EDCs, as contaminants, in the food chain. ORD and the EC have co-organized a number of international workshops. On the other side of the world, Japan has also had a keen interest in supporting research on EDCs. The National Institute on Environmental Studies under the Ministry of the Environment has built a separate laboratory devoted to research on EDCs. ORD has been collaborating with the Japanese Ministry of the Environment and the two agencies have also co-organized a number of workshops.

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, the OECD has the lead on the validation of several of the assays under consideration for implementation under the EDSP (i.e., Hershberger, uterotrophic, avian life cycle). Furthermore, ORD has the lead within the OECD in overseeing the validation of the Hershberger assay. 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 EDCs research program as well. ORD's participation in these efforts demonstrates the international recognition accorded to its scientists.

In addition to ongoing research across the US federal government and those of other countries, the chemical industry is also engaged in research in this area. 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. Under the auspices of an Endocrine Issues Science Forum, ACC supports an annual compilation of industry-sponsored endocrine research projects (www.endocrinescience.com/background.cfm). A number of these research efforts have been incorporated into GEDRI. They have ongoing research activities in many areas that complement EPA's intra- and extramural programs, e.g., studies on testing and testing methodology, mechanisms of action, epidemiology, animal toxicology, wildlife studies, aquatic toxicology, environmental exposure, and environmental chemistry. The American Water Works Association Research Foundation (AWWARF; http://www.awwarf.org), the Water Environment Research Foundation (WERF; http://www.werf.org), and the Global Water Research Coalition (GWRC; http://www.globalwaterresearchcoalition.net) are consortia of member organizations from the water utilities and industries. All three organizations issue solicitations to support research on issues of key interest to their industries. For example, they are supporting research efforts related to endocrine disruptors and/or other emerging contaminant, including pharmaceuticals and personal care products (PPCPs). AWWARF sponsors research to enable water utilities, public health agencies and other professionals to provide safe and affordable drinking water to consumers. They are in the process of developing a research strategy for endocrine disruptors and have invited ORD to participate in this effort. WERF supports research to enhance management of water resources, including in the areas of wastewater treatment and reuse, solids treatment, and watershed management. The GWRC consists of twelve research-supporting organizations from around the world that have formed an alliance to promote international

cooperation and collaboration in water-related research. AWWARF, WERF and ORD are among those organizations. Under the auspices of GWRC, we are all participating in a round-robin study to assess the ability of a number of screening assays (including one developed by ORD) to detect estrogenicity of effluents collected from wastewater treatment plants from their member countries from around the world.

Research conducted in EPA

The goals of the EDRP are to provide the Agency with the science and tools it needs to carry out its regulatory mandates. None of the other US or international ED research programs have similar goals, in terms of scope and mission. EPA's ED research is a multi-disciplinary approach to research that crosses all aspects of the risk assessment/risk management paradigm for both humans and wildlife. EPA is unrivaled in its ability to organize an extensive portfolio of ongoing research to provide methods, models, and data for reducing scientific uncertainty regarding EDCs. EPA's EDRP includes many areas that are of unique importance in helping the Agency meet its legislative mandates, such as developing screening and testing assays for OPPTS to assess the potential of chemicals to interact with the endocrine system, while other research areas are improving the basic scientific understanding regarding these agents that OPPTS and other parts of the Agency need to evaluate data submissions; and that EPA, other federal agencies, states, and other governments can use to conduct risk assessments and make informed management decisions. Furthermore, when resources are available, ORD's intramural program is complemented by an extramural program implemented through the Science to Achieve Results (STAR) program. The STAR program enables EPA to increase capability by providing grant funding in support of project areas that complement, and in some cases leverage, the expertise within ORD. 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 efforts are not duplicative, and expand research capability and capacity through collaboration.

Focus of EPA's contribution

In ORD's *Research Plan*, those areas where EPA could have the greatest impact and importance to the Agency were selected, taking into consideration the research activities of other agencies and organizations. Priorities for both the *Research Plan* and the MYP were assigned based upon an assessment of the importance of the research to the EPA program and regional 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 and, finally, the technical feasibility of conducting a successful project.

EPA's program strikes a balance between "problem-driven" and "core" research. It includes areas that are uniquely of importance to EPA in helping the Agency meet its legislative mandates and includes research areas that serve to improve the basic understanding of EDCs, in general, that is complementary to research programs conducted at other federal agencies, in other countries, or by industry. ORD has significant expertise in the areas of toxicology, endocrine

effects, behavioral sciences, and environmental exposures, relating to both humans and ecological systems and in providing solutions to solving environmental problems. ORD scientists are respected members of the scientific community and leaders in the field. Therefore, ORD can make a significant contribution in the areas of improving our understanding of endocrine disruptors, their impact on human health and the environment and the management of the risks they pose.

IV. PROGRESS TO DATE/CHANGES FROM PREVIOUS VERSION

Progress to Date

Major accomplishments of the EDRP are described in Appendix VI. Accomplishments have been aligned by Long Term Goals which are defined in Section V. Where available, a website where more detailed information can be accessed, is provided.

Changes from Previous Version

The ED MYP has undergone some changes since 2003 (USEPA, 2003), resulting from the following: 1) advances in the science that have taken place through ORD and other organizations and have consequently impacted the directions of the research, 2) addressing recommendations from external reviews of the EDRP, such as by the EDC Subcommittee of the BOSC, 3) improved collaborations with OW to better understand and ensure their needs are being addressed where possible, and 4) budget decreases to the budget (approximately $5 million) eliminating the extramural STAR program and reducing the intramural risk management research. A list of significant changes to the ED MYP is as follows:

- There are numerous changes to Annual Performance Goals (APGs) and milestones or Annual Performance Measures (APMs). The changes are largely due to the fact that a significant number of these goals and measures have been met and, therefore, have been deleted from this MYP. A few other goals and measures have been deleted because budget decreases forced the elimination of elements of the program. New goals and measures have been added that capture the current and future directions of the program and the timing for completion of some previous ones has been extended. Extensions in APG/APM timing are due to one of several reasons: reductions in resources (e.g., it will take us longer to complete an effort because there are fewer resources), the client offices' requesting additional research, or earlier investigations stimulating the need for additional research. This MYP strives to present the research in a more aggregated fashion than in the past and, therefore, overall there are fewer APGs and APMs. This is consistent with the guidance on MYP-development and from ORD's budget office.
- The number and content of the Appendices has been enhanced.
    - In response to one of the recommendations of the EDCs Subcommittee of the BOSC, the final MYP will attempt to describe its accomplishments in a more integrated and coherent fashion by aligning the accomplishment by APG. (This effort was is still under development and, therefore, the Appendix is not attached to this draft.) A separate document on Accomplishments has been developed which provides much greater detail on summarizing the research results from the

last decade conducted within our intramural laboratories and through the STAR grants extramural program.
    - To provide readers who may be interested in more detail on the research themes, an additional Appendix that identifies the goals and approaches, intended outcomes, and links to other research is included.
    - An Appendix that identifies the key ORD researchers for each of the themes has been added.
    - The previous version was cumulative (that is, included APGs and APMs from the first version of the MYP covering the years 2000-2012) whereas this version does not include past years' efforts and only covers the years 2007-2013.
- This version provides improved examples of where the EDRP is cross-linked to other ORD research programs in the descriptions of the research themes and in a new section in the body of the MYP.
- The MYP provides better descriptions of interagency collaborations and research supported/promoted by other countries and international organizations, and through industry-supported organizations.
- In the previous version we included the description of an integrated case-study that would be conducted using the expertise from all of ORD's Laboratories and Centers in the Appendix on what research would be conducted with potential additional resources. This case-study has been incorporated into the portfolio of research described in this version of the MYP.
- The titles of the Long Term Goals have been reworded to make them more "outcome-oriented" as a result of the PART review and recommendations by OMB. This has not impacted the overall intent of the research under each of the Long Term Goals.

V. LONG-TERM GOALS

The *Research Plan* is the road map that identifies the research that is needed to improve our understanding of endocrine disruptors. It should be referred to in order to understand the overall research program that was envisioned in 1998 and that has served to guide the EDRP's strategic directions ever since. This version of the MYP, as has been true for all of the previous ones, identifies the elements of the *Research Plan* that ORD will be working on in an integrated fashion, across branches, divisions, Laboratories, and Centers, over the next five to eight years.

Science questions from research plan

The *Research Plan* identified a number of key areas of uncertainty, in the form of questions that needed to be addressed. For the first version of the MYP in 1999, the Multi-Year Planning Committee considered the nine questions from the *Research Plan* as still valid and critical and augmented the list with an additional question to address risk assessment methodologies. The current Multi-Year Planning Committee still considers addressing these key science issues as critical to give the Agency program and regional offices the tools they need to meet mandates as related to EDCs. The key questions are not in any order of priority, but rather follow the order identified in the *Research Plan*.

- What effects are occurring in exposed human and wildlife populations?
- What are the chemical classes of interest and their potencies?
- What are the dose-response characteristics in the low-dose region?
- Do our testing guidelines adequately evaluate potential endocrine-mediated effects?
- What extrapolation tools are needed?
- What are the effects of exposure to multiple EDCs and will a TEF (toxicity equivalent factor) approach be applicable?
- How and to what degree are human and wildlife populations exposed to EDCs?
- What are the major sources and environmental fates of EDCs?
- How can unreasonable risks be managed?
- What approaches are needed to assess risks to humans and wildlife?

The Multi-Year Planning Committee used these questions in conjunction with the three previously identified overarching needs as a basis to derive three Long-Term Goals (LTGs). ORD's EDRP will lead to:

Long Term Goal 1: Reduction in uncertainty regarding the effects, exposure, assessment, and management of endocrine disruptors so that EPA has a sound scientific foundation for environmental decision-making.

Synopsis of research: Previously, ORD's research determined classes of chemicals that act as endocrine disruptors and their potencies. Having characterized modes of action, research is focused on the shape of the dose-response curve for specific modes of action and the development of approaches for assessing cumulative risk and extrapolating results across species. ORD is finalizing the next generation of assays to be used by the Agency's EDSP. To accomplish these goals and consistent with recommendations made by the Subcommittee of the BOSC, ORD is incorporating the new technologies broadly described as "genomics" or '-omics.' ***Ultimate Outcomes: OPPTS and other Program Offices, Regions, and outside EPA organizations are using this information to evaluate manufacturers' data submitted to the Agency through EDSP and/or from other sources, and developing integrated risk assessments on EDCs.***

Synopsis of research: Previously, ORD's research developed and evaluated through laboratory and small scale pilot field studies, molecular indicators of exposure and analytical methods for detecting certain EDCs in environmental samples. ORD is now focusing on applying its efforts to identify the key factors that influence human exposures to EDCs and major sources of EDCs entering the environment, such as from wastewater treatment plants (WWTPs), concentrated animal feeding operations (CAFOs), and drinking water treatment plants. ORD is also developing tools for risk reduction and mitigation strategies. ***Ultimate outcomes: These tools and data are applied in field studies by EPA and/or others to determine routes and pathways of exposure, the levels of exposure to EDCs in environmental media and the extent to which and efficacy with which they could be reduced or eliminated (e.g., LTG 2).***

Long Term Goal 2: Determination of the extent of the impact of endocrine disruptors on humans, wildlife, and the environment to better inform the federal and scientific communities.

Synopsis of research: This work focuses on application of ORD's research, in partnership with grantees and other federal agencies, in using the methods, models, and tools developed under LTG 1 and elsewhere to characterize the impact of environmental mixtures of EDCs on environmental media and aquatic organisms. Potential sources of EDCs to be examined include WWTPs, CAFOs, and drinking water plants. ***Ultimate outcomes: ORD's resources are leveraged with those of other organizations (consistent with recommendations of the Subcommittee of the BOSC) to characterize the impact of EDCs on the environment, wildlife, and humans.***

Long Term Goal 3: OPPTS is using endocrine disruptors screening and testing assays developed by ORD to create validated methods that evaluate the potential for chemicals to cause endocrine-mediated effects in order to reduce or prevent risks to humans and wildlife from exposure to endocrine disrupting chemicals.

Synopsis of research: Earlier ORD research has led to the development of standardized protocols for all of the *in vitro* and *in vivo* assays identified by OPPTS as viable candidates in their Tier 1 screening battery and the mammalian and invertebrate tests for Tier 2. ORD now is focusing on finishing the Tier 2 assays in the amphibian and fish models. Once this research is completed this LTG will be considered as being met and any further research on developing the next generation of EDSP assays will be conducted under LTG 1. ***Ultimate outcomes: ORD is developing, standardizing and finalizing assays that OPPTS and/or other national and/or international organizations will validate for screening and testing of chemicals for endocrine activity in the US and/or internationally***.

The three LTGs with the key areas of uncertainty (science questions) they address are as follows:

LTG 1 Providing a better understanding of the science underlying the effects, exposure, assessment, and management of endocrine disruptors

- Determine what are the dose-response characteristic in the low-dose region
- Determine what extrapolation tools are needed
- Determine what are the effects of exposure to multiple EDCs and will a TEF approach be applicable
- Determine how can unreasonable risks be managed
- Determine what approaches are needed to assess risks to humans and wildlife

LTG 2 Determining the extent of the impact of endocrine disruptors on humans, wildlife, and the environment

- Determine how and to what degree are human and wildlife populations exposed to EDCs

- Determine what effects are occurring in exposed human and wildlife populations
- Determine what are the chemical classes of interest and their potencies
- Determine what are the major sources and environmental fates of EDCs

LTG 3 Supporting EPA's screening and testing program

- Determine whether our testing guidelines adequately evaluate potential endocrine-mediated effects

It should be noted that the LTGs identified by the Multi-Year Planning Committee are consistent with not only the key data gaps and areas of research identified by EPA's *Research Plan*, but also assessments conducted by the following organizations: 1) the research needs framework and priority data gaps identified by the Endocrine Disruptors Working Group (Reiter et al., 1998) of the Committee on Environment and Natural Resources (CENR) under the President's National Science and Technology Council (NSTC), 2) research needs identified by the National Research Council in its 1999 report "Hormonally Active Agents Environmental Agents," (NRC, 1999), 3) research needs developed at a joint US-European Union workshop held under the auspices of US-EU Science and Technology Agreement (JRC, NIEHS, EPA, 1999), and 4) the internationally developed document *Global Assessment of the State-of-the-Science of Endocrine Disruptors* (WHO, 2002). Therefore, it is well-recognized and accepted that resolution of these goals are key to being able to: 1) improve our understanding of the science regarding EDCs - *specifically in the areas of effects, exposure, risk assessment, and risk management*, 2) determine the extent of the problem EDCs are causing to humans, wildlife, and the environment, and 3) develop scientifically valid methods to screen and test for agents in the environment that may be EDCs.

ORD is committed to addressing particular aspects of each of these LTGs. The attached flow diagrams for each of the LTGs (Figures 2-4) depict the time line for completion of the APGs and their interrelatedness and should help put the information in Section VI into simpler context. The research that will be conducted is reflected in the Annual Performance Goals (APGs) and their respective Annual Performance Measures (APMs) described in greater detail in Section VI and in attached Appendix III (Tables 1-3).

The degree of emphasis for each LTG is based on criteria similar to those used when assigning priorities in the *Research Plan*: 1) an assessment of the potential impact the research could have on EPA decision-making at the program/regional office level, 2) the magnitude of the uncertainties in the knowledge base and the ability of the research program to decrease them, 3) the sequence of research needed to obtain goals, 4) the likelihood that the research would result in a significant advancement of science, and improve the underlying information for risk characterization and risk management decisions , 6) the technical feasibility of reaching goals based upon the capabilities and capacity of ORD, and 7) time frames of legislative mandates. The following table summarizes the relative emphasis of each LTG over the period of FY2007 through 2013.

LTG	Emphasis from 2007 through 2013
1	Level through completion of LTG 3 with the potential to increase as those resources are freed and reallocated
2	Level through completion of LTG 3 with the potential to increase as those resources are freed and reallocated
3	Level through completion of screening and testing assays development when the goal will be completed and met and resources potentially redistributed across LTGs 1 and 2

The rationale behind the projected level of emphasis over time is as follows. By the end of FY2010, it is expected that ORD will have completed the amphibian and fish Tier 2 assays for transmittal to OPPTS. Additional targeted research in support of the EDSP, as with developing the second generation screening and testing assays and prioritization tools using newer technologies, could either be conducted under LTG 1 of the ED MYP, LTG 1 of the SP2 MYP or through the Computational Toxicology Research Program. Therefore, LTG 3 of the ED MYP will be declared met and will not be carried forward beyond 2011. Any remaining resources potentially would be reallocated to LTGs 1 and 2 of the ED MYP to address projected increasing needs in those areas. For example, under LTG 1 there will be an increased need for providing the necessary underlying science for OPPTS to use in interpreting data submitted from EDSP. Under LTG 2, additional resources will be needed to address projected increasing needs to better understand the impact of endocrine disruptors in the environment (e.g., Congressional and public pressure).

Successfully addressing these LTGs will require a highly coordinated effort. Some of the laboratories have developed implementation plans for the ED research (i.e., NHEERL's EDCs Implementation Plan, 2000, 2004; NRMRL's Risk Management Evaluation, 2003) that further facilitate the coordination and sequencing of the research elements to occur. The implementation plans are consistent with the *Research Plan* and the MYP. These documents, along with the Computational Toxicology Framework (www.epa.gov/comptox/publications/comptoxframework06_02_04.pdf) whose proof-of-concept pilot projects used EDCs as sentinel chemicals, and any other strategies or plans that may be developed around EDCs should be considered collectively when trying to understand the overall EDRP, what specific research the Laboratories and Centers will be carrying out, and when. Recognizing the dynamic nature of research, the Multi-Year Planning Committee revisits the priorities in the MYP, and in the 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. For example, this is the fourth version of the MYP since 1999.

The following section on Description of the Flow Diagrams lays out which specific aspects of the LTGs ORD plans to address. However, given the breadth of the goals and the complexity of the issue, it will require coordinated efforts across the federal government, academia, industry, non-governmental organizations, and with our international counterparts to fully address these goals. The mechanisms for these collaborations are highlighted in Section III.

VI. DESCRIPTION OF THE FLOW DIAGRAMS

The flow diagrams (Appendix II, Figures 2-4) 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 Appendix III (Tables 1-3).

In developing the MYP and determining the APGs, the research planning committee took the following into consideration when determining the APGs: 1) the key questions identified in the *Research Plan*, 2) the original 33 subissues identified in the *Research Plan*, 3) the research themes that were identified within laboratory implementation plans (i.e., NHEERL's Multi-Year Implementation Plan and NRMRL's Risk Management Evaluation) and the Computational Toxicology Implementation Plan, and 4) an assessment of other ongoing and anticipated ORD research efforts. As a result, 7 discrete research areas (APGs) were identified in which the ORD research program will result in a significant impact on advancing the state of the science to provide more effecting decisionmaking by EPA on risks from EDCs within the next 6 years. Schedules for these research areas were estimated based upon knowledge of: 1) existing resources, 2) intramural capacity and capability, 3) the complexity of the area, and 4) in several cases, regulatory-driven deadlines.

Each APG is supported by a number of APMs that represent major segments or milestones of research that together will result in achieving the overall goal of that APG. The APMs will help to determine progress made towards completing the APG. The APMs in the Tables represent the expected product(s) 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 as they may take the lead for that APM but the APM may reflect the work across more than one ORD Laboratory or Center. It should be noted that, for the most part, those that are attributed to NCER are products of STAR grants. It is important to note that the Tables capture the APGs and APMs currently anticipated. The Committee 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 seven APGs for the MYP are described below. For each APG, the objective, the significance/impact, and the schedule of the research are described. Completion of the several APMs will lead to the achievement of their associated APG. The research themes that support the APGs are identified. More detailed information on the themes can be found in Appendix IV. In some cases the research themes may be supporting more than one APG. Further the descriptions identify where there are linkages to other research themes in the EDRP or with other ORD research programs. Since the MYP identifies which areas of the *Research Plan* our efforts will focus on for the next 6 years, the descriptions below include the cross-reference to the relevant subissues in the *Research Plan*. For more detail and context, the reader should refer to the *Research Plan* itself.

A summary of the science issues and the research program objectives makes up most of the remaining body of this MYP. The body of the document concludes with sections on how this MYP is linked to other MYPs/research programs and how the results of the EDRP are communicated. A compendium of what research could be enhanced or expanded as more resources become available, 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 (Note: this is still under development is and, thus, is not included in this draft), and a list of acronyms can be found as Appendices.

Long Term Goal 1: Reduction in uncertainty regarding the effects, exposure, assessment, and management of endocrine disruptors so that EPA has a sound scientific foundation for environmental decision-making.

Ultimate Outcomes: OPPTS and other Program Offices, Regions, and outside EPA organizations are using data from ORD's EDRP to evaluate manufacturers' data submitted to the Agency through EDSP and/or from other sources, and develop integrated risk assessments on EDCs. Furthermore, the tools and data developed will be applied in field studies by EPA and/or others to determine the levels of exposure to EDCs in environmental media and the extent to which and efficacy with which they could be reduced or eliminated (e.g., LTG 2).
The LTG 1 research can be thought of, therefore, as basic or "core" research. Therefore, the results will be of value to most of EPA's program and regional offices, other federal agencies, agencies in other countries, and the scientific community at large.

Synopsis of research: ORD research is:

- determining classes of chemicals that act as endocrine disruptors and their potencies
- characterizing modes of action and the shape of the dose-response curve
- developing approaches for assessing cumulative risk
- developing approaches for extrapolating results across species

- applying 'omics approaches (consistent with recommendations made by the Subcommittee of the BOSC)
- developing molecular indicators of exposure and analytical methods for detecting certain EDCs
- identifying the key factors that influence human exposures to EDCs
- identifying and characterizing sources of EDCs entering the environment, focusing on: wastewater treatment plants (WWTPs), concentrated animal feeding operations (CAFOs), and drinking water treatment plants, and
- developing tools for risk reduction and mitigation strategies.

APG- Provide OPPTS, OW, the Regions and other organizations with improved data on the shape of the dose-response curve as a result of exposure to environmentally relevant levels of endocrine disruptors – FY 2010

Objective: To develop a more complete understanding of specific chemical mechanisms of action particularly for mechanisms that operate in the low end of the dose-response curve and in particular at levels that are of environmental relevance.

Significance/Impact: Understanding of how EDCs elicit toxicity through receptor-based interactions, membrane receptors, enzyme alterations, and other non-nuclear receptor-based pathways, will lead to improved methods to interpret data (particularly from the EDSP) and, thus, improved risk assessments. Most of what we know about endocrine disruptors is a result of laboratory studies or as a result of environmental spills/accidents where exposures in both types of studies were relatively high. Understanding how EDCs operate at the low end of the dose-response curve is particularly relevant to evaluating effects at ambient environmental levels of exposure and has become even more controversial in light of more recent studies and reviews **(provide some references).** Gathering this information in a variety of species will lead to improved methods for extrapolating data across species.

Schedule: Because data from this research are critical to help interpret the results from the screening and testing program, this research is ongoing now in both our intramural and STAR programs. Because of the complexities associated with elucidating mechanisms of action and characterizing effects at environmentally relevant levels, the ongoing research that is addressing this APG will not be completed until FY10, longer than originally anticipated. One of the reasons is that it was determined that this topic warranted issuing a STAR solicitation **(add URL)** for additional research in this area, based upon the recommendations that came out of the "Low-Dose Peer Review Panel on Endocrine Disruptors" sponsored by EPA and NIEHS. The time to complete the research through the awarded proposals has resulted in extending the timeframe for this APG. Two of the three awards made under this solicitation were cooperative agreements and the extramural scientists are working closely with intramural scientists.

MYP Research Themes: 1.1.1 and elements of 1.3.2

Linkages to other themes in EDCs MYP or to other ORD research programs: 1.2.1

Research Plan Subissue: EFF.3.1

APG - Provide OPPTS, OW, the Regions and other organizations with systems and models to test and predict vulnerability of the neuroendocrine system to contaminant-induced effects - FY 2013

Objective: To develop a pharmacokinetic and pharmacodynamic (PBPK/PBPD) models that improves cross-species and low-dose extrapolation and relates adverse developmental consequences to short term *in vivo* screens.

Significance/Impact: Research is addressing three of the previously identified critical areas of uncertainty: **What are the dose-response characteristics in the low-dose region?** The ability to predict low-dose effects in rodent models is a major source of uncertainty in risk assessment. Research will develop dose-response data for a number of thyroid disrupting chemicals to determine the effects of marginal or mild hypothyroidism on sensitive indicators of thyroid axis disruption, alterations in brain structure, and impact on brain function. **To what extent can data from rodent models extrapolate risk to humans?** Extrapolating effects of thyroid disrupting chemicals in rodents to humans is controversial. Pharmacokinetic and pharmacodynamic models provide a framework for more accurate cross-species extrapolation of thyroid hormone disruptors. Development of these models will improve extrapolation from animal models to humans by linking mechanistically based biomarkers of cellular events to the functional outcomes of concern in children exposed to thyroid disrupting chemicals. **What are the effects of exposure to multiple thyroid disrupting chemicals and will a TEF approach be applicable?** The current Agency default for predicting the effects of mixtures is to assume dose addition. There is a critical need to determine if this assumption accurately predicts the empirical effects of mixtures of thyroid disruptors, especially for mixtures of chemicals with different mechanisms of action.

Schedule: Given the complexity and multidisciplinary needs of this research, this effort involves ORD intramural scientists working across Divisions and in collaboration with extramural scientists through a number of efforts. There are two cooperative agreements with STAR grantees. In addition, this research is aligned with efforts in another ORD Division through research under the SP2 program that is partly funded through the Comp Tox program. Additional collaborations include research on structural and functional changes in brain with early thyroid insufficiency and research on mixtures of thyroid disrupting chemicals. Together, these efforts will provide the framework for a cross-species model of thyroid hormone disruption that will enable more accurate human extrapolations of animal data. In addition, data generated by this project will be used to inform the "Virtual Liver Project" in the Human Health MYP. This goal is not anticipated to be met until FY 2013.

MYP Research Theme: 1.2.1

Linkages to other themes in EDCs MYP or to other ORD research programs: 1.1.1, SP2 LTG 1, Human Health virtual liver, Comp Tox framework

Research Plan Subissue: LNK.2.3; EFF.4.2; EFF.3.1; EFF.3.4; EFF.5.3, EFF. 6.1

APG - Provide OPPTS, OW, the Regions and other organizations with data from the development and application of high-throughput and molecular approaches, including 'omics , and computational tools for defining mechanisms of action, extrapolation across species and improving assessments for EDCs – FY 2013

Objective: To develop and apply newer technologies with the ultimate goal of improving Agency human health and ecological risk assessments.

Significance/Impact: The aggregate body of research aligned under this goal is using high-throughput and molecular approaches, including 'omics, and computational tools as a means to address a number of key scientific questions and proposing ways in which these data can be incorporated into Agency assessments:

defining mechanism of action following exposures to single chemicals as well as to mixtures of EDCs - As noted previously understanding how EDCs elicit toxicity through receptor-based interactions, membrane receptors, enzyme alterations, and other non-nuclear receptor-based pathways is important. This is true in characterizing exposures to single chemicals as well as in trying to understand how exposures to multiple chemicals with similar and/or different mechanisms of action will impact organisms. Furthermore, it is critical to develop approaches to facilitate incorporation of these data into risk assessments.

determining the critical factors that account for exposures during development resulting in toxicities occurring later in life (e.g. windows of vulnerability, developmental tissue dosimetry, modes of action) - Development is a period when hormone-mediated changes in gene expression can have permanent consequences that may not be apparent until later in life because functional changes do not occur until puberty or adulthood and during which extraordinary changes occur. Thus, the developing organism may be more vulnerable to toxic effects at lower doses than would produce adverse effects in adults. It also has been suggested that there are processes for which there may be no apparent threshold due to limitations in the kinds of regulatory, surveillance, and repair processes that create biological thresholds in adults. Research in this area will provide the critical information needed for assessing the potential consequences of *in utero* and childhood exposures, both representing periods of sensitivity of human and wildlife populations. Since, EDCs research focuses on

the mode of action of a compound, the impact of these effects on developmental processes is critical to risk assessment. The determination of developmental anomalies caused by a compound, the identification of the dose required to produce such effects and an understanding the mode and mechanism of action involved provides information that can allow for informed decisions on the potential risk to the developing human and wildlife species. Furthermore, mode of action analyses of the low-dose issue could have a major influence on the Agency's approach to risk assessment of endocrine disruptors.

determining the degree to which the effects of EDCs with defined mechanisms/modes of action (MOAs) can be extrapolated across classes of vertebrates – This research is needed: 1) To reduce the uncertainty associated with extrapolating effects of chemicals across species. 2) To understand the degree to which quantitative extrapolation is defensible/possible, comparative toxicological studies using chemicals with well-defined MOAs are necessary. The EDC program, because of its focus on systems controlled by endocrine function which appear to be highly conserved and, in some cases, reasonably well-characterized across species, offers a logical opportunity around which to formulate and test hypotheses related to across-species extrapolation of chemical toxicity. Research will have a direct and, potentially, relatively immediate impact on the regulation of EDCs in terms of defining the degree to which screening and testing based on an assumption of similar MOAs across species is technically and scientifically defensible. Of broader significance, the development of approaches to evaluate and conduct inter-species extrapolation research should ultimately help reduce uncertainties in both human health and ecological risk assessments and reduce the number of animals needed for testing.

developing biomarkers and the next generation of assays for screening chemicals for their potential endocrine disruption - For the last ten years, ORD's research program in support of EDSP has largely focused on providing OPPTS with protocols consistent with EDSTAC recommendations and early Agency decisions as to which types of assays and tests should be developed for the first round of testing through EDSP. In recent years, there has been tremendous growth in the development of newer molecular approaches that are amenable for application to the endocrine disruptors issue in general, and to the development of newer screening methods, specifically. ORD is taking advantage of these opportunities and developing predictive biomarkers and the next generation of assays for possible use in subsequent rounds of EDSP. The main advantage of these assays is that they often take less time to evaluate chemicals for their ability to interact with the endocrine system, cost less than other more conventional assays and test, and reduce (and in some cases eliminate) the use of whole animals. These latter elements are consistent with the recently issued NAS report on recommendations for a new testing paradigm in the 21st century.

Schedule: Because of the importance of addressing these multiple uncertainties, the breadth of the research that is needed, and the complexity of the issues, additional time and resources are still needed. Research has been ongoing for awhile in these areas and the Accomplishments Appendix should be referred to in order to see where we have made a significant impact in this area. This is largely due to our in-house expertise, which is highly capable of addressing these issues as they relates to both human health and wildlife. Some of this research was anticipated to have been "completed" by now; however, research by EPA and other scientists has stimulated the need to continue efforts, at least until FY 2013. Data from this research are critical to help interpret the results from the EDSP and to determine the future generations of assays that would be needed for subsequent EDSPs. The conduct of this research should be evaluated periodically as data become available from EDSP to ensure that it focuses on questions that require addressing. With the advent of new molecular approaches since this effort began, this research should advance more quickly. However, it will be important, as has been noted in the previous MYPs, to re-evaluate the progress made, the state of the science, and the development of newer tools, to determine whether further study will be needed beyond the currently estimated timeframe. As issues get addressed it will be important to develop the approaches that will enable incorporation of the information into risk assessments for human and ecological health. A first step is taken with this MYP where a case-study is described as a model as to how toxicogenomics information could be incorporated into an assessment. Similarly, the development of approaches for integrated cumulative risk assessments for chemicals that have similar and/or different mechanisms of action will also need to be developed (although not addressed in this MYP). The laboratory studies are focused on evaluating the impacts of combinations of chemicals that interfere with the androgen system and the thyroid system. Complementary efforts in assessing impacts in the field from exposures to environmentally-relevant mixtures are ongoing under APG 2-2 in LTG 2. Methods to incorporate results from across species into Agency assessments will also be needed (again although not addressed in this MYP) that would use as a basis the data from the several efforts currently ongoing in this area.

MYP Research Themes: 1.3.1, 1.3.2, 1.3.3, 1.3.4, 1.3.5, 1.3.6,

Linkages to other themes in EDCs MYP or to other ORD research programs: 1.2.1, 2.2.1, 2.2.2, 2.2.3, 3.1.1 ; Comp Tox research program

Research Plan Subissue: EFF.1.1; EFF.2.1; EFF.2.3; EFF.3.1; EFF. 4.2; EFF.5.1; EFF.6.1; LNK. 2.3; LNK.4.1; LNK.4.2

APG - Provide OPPTS, OW, the Regions and other organizations with new exposure assessment and risk management tools to characterize and reduce exposure to EDCs – FY 2013

Objective: Develop and evaluate methods for characterizing environmental EDC exposures and determine whether existing risk management tools can be applied to major sources of EDCs to mitigate exposures.

Significance/Impact: This cross-ORD laboratory research is addressing two key questions: **What are the major sources and environmental fates of EDCs?**
How can unreasonable risks be managed? Exposure research through the EDRP is designed to develop and/or refine chemical and molecular methods for selected EDCs and endocrine-active pharmaceuticals that can be used to qualitatively and/or quantitatively measure indicators of exposure in real-world aquatic environments. The resulting exposure data produced using these exposure methods will be used to characterize selected sources, the fate of the chemicals once released in the environment, and to develop tools for reconstructing exposures and apportioning these exposures to relevant EDCs sources. ORD will develop chemical and molecular indicators of exposure on the highest priority endocrine-active pharmaceuticals identified in the bioinformatics report "An Informatic Approach To Estimating Ecological Risks Posed By Pharmaceutical Use."

There are a number of existing risk management tools that possibly could be applied to reduce exposures to EDCs. ORD's risk management research is determining the efficacy of existing risk management approaches to minimize exposure to suspected EDCs and developing new risk management tools where needed. If technologies exist that can be applied to major sources of exposure, the impact could potentially be a major reduction of EDC release to the environment. As a result of previous research to identify and evaluate potential major sources of EDCs and because of reductions in resources current efforts are focusing on the following sources: wastewater treatment plants (effluents and biosolids); drinking water plants; and CAFOs.

Schedule: This research builds upon previous efforts that resulted in the development of a molecular biological indicator to measure exposure of aquatic species to single and multiple EDCs (see Accomplishments Appendix). In the 2003 MYP we anticipated that the exposure, effects, and risk management research programs will develop in an iterative approach with future tools defined through the analysis of past and current research results. Currently chemical methods are being developed and controlled laboratory exposures conducted to identify molecular markers of exposure. Chemical methods are also being developed to characterize EDCs and endocrine-active pharmaceuticals in water, sediment, and biological fluids/tissues. Current pilot field measurement projects designed to evaluate EDC exposure methods under real-world environmental conditions (i.e., CAFOs, wastewater treatment plants, experimental stream facility exposures, Ohio River, South Branch and mainstream of the Potomac) will be completed during the period 2007-2008. Future pilot studies are being planned for validating the new/refined methods. Thus, this effort is viewed on successively building upon previous methods and applying new approaches to assess ambient environmental conditions for the presence and impact of endocrine active agents under LTG 2. The exposure and risk management

research is conducted jointly and includes a number of collaborators from other ORD research programs, from EPA's regional offices, from other federal agencies, from academia (through the STAR program), and from the water industry. As more information becomes available on sources of EDCs, research for this APG will be evaluated to ensure it is focused on the most appropriate sources. Given the current sources of concern, it is anticipated that research to address this APG will be completed in FY 2013.

MYP Research Themes: 1.4.1, 1.4.2, 1.4.3, 1.4.4, 1.4.5

Linkages to other themes in EDCs MYP or to other ORD research programs: 2.2.1, Drinking Water Research Program, Water Quality Research Program

Research Plan Subissue: EXP.2.3; LNK. 4.2

Long Term Goal 2: Determination of the extent of the impact of endocrine disruptors on humans, wildlife, and the environment to better inform the federal and scientific communities.

Ultimate outcomes: ORD's resources are leveraged with those of other organizations (consistent with recommendations of the Subcommittee of the BOSC) to characterize the impact of current environmental exposures to EDCs on the environment, wildlife, and humans.

As noted above, the methods, approaches, and tools developed through LTG 1 and elsewhere are being applied under LTG 2. The LTG 2 research can be thought of, therefore, as more "applied" research. The results of this research will be of value to most of EPA's program and regional offices, other federal agencies, agencies in other countries, and the scientific community at large.

Synopsis of research: ORD research, alone and in partnership with grantees and other federal agencies, is:

- Applying methods, models, and tools developed under LTG 1 and elsewhere to characterize the impact of environmental mixtures of EDCs on environmental media and aquatic organisms and develop methods for risk management. Sources of EDCs being evaluated include WWTPs, CAFOs, drinking water plants, and combustion byproducts.
- Completing epidemiology studies on the impacts of EDCs on human development and reproduction.

APG - Provide OPPTS, OW, the Regions and other organizations with data to determine the potential for adverse developmental/reproductive effects in human populations following exposures to EDCs – FY 2011

Objective: To determine the extent to which human development/reproduction is being adversely affected by exposure to EDCs

Significance/Impact: To address one of the biggest unanswered questions related to EDCs, that is, whether humans are being adversely impacted by exposure to EDCs. Given that development and reproduction appear to be highly sensitive endpoints in laboratory animal and wildlife studies and that there are reported alterations in particular endpoints (e.g., hypospadias, cryptorchidism, sperm quality), if any adverse effects are to be found, then evaluating these endpoints in humans appears to be logical.

Schedule: One of the biggest unanswered questions related to EDCs is whether they are impacting human health. Work under this area is, by nature, exclusively through the STAR program. Five epidemiology studies that are the result of an FY00 joint solicitation with NIOSH/CDC, NIEHS, and NCI are close to completion. The studies are examining the effects of exposures of a variety of environmental contaminants on a variety of endpoints. Specifically they are evaluating the effects of: 1) heptachlor on reproduction and immune function, 2) phthalates on breast and genitalia development, 3) dioxin on male reproductive development, 4) organochlorines and PCBs on endometriosis, and 5) PBDEs on thyroid function. The other federal partners are funding an additional total of seven studies as a result of the joint RFA. This group of 12 grants from the same solicitation will be followed closely. Given length of time to conduct studies in human populations and prepare peer reviewed publications, significant impacts in this area are not anticipated until at least FY11.

MYP Research Theme: 2.1.1

Linkages to other themes in EDCs MYP or to other ORD research programs: Human Health Research Program

Research Plan Subissue: LNK.2.3; LNK.2.4

APG - Provide OPPTS, OW, the Regions and other organizations with information from the application of cross-disciplinary tools to characterize the occurrence and potential biological effects from and develop management approaches for EDCs in complex environmental media – FY 2012

Objective: To characterize the occurrence and effects of endocrine active compounds in environmental media and develop management approaches to mitigate unreasonable risks.

Significance/Impact: Researchers are working in multi-disciplinary teams to determine: how and to what degree human and wildlife populations are exposed to EDCs, what effects are occurring in exposed wildlife populations, what are the major sources and environmental fates of EDCs and how unreasonable risks can be managed. It is

important to understand the extent of EDC exposures and the factors influencing the source-to-exposure-to-dose relationships in order to develop effective risk management strategies. Gaining improved understanding regarding the fate and transport processes, the interactions of EDCs from the source to the receptor, and collecting high quality exposure data for the development of multimedia, multi-pathway models are critical for ecological and human health risk assessments. Application of biological indicators of exposure to the study of components of mixtures offers the potential to validate and refine these models.

Schedule: Characterizing the occurrence and impact of endocrine active chemicals in the environment are complex issues that require the attention of experts with diverse training working cooperatively toward the common objective. ORD scientists from the effects, exposure and risk management laboratories are combining resources to specifically evaluate the impact of CAFOs. ORD's intramural expertise is complemented by that of awardees through the STAR extramural program. In some instances, the cross-disciplinary teams of ORD scientists are working through cooperative agreements with STAR awardees. This research is also being done in collaboration with scientists from other ORD research programs, from the regional office, other federal agencies, and international coalitions. The research includes field and laboratory studies, development and optimization of suites of *in vitro* screening and analytical methods to identify and quantify compounds responsible for endocrine activity, the identification of ecologically-relevant biomarkers in aquatic species, characterization of environmental fate and transport of endocrine active agents in the environment, and evaluation of current risk management technologies and development of new mitigation tools. This research is expected to continue through FY12.

MYP Research Themes: 2.2.1, 2.2.2, 2.2.3

Linkages to other themes in EDCs MYP or to other ORD research programs: 1.4.5, Water Quality Research Program

Research Plan Subissue: LNK.4.1; EXP.2.1

Long Term Goal 3: OPPTS uses endocrine disruptors screening and testing assays developed by ORD to create validated methods that evaluate the potential for chemicals to cause endocrine-mediated effects in order to reduce or prevent risks to humans and wildlife from exposure to endocrine disrupting chemicals.

. ***Ultimate outcomes: ORD will develop, standardize and finalize assays that OPPTS and/or other national and/or international organizations will validate for screening and testing of chemicals for endocrine activity in the US and/or internationally****. This research is critical to OPPTS and OW in their meeting the Congressional mandates in FQPA and SDWAA,*

respectively, for development of a screening and testing program. Development of protocols will also be of value to other federal agencies, agencies in other countries, and international organizations, such as the OECD under which protocol development for industrial countries are harmonized. Furthermore, research under this LTG will result in the development of improved methods for risk assessment that will also be of particular value to OPPTS and OW, as well as to other EPA program and regional offices, other federal agencies, agencies in other countries and the scientific community at large.

Synopsis of research: ORD research is:

- finalizing the development of the first generation of Tier 2 assays in the amphibian and fish models
- finalizing the next generation of assays to be used by the Agency's EDSP, using newer technologies (consistent with recommendations of the Subcommittee of the BOSC). The development of second generation screens will have a profound impact not only on the future testing paradigm used in EDSP but also will serve as a model for other toxicity testing programs.

APG: Provide OPPTS with standardized protocols for testing chemicals for their potential endocrine mediated effects to meet FQPA requirements – FY 2011

Objectives: To develop Tier 2 level methods to test chemicals for their potential to elicit endocrine-mediated effects.

Significance/Impact: To support the legislative mandates of the Food Quality Protection Act (FQPA) and the Safe Drinking Water Act Amendments (SDWA) of 1996 that require EPA to develop and implement a screening and testing program for endocrine disruptors. This research is addressing the recommendations made by EDSTAC (1998) regarding the need for Tier 2 tests in a variety of species, i.e., mammalian, avian, fish, amphibian, and invertebrates. The research complements that conducted and concluded on the evaluation of existing protocols for their adequacy to assess chemicals for the potential to interact with the endocrine system and consistent with the EDSTAC recommendations the research leading to the development of Tier 1 assays. Collectively this part of the EDRP is leading to the development of protocols critical to the success of the Agency in fulfilling its Congressional mandates to develop and implement a screening and testing program. After the development, standardization and validation, these screening and testing protocols will be used not only by the USEPA to require the testing of chemicals, but also internationally through the OECD's test guidelines program and possibly by other regulatory agencies. The process to develop and implement screening and testing program has had a high profile and the products are closely scrutinized by the US Congress, stakeholders, and the scientific community within the US and internationally.

Schedule: Because of the timetables of the Congressional mandates, the research for this APG has been (since 1997) and will continue to be of highest priority until achieved in FY11. Research is ongoing intramurally to finalize the Tier 2 assays in fish and

amphibians. This research is expected to be completed in FY10 with the resultant protocols handed off to OPPTS for validation for use in EDSP. Once this particular effort is completed, the LTG will be considered as being "met" and any new research on developing next generation testing approaches will be conducted either under LTG 1 (see theme 1.3.4) of the EDCs MYP (if it addresses an endocrine mode of action), LTG 1 of the SP2 MYP or through the Computational Toxicology Research Program. An important role of ORD scientists is to not only develop data and testing methods but also to support the regulatory programs by transferring the technology, interpreting the science, and assisting in the implementation of new testing methods by the latter. This scientific and technical support is critical for the Agency to meet the Congressional mandate to develop and implement an EDSP that can identify environmental estrogens and other hormonally active substances. Because this activity is so crucial and resource-intensive it is specifically captured as a "research theme" with an APM in this MYP

MYP Research Theme: 3.1.1, 3.1.2, 3.1.3

Linkages to other themes in EDCs MYP or to other ORD research programs: 1.3.4, SP2 LTG 1 and 2

Research Plan Subissue: EFF.1.1; EFF2.1; EFF.5.1

VII. RELATIONSHIP TO OTHER MULTI-YEAR PLANS

As noted earlier, there are a number of high priority science needs in OPPTS, OW and the Regional Offices 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/risk management issues can be found in the Sustainability Research Program and its MYP, and therefore, are addressed in the EDRP only as related to endocrine disruptors. Some of the risk management research in the area of endocrine disruptors is leveraged with that going on in other research programs (e.g., water quality, drinking water).
- The scientific gaps in our capability to assess cumulative and aggregate risks, susceptible sub-populations amongst human or other vulnerable species, and stochastic exposure comprise additional core ORD research efforts under the Human Health MYP.
- The need for prioritization tools is also being addressed by ORD's Computational Toxicology, SP2, and to a lesser extent, the Human Health Research Programs.
- The information and methods needed by decision makers to assess the benefits of ecosystem goods and services to human well-being for inclusion in management alternatives is being provided through the Ecological 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' and OW's 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 (http://www.epa.gov/osa/nanotech.htm).
- OPPTS is using the results of ORD's SP2 research program as scientific foundation for: 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 pesticide 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; and 3) decision-making related to products of biotechnology (www.epa.gov/osp/myp/sp2.pdf).
- OW's research needs are directly supported by the Drinking Water and Water Quality Research Programs. Under the Drinking Water program, ORD is developing data and approaches to assess and manage risks to waterborne pathogens and chemicals and providing tools and technologies to support management decisions to protect source water and the quality of water in the distribution system ((www.epa.gov/osp/myp/dw.pdf). The Water Quality program is providing approaches to: develop and apply criteria for support of aquatic systems, diagnose the causes and sources of impairment in aquatic systems, restore and protect impaired aquatic systems, and assess and reduce risks from biosolids ((www.epa.gov/osp/myp/wq.pdf).

In addition, the EDRP while most closely developed to support OPPTS needs (and OW and Regions to a lesser extent) may also provide indirect benefits to other Agency Goals and ORD Research Programs. A few of the examples where endocrine disruptors research will be of value to other Agency Goals and MYPs are provided in the following table.

Examples of EDCs Research - Goal 4.4	**Goals and MYPs/Research Programs that benefit indirectly**
Exposures to multiple endocrine disruptors	Goal 4 aggregate exposure and cumulative risk issues – Human Health, Ecological, and Human Health Risk Assessment MYPs
Development of protocols for screening and testing and prioritization tools	Goal 4 test methods development program - Safe Pesticides/Safe Products (SP2), Human Health MYPs, Computational Toxicology Research Program
Understanding critical biological factors during development	Goals 2 and 4 sensitive subpopulation issues - Drinking Water, SP2, Human Health, and Human Health Risk Assessment MYPs

Understanding impacts of endocrine disruptors on wildlife	Goals 2 and 4 ecological methods, models, and measures research - Water Quality, SP2, and Ecological MYPs
Developing integrated risk assessment methods	Goal 4 risk assessment methods development programs – SP2, Ecological, Human Health, and Human Health Risk Assessment MYPs
Developing risk management approaches, including pollution prevention methods	Goal 5 pollution prevention/risk management methods - Sustainability MYP

It should be recognized that, as other related ongoing research has been noted previously, that there is a need to coordinate the EDRP 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) 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 are used by two separate groups to evaluate the EDRP. On December 12-14, 2004 a Subcommittee of ORD's BOSC first met to address a number of charge questions, including being asked to comment specifically on how the program meets the R&D investment Criteria (http://www.epa.gov/osp/bosc/subcomm-edcs.htm). Earlier in 2004 the Office of Management and Budget (OMB) used the Criteria in the joint evaluation of EDRP and

EDSP using the Program Assessment Rating Tool (PART) (http://www.whitehouse.gov/omb/expectmore/summary/10002280.2004.html). The BOSC Subcommittee will be holding a mid-cycle review of the EDRP on September 18, 2007. The date for a subsequent PART review has not been set.

IX. COMMUNICATIONS

Because of the breadth of the EDRP, 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.
- Work through the interagency working group on endocrine disruptors and researcher-to-researcher contacts to coordinate/collaborate with other federal agencies.
- Use international workshops and researcher-to-researcher contacts to coordinate/collaborate with programs in other countries.

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 and the ORD-OW seminar series ongoing since 2006. Teleconference lines are always available for remotely located interested parties, e.g., Regional, state scientists.
- 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.
- Work through the interagency working group on endocrine disruptors and researcher-to-researcher contacts to coordinate/collaborate with other federal agencies.
- Use international workshops and researcher-to-researcher contacts to coordinate/collaborate with programs in other countries.

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 (or a series of APGs are) 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(s).

To facilitate communication within the Agency and with the public, an EDRP website is under development and will be maintained.

X. REFERENCES [Being Updated]

Ankley, GT, RD Johnson, G Toth, LC Folmar, NE Detenbeck and SP Bradbury. 1997. Development of a Research Strategy for Assessing the Ecological Risk of Endocrine Disruptors. Reviews in Toxicology 1:71-106.

Chemical Manufacturers Association. 1999. Endocrine Issues Research Summary.

CRS Report for Congress. Summaries of Environmental Laws Administered by the Environmental Protection Agency. M.R. Lee, Coordinator. Congressional Research Service. The Library of Congress. January 3, 1995.

EDSTAC. 1998. Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) Final Report.

Joint Research Centre European Commission, National Institute of Environmental Health Science, Environmental Protection Agency. 1999. Expert Panel Meeting on Opportunities for Collaborative EU/US Research Programmes on Endocrine Disrupting Chemicals.

Kavlock, RJ, GP Daston, C DeRosa, P Fenner-Crisp, LE Gray, S Kaattari, G Lucier, M Luster, MJ Mac, C Maczka, R Miller, J Moore, R Rolland, G Scott, DM Sheehan, T Sinks, and HA Tilson. 1996. Research Needs for the Risk Assessment of Health and Environmental Effects of Endocrine Disruptors: A Report of the U.S. EPA Sponsored Workshop. Environmental Health Perspectives 104 (Supplement 4):715-740.

National Research Council. 1999. Hormonally Active Agents in the Environment. National Academy Press: Washington, DC.

National Research Council. 2007. Toxicity Testing in the Twenty-first Century: A Vision and a Strategy. National Academy Press: Washington, DC.

Reiter, LW, C DeRosa, RJ Kavlock, G Lucier, MJ Mac, J Melilo, RL Melnick, T Sinks, and B Walton. 1998. The U.S. Federal Framework for Research on Endocrine Disruptors and an Analysis of Research Programs Supported during Fiscal Year 1996. Environmental Health Perspectives 106(3):105-113.

USEPA. 1996. Strategic Plan for the Office of Research and Development. Washington, DC, EPA/600R-96-059.

USEPA. 1997. 1997 Update to ORD's Strategic Plan. Washington, DC, EPA/600/R-97/015.

USEPA. 1998. Research Plan for Endocrine Disruptors. Office of Research and Development. Washington, DC. EPA/600/R-98/087.

USEPA. 2000. 2000 Update to ORD's Strategic Plan. (http://www.epa.gov/ocfo/plan/plan.htm)

WHO. 2002. Global Assessment of the State-of-the-Science of Endocrine Disruptors. Damstra, T, Barlow, S, Bergman, A, Kavlock, R, Van Der Kraak, G (eds.).

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

In the event that additional resources become available, the EDC research program would accelerate and/or expand the conduct of research already captured in the 2007-2015 timeframe. In the 2003 version of the MYP, we identified a number of different methods, tools, and approaches that would move the EDCs program a lot more forward. In addition, we identified a specific case study where we proposed the application of the methods, tools, and approaches to work on in an integrated cross-Laboratory/Center manner on a real-world situation.

In fiscal year 2005, the EDCs STAR program was eliminated in the President's Budget. However, in their appropriations for fiscal years 2005-2007, Congress provided additional appropriations above those in each year's President's Budget, that partially made up for the loss in resources. The EDCs research program has applied those additional resources toward the integrated cross-Laboratory/Center study to support intramural and extramural research. The definition of the cross-Laboratory/Center study has been enhanced since the 2003 MYP but the concept of having all of the ORD organizations and scientists from the STAR program work in collaboration on a real-world source of EDCs to characterize the occurrence, determine the biological effects, and develop risk management approaches has remained the aim. This effort is described under Theme 2.2.1 in Appendix IV.

With the updated MYP, we have identified several options for application of additional resources:

The first, and the most logical, is to apply those resources to accelerate and possibly expand the integrated cross-Laboratory/Center study on CAFOs. A lot of effort has gone into defining this project, building collaborations across our intramural organizations, preparing and issuing an RFA and making seven awards (some in the form of cooperative agreements), holding a workshop that brings together scientists from ORD, our grantees, the EPA Program and Regional Offices, and from other federal agencies also interested in research on CAFOs. The Office of Water, Regional Offices, other federal agencies and state and local environmental agencies, and the public will use the results of this case-study to develop improved risk assessments and risk management strategies for EDCs associated with CAFOs (See theme **2.2.1** for more detail).

A second option is to take the approaches and collaborations that have been used to bring together intramural ORD, extramural, and scientists from other federal agencies to work on CAFOs and apply them to a different real-world situation. A key example and critical need is to understand the occurrence of endocrine activity in effluents from WWTPs, to characterize the biological effects of these complex mixtures on wildlife and humans, and to develop improved methods to reduce or prevent the release of endocrine active compound into WWTP effluents. The completion of this new application of ORD expertise would deliver important risk assessment and management information to key environmental stakeholders. The Office of

Water, Regional Offices, interested industries, state and local environmental agencies, and the public will use the results of this case-study to develop risk management strategies for EDCs associated with WWTPs.

The research program for EDCs should provide the Agency with the tools it needs for proper evaluation of EDC risks. Developing guidance for EDC risk assessment had been identified, through the development of the *Research Strategy*, as high priority by program and regional offices to support their risk management decisions. Additional resources applied to addressing issues we have been studying for awhile would help accelerate our being able to make significant advancements in reducing uncertainty and developing improved approaches for risk assessment in the following areas:

"Low Dose" Issues

One of the key unresolved issues with EDCs is determining the shape of the dose response curve at low (environmentally relevant) levels of exposure. The 2004 RFA on this topic resulted in three grants but clearly this work is not enough to address the enormity of this issue and the impact that results in this area could have on how EPA and others conduct risk assessments on EDCs. This issue has not been resolved. Research on developmental and reproductive effects of chemicals continues to report non-monotonic dose-response relationships for outcomes. The mechanisms for these effects have not been determined and the implications for risk assessment have not been adequately considered.

With additional resources, ORD could analyze existing data sets and help them better inform the design of targeted experiments to fill identified data gaps. Since this topic has broad application and implication across international boundaries, this effort could include convening an international workshop on the topic to examine existing data and develop a collaborative plan to address research gaps/needs.

Integrated Risk Assessment Case Studies

One of the areas that the BOSC, in its 2004 review, recommended us to focus on, given our expertise and the fact that we have cases with existing data, is to develop ways to integrate human health and ecologic data into risk assessments. A case study was previously done for bisphenol A that provided useful data that could be used to predict the mode of action for related species for which data do not exist.

ORD could use the analysis for BPA as a model to identify additional chemicals amenable to case study. The focus would be on developing a generic method for conducting an integrated human health/ecological risk assessment. The 2003 version of the MYP identified that a framework for integration of human and ecological information into risk assessments and a guidance document would be developed. However, limited resources and insufficient staffing resulted in the elimination of the assessment component of the EDCs research program. Development of the framework would require expertise from across ORD Laboratories/Centers and scientists from the Program/Regional Offices. Progress could be made by holding a workshop or series of workshops to engage expertise from outside EPA to provide input on

methods and guidelines for integrated assessments and to assure proper development and acceptance of Agency assessment approaches.
Successful completion will provide the Agency with a consistent approach which supports domestic and international EDC risk assessment activities.

Cross Species Extrapolation
Progress in the ability to sequence the human genome has led to a rapid development of laboratory methods to profile the expression of mRNAs and proteins. cDNA microarray and proteomics technologies that assess gene expression on a genome-wide basis may provide a "global" perspective about how an organism responds to specific stressors, such as exposure to endocrine disruptors. This information can define cellular networks or response genes, identify target molecules or toxicity, provide future biomarkers and alternative test procedures, and identify individuals with increased susceptibility to endocrine disruptors. Measuring specific changes in gene expression in humans and other species that are exposed to endocrine disruptors could lead to a "signature" for a given pathway of toxicity. Comparison of effects from animal and human assays will permit a direct assessment of interspecies extrapolation. This is important because there is significant uncertainty about how to extrapolate data from many current *in vitro* assays and rodent bioassays to humans.

The overall approach of this research program will be to develop microarray technologies to assess changes in expression of genes for high priority target sites such as the estrogen or androgen receptors. This effort represents and is consistent with a part of the new Computational Toxicology research program as it relates to endocrine disruptors. Specific changes in gene expression in humans and other species exposed to endocrine disruptor and other environmental chemicals will be evaluated to identify patterns of changes for a given pathway associated with endocrine disruption. Once these patterns of change have been identified, more focused arrays will be developed to assess the potential toxicity of chemicals in a rapid, prospective manner. This research could result in better interspecies extrapolation, greater confidence in animal models, a reduction in the number of animals needed for testing, and insights into pathways of toxicity and mechanisms of endocrine disruption. Results from these studies could also lead to the development of new tools for human exposure assessment. Using patterns of changes from genomic or proteomic studies could help identify the agent and dose to which individuals or populations have been exposed. Surveillance programs could result for humans and animals where exposure to and/or contamination by endocrine disruptors are suspected.

Collaboration between intramural and extramural research scientists will be given a high priority. Workshops involving ORD and extramural scientists will be needed to develop principles for the selection of appropriate endpoints to be included in microarrays. Basic research on microarray technology and approaches to the statistical analysis of patterns of changes observed in genomic and proteomic studies will be supported intramurally, as well as by grants through the STAR program. Workshops involving ORD scientists and regional and program office scientists will also be needed to explore approaches and principles for the use of genomic and proteomic data in a risk assessment context.

Discussions through the IWG on PiE will result in the development of two research needs' documents. The Federal agencies will individually and collectively determine what research gaps they each would be willing and able to address. Some of these research needs could be addressed through the EDCs, Water Quality, or Human Health Research Programs and could be leveraged across multiple research programs. Therefore, another option for use of additional funds is to address research the forthcoming needs from the interagency activities. One such effort has been preliminarily scoped-out, as an example:

Determination and Prioritization of Potential Risk from the Disposal of Pharmaceutical and Personal Care Products (PPCPs)

PPCPs have been detected in the effluent of waste water treatment plants with suspected impacts upon aquatic species[1,2,3]. As a result, federal government and state agencies have issued new recommendations for the disposal of unused pharmaceuticals in landfills to avoid sewage disposal. Despite these policies, the disposal of PPCPs in solid waste may result in the transmission of PPCPs from landfills to wastewater treatment plants and the environment because landfill leachate is commonly managed via waste water treatment plants. To date, the technical literature regarding analysis of PPCPs in landfill leachate is lacking[4]. Therefore, it is unclear if PPCP disposal in landfills will have an impact on landfill leachate and its treatment.

Proposed research would focus on methods development to target PPCPs of common chemical composition to maximize method effectiveness. Methods development would include solid phase extraction and other concentration techniques to determine and achieve lowest possible detection levels (ppb-ppt range) in complex matrices. Landfill leachate would be analyzed to determine its potential contribution of PPCPs to waste water treatment systems. This information is vital to the development of models and methods for the risk assessment of discarded pharmaceutical and personal care products. A national survey of landfill leachate would also be conducted. Through cooperation with leading solid waste industry corporations and other volunteer landfill sites, a minimum of forty landfills would be sampled. Analytical techniques developed in prior research will also be utilized. Many factors affecting PPCP disposal and concentrations in the landfill leachate (e.g., age of the population, urban vs. rural community, community access to healthcare, types of waste disposed and landfill age) will be investigated in determining risk of exposure to PPCPs.

This research would enhance our understanding of the proper management and appropriate disposal methods for PPCPs through the development of analytical methods and management practices for these compounds.

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[1] Hirsch, R., Terner, T., Haberer, K., Melchlich, A., Ballwanz, F., Kratz, K. J. *Chromatogr. A.*, **1998,** 815,213-223.
[2] Wiegel, S., Berger, U., Jensen, E., Kalenborn, R., Thorensen, H., Huhnerfuss, H., *Chemosphere*, **2004**, 56, 583-592.
[3] Carballa, M., Omil, F., Lema, J., Llompart, M., Garcia-Jares, C., Rodriguez, I., Gomez, M., Ternes, T., *Wat. Res.*, **2004,** 38, 2918-2926.
[4] Schwarzbauer, J., Heim, S., Brinker, S., Littker, R. *Wat. Res.*, **2002,** 36, 2275-2287.

APPENDIX II

FLOW DIAGRAMS

Figure 2 - Linkage and Timeline for APGs to Meet EDCs Long-Term Goal 1

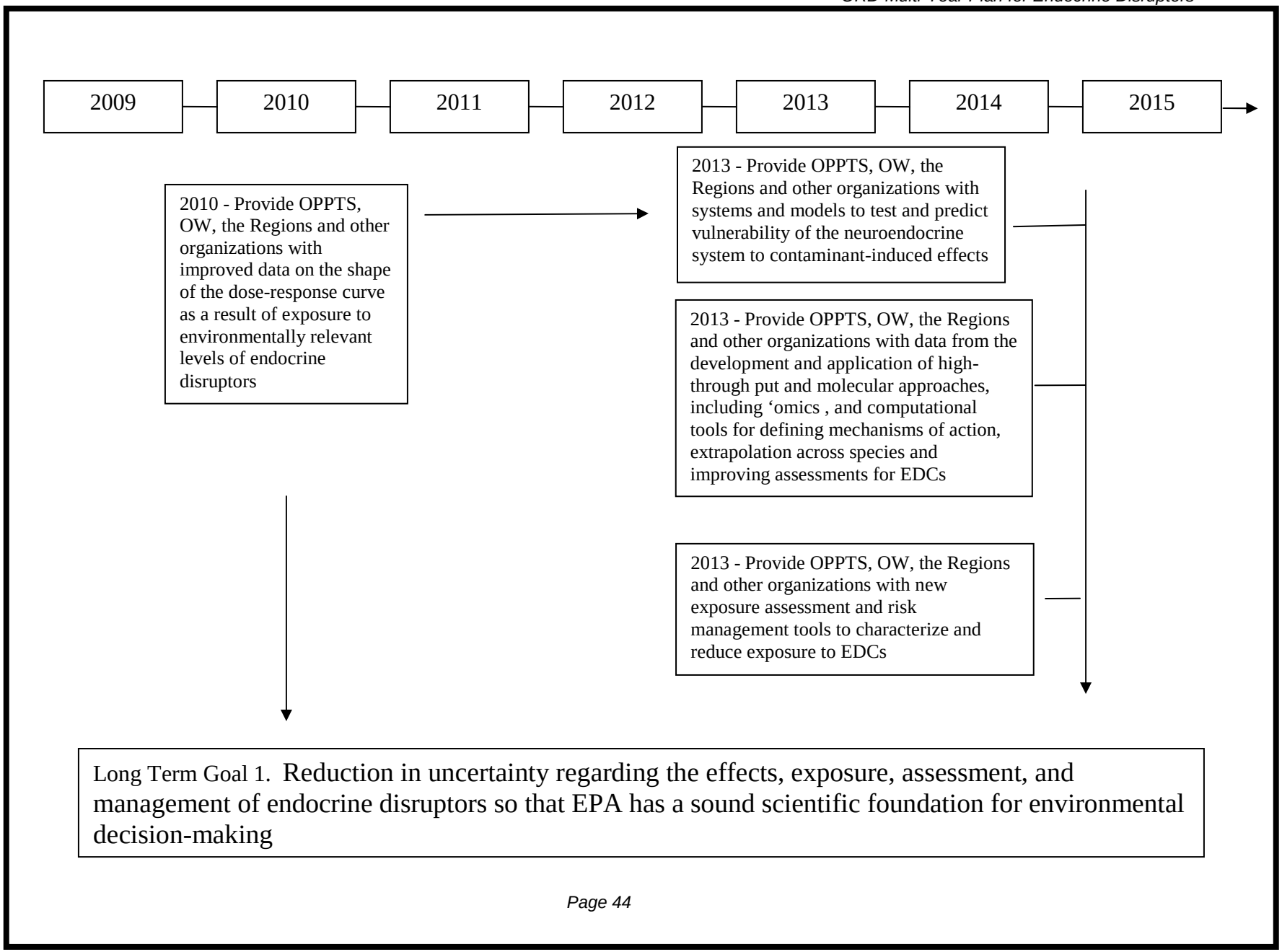


Figure 3 - Linkage and Timeline for APGs to Meet EDCs Long-Term Goal

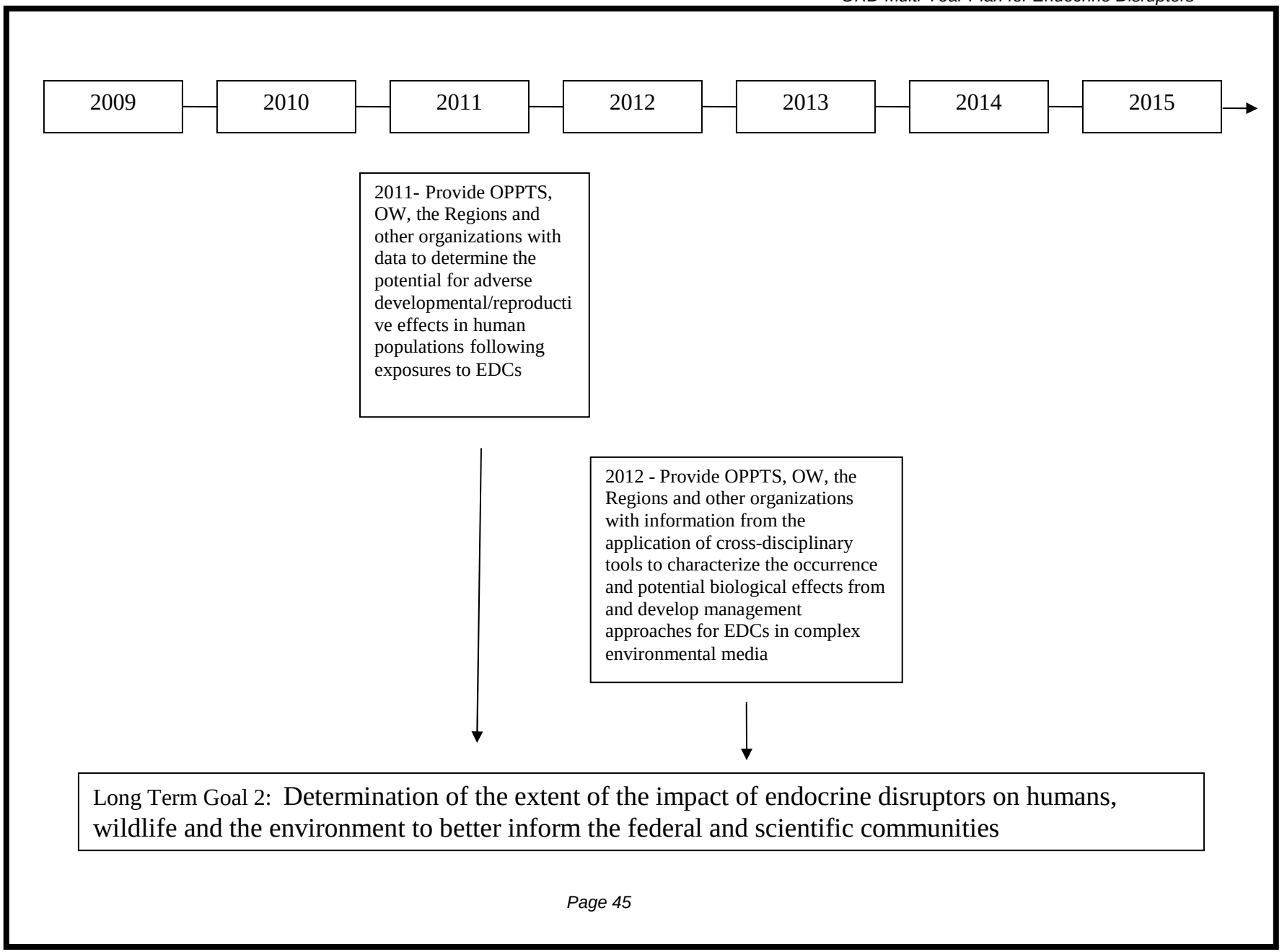
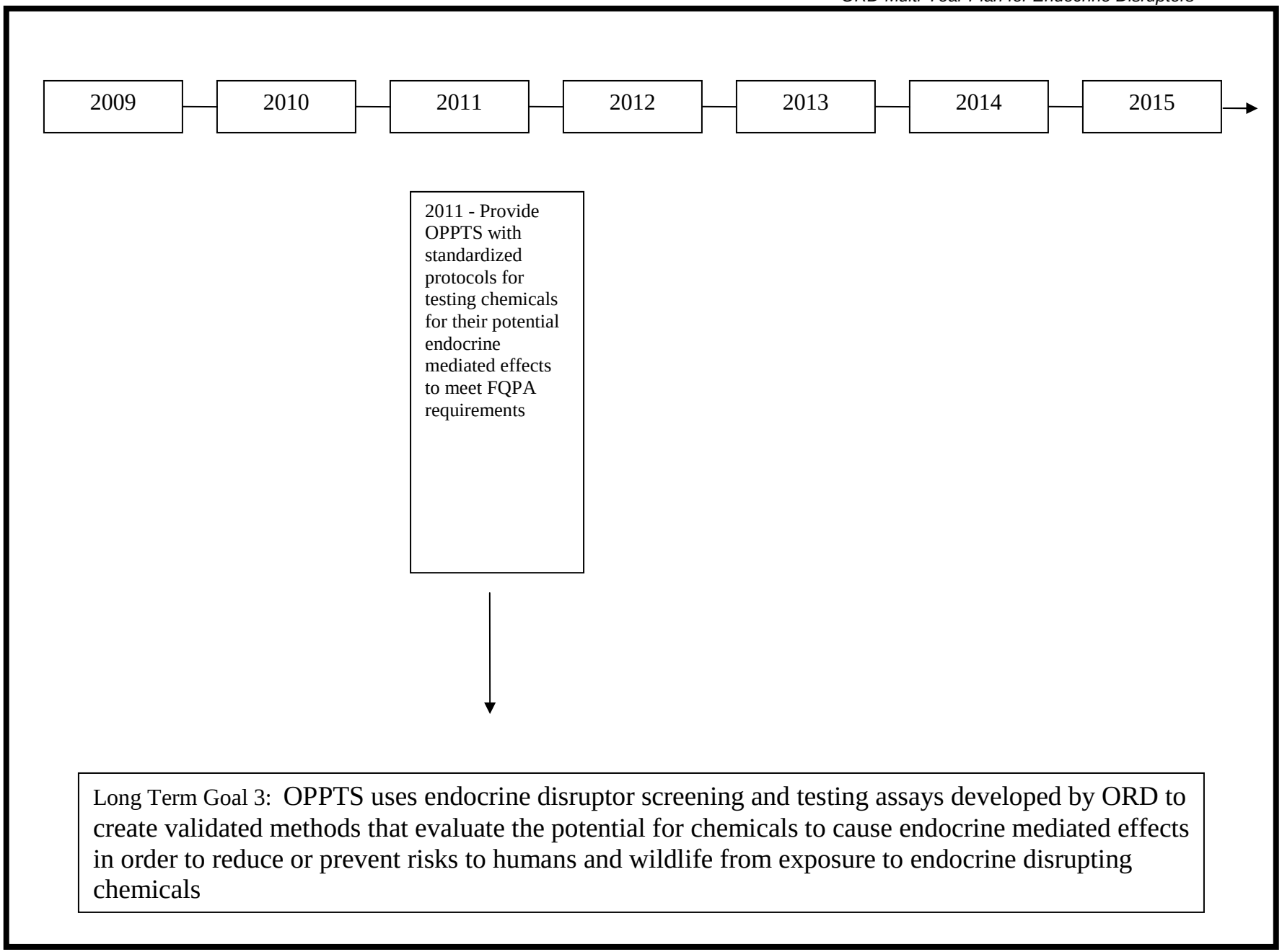


Figure 4 - Linkage and Timeline for APGs to Meet EDCs Long-Term Goal 3



APPENDIX III

Annual Performance Goals/ Annual Performance Measures TABLE 1.

Long Term Goal 1. Reduction in uncertainty regarding the effects, exposure, assessment, and management of endocrine disruptors so that EPA has a sound scientific foundation for environmental decision-making

	Annual Performance Goals and Measures	Year	Lab/Center
APG	**Provide OPPTS, OW, the Regions and other organizations with improved data on the shape of the dose-response curve as a result of exposure to environmentally relevant levels of endocrine disruptors**	**2010**	
APM	1. Phthalate Mixture publications with pairs of chemicals; 2. Mixture effects of 7 chemicals on the reproductive tract of male rats 3. Publication of low dose effects of environmental estrogens on reproductive tract and behavior 4. Publication of low dose in utero and pubertal effects of DEHP (2 papers) 5. Publication of effects of prochloraz on puberty and steroidogenesis in the male rat. (2 papers)	2007	NHEERL
APM	Report on low dose effects of thyroid toxicants on neurodevelopment	2009	NCER
APM	Report on low dose effects of *in utero* exposure to cadmium on puberty	2009	NCER
APM	Report on development of a BBPK model for the thyroid axis in the pregnant rat and fetus for the dose response analysis of developmental neurotoxicity	2009	NCER
APG	**Provide OPPTS, OW, the Regions and other organizations with systems and models to test and predict vulnerability of the neuroendocrine system to contaminant-induced effects**	**2013**	
APM	Provide initial assessment of cumulative risk from thyrotoxic environmental chemicals (APM 254)	2008	NHEERL
APM	Examination of the predictive power of thyroid hormone disruption during development on neurophysiological, auditory and cognitive function (APM 255)	2008	NHEERL
APM	Comparison of ability of short-term in vivo screens to predict developmental neurotoxicity of thyrotoxic agents (APM 58)	2010	NHEERL
APM	Proposed approach for assessment of cumulative risk from thyroid hormone disruptors acting through multiple mechanisms (milestones for ‘10 and ‘11)	2012	NHEERL
APM	Quantitative dose-response model in pregnant animal and developing	2012	NHEERL

	fetus/neonate based on a description of the cellular and molecular biomarkers of effects for thyroid hormone disruption (Collaboration with cooperative agreement scientists) (milestones in '10 and '11)		
APG	**Provide OPPTS, OW, the Regions and other organizations with data from the development and application of high-through put and molecular approaches, including 'omics , and computational tools for defining mechanisms of action, extrapolation across species and improving assessments for EDCs**	**2013**	
APM	Report and manuscript on the approach to utilizing genomics in EPA risk assessments and the DBP case study findings.	2007	NCEA
APM	Manuscripts describing effects of EDCs on aromatase activity in the fish and rat. (APG 119 APM 30)	2007	NHEERL
APM	Report on fish versus human AR binding affinities with 15 chemicals	2007	NHEERL
APM	Report on chimp AR binding assay	2008	NHEERL
APM	1. Publication of seven individual phthalates on male rat fetal testosterone production and gene expression. 2. Publication of effects of an environmental androgen on female rat reproductive development	2008	NHEERL
APM	Characterization of atrazine and metabolites in diverse species and utility in species extrapolation	2009	NHEERL
APM	Characterization of proteomic alterations following phthalate exposures to inform the mode of action analysis	2009	NHEERL
APM	Report on the development of an abbreviated amphibian thyroid axis screen based on the use of diagnostic molecular and biochemical endpoints	2009	NHEERL
APM	Report on how predictive in vitro assays of aromatase activity are of in vivo results.	2010	NHEERL
APM	Correlations between alterations in testis and sperm proteomes and observed phenotypes following phthalate exposures	2010	NHEERL
APM	1. Publication of the effects of a mixture of seven phthalates on male rat fetal testosterone production and gene expression and adult offspring latent effects. 2. Mixture publication with broader modes of action.	2010	NHEERL
APM	1. Recombinant ER binding assays for multiple vertebrate species 2. Evaluate the utility of ER and AR gene expression assays for multiple species of vertebrates	2010	NHEERL
APM	Report on the activity of selected chemicals using an abbreviated thyroid axis screen	2011	NHEERL
APM	Report on the relationship between changes in aromatase and adverse physiological effects across diverse species.	2011	NHEERL
APM	Path to phenotype predictions based on gestational phthalate exposures: selection of biomarkers to permanent testicular alterations following developmental phthalate exposure	2011	NHEERL
APM	Validation of novel biomarkers of testicular dysfunction	2012 and beyond	NHEERL
APM	1. Develop assays for additional receptors beyond and pathways ER	2012	NHEERL

	and AR. 2. Develop better in vitro assays of steroidogenesis.		
APM	Publication of a framework for cumulative risk assessments with chemicals with diverse modes of action that disrupt reproductive development	2012	NHEERL
APG	**Provide OPPTS, OW, the Regions and other organizations with new exposure assessment and risk management tools to characterize and reduce exposure to EDCs**	**2013**	
APM	Screening California surface waters for estrogenic endocrine disrupting chemicals (EEDC) with a juvenile rainbow trout liver vitellogenin mRNA Procedure V. de Vlaming1	2007	NERL
APM	Training course on molecular methods – program office upper managers & another for Regions	2007	NERL
APM	Analytical methods development for conjugated hormones in CAFOs	2008	NRMRL
APM	Methods development to quantify the EDCs and other emerging contaminants	2009	NRMRL
APM	CAFOs as a source for contaminating groundwater	2009	NRMRL
APM	Determine treatability of selected EDCs	2009	NRMRL
APM	Provide the Program Offices and Regions with an improved method that can be readily employed for the detection and isolation of exposure induced, differentially expressed proteins in adult embryo and juvenile fish.	2009	NERL
APM	Provide the Program Offices and Regions with a report summarizing the development and evaluation of diagnostic chemical and omics-based biomarkers for characterizing estrogenic and androgenic exposures to aqueous and solid CAFO effluents	2009	NERL
APM	Field scale studies to characterize wastewater treatment plants as sources of EDCs to the environment	2010	NRMRL
APM	Report on the transport and fate of selected EDCs and other emerging contaminants during land application of biosolids	2010	NRMRL
APM	Field scale studies to characterize the management of EDCS during onsite WWT	2011	NRMRL
APM	Field scale studies to characterize the management of EDCs in unit operations of wastewater treatment plants	2012	NRMRL
APM	Characterizing full-scale water treatment unit operations and integrated plant treatment through distribution for EDC removal.	2012	NRMRL

APG/APM TABLE 2.

Long Term Goal 2. Determination of the extent of the impact of endocrine disruptors on humans, wildlife, and the environment to better inform the federal and scientific communities

	Annual Performance Goals and Measures	Year	Lab/Center
APG	**Provide OPPTS, OW, the Regions and other organizations with data to determine the potential for adverse developmental/reproductive effects in human populations following exposures to EDCs**	**2011**	
APM	Report on latent effects of gestational exposure to heptachlor	2009	NCER
APM	Report on study of phthalates in pregnant woman and children	2009	NCER
APM	Report on persistent organic pollutants and endometriosis risk	2009	NCER
APM	Report on dioxins, male pubertal development and testis function	2009	NCER
APM	Report on endocrine disrupting chemicals and thyroid outcomes	2010	NCER
APG	**Provide OPPTS, OW, the Regions and other organizations with information from the application of cross-disciplinary tools to characterize the occurrence and potential biological effects from and develop management approaches for EDCs in complex environmental media**	**2012**	
APM	Report on contribution of estrogen conjugates to total estrogen load in CAFO swine lagoon effluents	2007	NRMRL
APM	Develop strategy for efficiently screening environmental samples for contaminants with androgenicity and estrogenicity activity	2008	NHEERL
APM	Provide the Program Offices and Regions with a report characterizing the presence, fate, and potential environmental exposures to EDCs in aquatic environments influenced by CAFOs	2009	NERL
APM	Report on development and validation of a rapid detection method for trace concentrations of endocrine disrupting chemicals in natural waters	2009	NCER
APM	Report on development and linkage of activity-based in vitro assays with chemical analysis and bioassays	2009	NCER
APM	Report on synergistic interactions of mixtures, assembly of a biologically relevant effects matrix, and neuroendocrine endpoints for rapid assessment	2009	NCER
APM	Report on a fully integrated microfluidic device that will separate and quantify EDCs in environmental samples	2009	NCER

APM	Report on development of a gene-expression based, whole-organism approach to evaluating cumulative exposure to EDCs	2009	NCER
APM	Identification of specific chemicals responsible for estrogenic and androgenic effects of complex mixtures associated with CAFOs (contingent upon availability of extramural funds)	2009	NHEERL
APM	Analytical and biological determination of the spatial and temporal occurrence of estrogenic and androgenic substances in surface and ground waters from CAFOs	2009	NRMRL supported by NERL
APM	An integrated assessment of the ecological risk of endocrine-active substances from representative beef and dairy CAFOs	2010	NHEERL supported by NERL and NRMRL
APM	Establish extramural contract to monitor waste and drinking water for EDC activities	2010	NHEERL
APM	Report on environmental transport and fate of natural and synthetic steroid hormones that accompany discharges and the disposal of animal wastes from cattle, dairy, swine and poultry CAFOs and how different management practices may affect fate and transport. (U. of Wisconsin)	2012	NCER
APM	Report on assessing the relative amount of hormones discharged from tile-drained agricultural fields under different manure and lagoon effluent application practices, hormone persistence in fields under these conditions, and the impacts of hormone loads on aquatic organisms. (Purdue U.)	2012	NCER
APM	Report on fate and transport of exogenous and endogenous hormones in cattle manure within the feedlot and after application to crop land. (U. Nebraska)	2012	NCER
APM	Report on evaluating and modeling occurrence, fate, and transport of synthetic and endogenous steroid hormones released by cattle and cows from CAFOs into ground water and surface water. (U. CA, Berkeley)	2012	NCER
APM	Report on occurrence and fate, and biological effects of steroids from agricultural application of poultry litter and the effects of different agricultural practices on mitigating environmental steroid loads. (U. Maryland)	2012	NCER
APM	Report on developing a total facility estrogen budget a swine farrowing CAFO and effects and transport of estrogens following field applications. (Duke U.)	2012	NCER
APM	Report on levels of hormones in broiler waste from poultry CAFOs, the effect of waste storage on hormone concentrations, and how common land application practices affect the fate of hormones found in poultry waste in soil and surface run-off. (U. Georgia)	2012	NCER

APG/APM TABLE 3.

Long Term Goal 3. OPPTS uses endocrine disruptors screening and testing assays developed by ORD to create validated methods that evaluate the potential for chemicals to cause endocrine-mediated effects in order to reduce or prevent risks to humans and wildlife from exposure to endocrine disrupting chemicals

	Annual Performance Goals and Measures	**Year**	**Lab/Center**
APG	**Provide OPPTS with standardized protocols for testing chemicals for their potential endocrine mediated effects to meet FQPA requirements**	2011	
APM 324	Report on amphibian-based reproductive and developmental toxicity test method for endocrine disruption	2007	NHEERL
APM	Report on the evaluation of the amphibian full life cycle test using model endocrine active agents	2009	NHEERL
APM	*Complete development of testing methods for evaluating toxicant effects on fish development, growth and reproduction. (APM is in SP2 MYP)*	2010	NHEERL
APM	Established approach to support informing the validation and implementation process of Tier I and Tier II screening and testing in support of EDSP	2011	NHEERL

APPENDIX IV
DETAILS ON RESEARCH THEMES

Long Term Goal 1:
Reduction in uncertainty regarding the effects, exposure, assessment, and management of endocrine disruptors so that EPA has a sound scientific foundation for environmental decision-making

APG - Provide OPPTS, OW, the Regions and other organizations with improved data on the shape of the dose-response curve as a result of exposure to environmentally relevant levels of endocrine disruptors – FY 2010

1.1.1 Development and Characterization of Biological Systems for Studying Low Dose Effects of Endocrine Disrupting Chemicals

Research Goals and Approaches: ORD is researching ways to design more appropriate toxicology studies of *in utero* or early postnatal exposures to endocrine disruptors and to better understand dose-response relationships. A request for applications (RFA) was released and three STAR awards were made in 2004 to support research in the area of Development and Characterization of Biological Systems for Studying Low Dose Effects of Endocrine Disrupting Chemicals. "Low-dose" refers to doses below those that are typically used in standard laboratory animal toxicity testing or that approximate environmentally relevant exposures.

1. STAR Grant- Georgetown University
Researchers are examining the hypothesis that *in utero* exposure to low doses of cadmium on estrogen-like effects in the hypothalamic-pituitary-gonadal axis and consequently alters the onset of puberty, predisposes to obesity, and accelerates the development of the mammary gland in female offspring. First, the dose-response relationship between *in utero* exposure to cadmium on the onset of puberty, weight gain, and mammary gland development are being determined. Then the mechanisms by which *in utero* exposure to cadmium alters the onset of puberty, alters weight gain, and accelerates the development of the mammary gland will be determined by evaluating biochemical and molecular signals.

Collaborative research on exposure and effects of thyroid toxicants on brain development
Two STAR investigators are examining the dose-response relationships of thyroid toxicants and are working collaboratively under cooperative agreements with ORD scientists. (See details on linked theme **1.2.1**)

2. STAR Cooperative Agreement: University of Massachusetts - Amherst
Researchers at UMass and ORD are identifying the critical factors affecting the shape of the dose-response relationship between thyroid hormone and developmental endpoints of thyroid hormone action. The working hypothesis is that small changes in circulating levels of thyroid hormone produce non-linear dose-dependent effects on specific endpoints of thyroid hormone action in the developing brain, heart and liver. Experiments are examining the sensitivity of endpoints to thyroid hormone insufficiency, comparing the dose-response of endpoints in different tissues to the same toxicant, and determining whether changes in tissue metabolism of thyroid hormones can account for the differences in dose-response.

3. STAR Cooperative Agreement: University of Georgia

Researchers at UGA and ORD are combining experimental results (U. Mass, ORD) into biologically-based pharmacokinetic (BBPK) models for the thyroid hormone axis in developing rats, adult male rats, and humans. This research is expanding a recent modeling effort to describe inhibition of uptake of radiolabeled iodide in the pregnant rat/fetus by including the thyroid axis feedback systems. Model predictions for effects of low doses of thyroid toxicants are being correlated with experimentally determined dose-response data on serum and tissue markers of thyroid status, developmental neurotoxicity endpoints, thyroid hormone levels in serum and tissue, and compensatory mechanisms of thyroid hormone secretion/metabolism by the thyroid, brain, and liver.

Impact and Outcomes: Research on the nature of dose-response curves in the low-dose region for exogenous endocrine active substances is needed to make more informed judgments about appropriate toxicology study designs and how to apply these data in risk assessments. An improved understanding of the mechanisms of action by which endocrine-active agents exert their effects will help determine whether existing toxicological testing protocols are valid. Data and models generated by this research will improve extrapolation of animal data to humans and identifying a set of endpoints that could serve as a general screen for endocrine toxicity of estrogen or thyroid disruptors. Overall, the results of this research will increase the understanding of how environmentally relevant concentrations of endocrine toxicants relate to human health and disease.

Cellular And Molecular Mechanisms For Abnormal Development (see theme description below for 1.3.2 which has elements that fit under this APG as well)

APG - Provide OPPTS, OW, the Regions and other organizations with systems and models to test and predict vulnerability of the neuroendocrine system to contaminant-induced effects – FY 2013

1.2.1 *Low Level Thyroid Hormone Disruption: Nervous System Development and Environmental Mixtures*

Research Goals and Approaches: Research is using a multidisciplinary approach to address a number of uncertainties in the risk assessment of chemicals that disrupt the thyroid axis. The overall goal is to develop animal models that mirror the neurodevelopmental outcomes of concern in humans by assessing the risk at low levels of exposure. Quantitative and qualitative relationships between developmental thyroid hormone insufficiency, brain structure and function, short-term *in vivo* screens, and adverse outcomes in humans will be discerned. Specific aims of this theme include: 1) defining degree of TH insufficiency associated with adverse neurodevelopmental outcomes, 2) determining the ability of short-term dosing models to predict the relationship between alterations in serum hormones and consequent developmental neurotoxicity, 3) characterizing and quantify the degree to which animal model systems predict thyroid hormone disruption in humans, 4) determining if risks associated with exposures to mixtures of thyroid hormone disruptors are cumulative, and 5) developing biologically-based pharmacokinetic and pharmacodynamic models for thyroid hormone disrupting chemicals.

Research is addressing adverse health outcomes induced by thyroid hormone insufficiency during early development. The overall goal is the development of pharmacokinetic and pharmacodynamic (PBPK/PBPD) models that improve cross-species and low-dose extrapolation and relate adverse developmental consequences to short term *in vivo* screens. ORD's intramural research is conducted in collaboration with STAR grantees (see theme **1.1.1**). Through the cooperative agreement ORD and grantees are evaluating the utility of biomarkers of thyroid hormone responsiveness in the developing brain as indicators of developmental neurotoxicity by prototype thyroid hormone disruptors, and placing these in

the context of a biologically-based dose-response (BBDR) model of thyroid hormone disruptors. In this project, STAR grantees measure molecular changes and serum and tissue hormone levels, while ORD evaluates behavioral, physiological, and anatomical effects. This mammalian work is aligned with efforts in an ORD ecological laboratory in which molecular and biochemical methods are under development to assess thyroid gene expression in an amphibian model, a research theme in the SP2 research program (SP2 theme **1.2.3**) that is partly funded through the Comp Tox research program.

Additional collaborations include research on structural and functional changes in brain with early thyroid insufficiency and research on mixtures of thyroid disrupting chemicals. Together, these efforts will provide the framework for a cross-species model of thyroid hormone disruption that will enable more accurate human extrapolations of animal data. In addition, data generated by this project will be used to inform the "Virtual Liver Project" in the Human Health research program (LTG 1 research track 1).

Impact and Outcomes: This research program will directly address a number of issues important to the EDCs research program as well as other Agency programs, as described below.

Reducing Uncertainties of Low Level Thyroid Hormone Disruption: This project will identify sensitive and predictive biomarkers of effect. Defining the degree of TH insufficiency associated with changes in these biomarkers will provide a benchmark response for determining a point of departure for use in risk assessment.

Mixtures Research: Initial work determined that additivity theory (e.g., TEF-like approaches) predicts the effects of a complex mixture of EDCs on thyroid hormones. These findings support the Agency's default assumption that cumulative risk is predicted by dose addition holds true for thyroid hormone disrupting chemicals. This work also informs a similar concern in other research and regulatory programs (e.g., Human Health research program, Office of Pesticides, Office of Water).

Extrapolation: Use of homologous functional endpoints in rodents to model the effects of concern in humans provides a common basis for comparison of effects across species. Results will inform predictive quantitative models relating hormone disruption to neurological outcome at different life stages in rats and humans (see also HH LTG 1 research track 1, Virtual Liver Project).

APG - Provide OPPTS, OW, the Regions and other organizations with data from the development and application of high throughput and molecular approaches, including 'omics , and computational tools for defining mechanisms of action, extrapolation across species and improving assessments for EDCs – FY 2013

1.3.1 An Approach to Using Toxicogenomics Data in Risk Assessment: A Dibutyl Phthalate Case Study

Research Goals and Approaches: Research is developing an approach for using toxicogenomics data in an EPA health assessment and performing a case study based on this approach. A case study is being performed on dibutyl phthalate (DBP), focusing on the male reproductive developmental effects. Using EPA's IRIS external peer review draft DBP assessment as the starting point, the case study focuses on two questions: Does the DBP toxicogenomics data 1) further define the mode(s) or mechanism(s) of action for the male reproductive developmental effects? and 2) inform interspecies extrapolation? The modes of action that explain some of the male reproductive developmental effects observed after *in utero* DBP exposure in rodents are reduced fetal testicular testosterone production and *insl3* gene.

Recognizing the future use of toxicogenomics data in risk assessments, as well as the limitations to the technologies, the research in this theme begins to address how toxicogenomics information can be used in risk assessments, whether toxicogenomics data inform one or more steps (e.g., dose-response) in the risk assessment process, how the current issues with microarray technologies (e.g., reproducibility) can be taken into account, and how toxicogenomics data can be used in conjunction with other types of information (e.g., toxicity data).

Impact and Outcomes: Results of this research are developing generic approaches for utilizing toxicogenomics data and further mining the raw data for use in risk assessment defined in this study that can be applied to new EPA (or other organizations) chemical assessments. Further research will be stimulated as a result of identifying specific needs for toxicogenomics studies for use in risk assessment. The specific case study used improves the Agency's IRIS assessment on DBP by incorporating the toxicogenomics data.

1.3.2 Cellular and Molecular Mechanisms of Abnormal Reproductive Development

Research Goals and Approaches: Research is characterizing the cellular and molecular mechanisms of abnormal reproductive development following exposures to EDCs through a series of integrated studies that have been designed to address a number of the well-recognized data gaps including to: 1) identify low dose effects, 2) characterize the shapes of the dose response curves (this element of the research fits under the APG for this topic above), 3) identify sensitive endpoints that are diagnostic of specific modes of action, 4) identify critical endpoints for risk assessments, 5) identify cellular and molecular mechanisms of action that can be linked to the adverse effects, 6) develop protocols to assess alterations of reproductive development that are more sensitive and predictive than current screening and testing assay, and 7) study the effects of mixtures of chemicals with both common and different modes of action when administered during critical developmental states of life. Some of these efforts are building upon previous research conducted through the laboratory (see Accomplishments Appendix). The culmination of these studies is the development of a framework for cumulative risk assessments with chemicals with diverse modes of action that disrupt reproductive development.

Impact and Outcomes: Results of these studies are providing OPPTS, OW, the Regions and other organizations with data needed to address some critical areas of uncertainty regarding EDCs and how the data should be incorporated into human and ecological risk assessments. The research in this theme is building upon a long history of successful data that have already been used in Agency risk assessments (see Accomplishments Appendix). Results from upcoming studies on mixtures using compounds will be used to develop a framework for cumulative risk assessments with chemicals with diverse modes of action that disrupt reproductive development. This will provide the Agency and other risk assessment organizations with a model to integrate data from multiple exposures and possibly multiple modes of action.

1.3.3 Biomarker Identification in the Testis and Epididymis following EDC Exposure during Reproductive Development

Research Goals and Approaches: Research is identifying new downstream (i.e., post steroidogenesis) biomarkers in the 'pathways to phenotype' observed following low dose gestational exposure to phthalates by conducting proteomic evaluations during reproductive development. Ideally, these biomarkers of effect will be sensitive and predictive of reproductive alterations (i.e., Leydig cell hyperplasia, CIS cell formation, decreases in sperm quality, reduced fertility, germ cell depleted seminiferous tubules) seen in the adult animal long after exposure ceases. The underlying hypothesis is that the Leydig cell hyperplasia and incomplete spermatogenesis observed following phthalate exposure is due, at least in part to, alterations in

paracrine factors in the testis. As such, it is anticipated that this project will elucidate low dose proteomic effects of phthalate exposures on the Leydig cells, and that one or more of the protein alterations will be attributed to protein secreted by the Leydig cell and/or the seminiferous epithelium.

Following an initial in vivo 'discovery' phase, the target cells/tissues will then be established in vitro, i.e., Leydig cell cultures, seminiferous tubule cultures, epididymal tubule fragment cultures. This in vitro effort parallels the in vivo effort and will essentially serve to 'validate' protein bio-indicators of effect, as well as provide the groundwork for an in vitro model(s) for early screening of EDC activities. Once protein bio-indicators of effect are identified following quantitative 2D SDS-PAGE and mass spectrometry, antibodies will be generated. These antibodies can then be incorporated into either in vivo or in vitro screening protocols. For example, cell lines which express these proteins can be 'screened' using new whole cell western technology.

Impact and Outcomes: Research from this theme will provide completely new information regarding the fate of phthalate exposures in the male reproductive tract alterations following low and moderate exposures to two phthalates on EDC modes of action that can be used by OPPTS and the general scientific community to improve risk assessments for these chemicals and possible other male reproductive toxicants. The research will help elucidate whether it may be that decreased testosterone is pivotal to some aspects of the phenotype, while events secondary to the decrease in testosterone (i.e., paracrine signal alterations) are pivotal to other aspects of the phenotype. It is expected that the proteomic evaluation will better clarify the 'pathway to phenotype' that may be common to developmental (i.e., Leydig cell hyperplasia) and adult phenotypes (i.e., incomplete spermatogenesis) and that are likely a function of exposure levels to these EDCs. Finally, this research will help address the uncertainty regarding the consequence of either low or moderate exposures prenatally on sperm function in the adult.

1.3.4 Short Term Methods for Evaluating Thyroid Hormone Disruption Using Molecular and Biochemical Endpoints in an Amphibian Model

Research Goals and Approaches: Research is developing a more efficient and diagnostic test of thyroid hormone disruption using molecular and biochemical methods. Previous work by this laboratory, in collaboration with international laboratories, has led to the development of a 21 day assay for thyroid hormone disruption using *Xenopus laevis*. This assay relies primarily on alterations of development and thyroid histology as its primary endpoints. It is currently undergoing validation through the OECD's efforts to harmonize testing guidelines for endocrine disruptor screening and testing. Subsequently, this laboratory has been working on developing more in depth information at the molecular and biochemical levels as part of an effort funded by the Computational Toxicology initiative to establish a basic thyroid systems model (SP2 MYP theme **1.2.3**). One of the major objectives of this work is to establish molecular and biochemical endpoints that would be amenable for use in an abbreviated assay format that would reduce costs, improve throughput, and improve diagnostic specificity.

The premise of this work is that several candidate molecular and biochemical endpoints have been identified through previous and on-going research efforts that may serve as a basis for an abbreviated testing format for thyroid hormone disruption. ORD has developed methods to assess thyroid gene expression by quantitative PCR and microarray analysis, to measure circulating and pituitary TSH, to measure circulating T3 and T4, and to measure iodinated chemicals in the synthetic pathway of thyroid hormone. These measurements are being applied to samples from *in vivo* exposures to selected thyroid hormone synthesis inhibitors to determine the temporal behavior and magnitude of change of these various endpoints. These studies will establish the time course and limits of the abbreviated protocol. Subsequently, a list of chemicals will be identified that potentially disrupt normal thyroid hormone synthesis, based upon

the literature and structural considerations. These chemicals will be tested using the abbreviated protocol to determine if they elicit responses that suggest anti-thyroid activity.

Impact and Outcomes: OPPTS is completing the validation of the initial Tier 1 screening assay developed by ORD for thyroid hormone disruption for consideration as part of the EDSP battery of assays. However, the duration and cost of the initial screening assay suggest that its use may be limited. Therefore, a quicker, cheaper, yet more diagnostic alternative is needed. When completed, products of this theme may provide the Agency and the international regulatory community with such an alternative.

1.3.5 Interspecies Extrapolation: Species Diversity in Ligand Binding and Gene Expression using Recombinant-derived Steroid Hormone Receptors

Research Goals and Approaches: Research is being conducted to characterize species diversity in ligand binding and gene expression with steroid hormone receptors to address the extent to which cross-species extrapolation is relevant and appropriate. Research is initially determining the extent to which ER and AR are present in a variety of animal species and if so the extent to which they bind endocrine disruptors.

Previous ORD research led to the development of estrogen and androgen-responsive stable cell lines which contain an ER (T47D-KBluc) or AR (MDA-KB2) inducible luciferase reporter gene. Because the stable cell line methods have ease of use advantages and have the potential to be used in a high through-put mode they are being further assessed, through a contractor, to evaluate their use, along with ER and AR competitive binding assays, in a prescreening strategy which might be used to prioritize chemicals for additional testing. .

Research is also developing steroid receptor binding and gene expression assays using recombinant derived ER and AR in order to eliminate the need to isolate these receptors from animal tissues. ORD research is developing cell based gene expression assays using recombinant human receptors which can distinguish estrogens from anti-estrogens and androgens from anti-androgens (see Accomplishments Appendix). Furthermore, ORD is the lead laboratory on the development on a binding assay protocol using recombinant ER in an international effort between OPPTS, ECVAM, OECD and Japan (CERI).

Impact and Outcomes: Through this theme, ORD is trying to develop AR and ER binding assays from representative species from several classes of vertebrates and invertebrates. Data will help determine to what extent there is homology across the species. These results will enable EPA and other regulatory programs around the world make better informed decisions regarding which species should be used as test models for EDCs and to what extent results from those models could be extrapolated to other species. These data could lead to the reduction of animals and species that would need testing. Further, these cross species comparisons will facilitate the identification of EDCs that may differentially affect specific species and aid in the development and support of future EPA risk assessment decisions.

1.3.6 Regulation of Aromatase (CYP19) as a Mode of Action for Endocrine Disrupting Chemicals (EDCs) in Rats and Fish

Research Goals and Approaches: Research is investigating whether modulation of the enzyme aromatase is a basic toxic pathway by which EDCs exert reproductive effects. Aromatase, a product of the CYP19 gene, is the enzyme that catalyzes the conversion of androstenedione and testosterone to estrone and estradiol during steroidogenesis. To date, the range of chemicals that may cause abnormal regulation of aromatase activity, as well as the physiological impact in multiple species, have not been determined. This

research theme builds upon the results of previous research conducted in ORD's laboratories (see Accomplishments Appendix).

In the short-term, research is using atrazine as a case study for an EDC that appears to modulate aromatase activity differently in different species and tissues. The central focus is on answering why differences in aromatase activity occur and what they might mean for species susceptibility to adverse effects from exposure to atrazine. Research is also investigating the role that species' differences in metabolism of atrazine and related pesticides (simazine, propazine) play in the ultimate effects on reproductive processes in diverse vertebrate species. In the long-term, research is focusing on providing insight into the importance of modulation of the aromatase enzyme as a common toxic pathway of EDC action in different tissues and across diverse species (*i.e.*, rat and fish) and to determine whether changes in aromatase activity are predictive of reproductive impacts.

Impact and Outcomes: Results of this research will elucidate issues that need to be considered when doing *in vitro* to *in vivo* extrapolations and across-species risk assessments. It is important for the Agency to understand the implications of new reports that some environmental chemicals may up-regulate aromatase activity in fish and frogs for two reasons: (1) an up-regulation of aromatase gene expression or alteration in substrate availability is a newly characterized EDC mode of action that will likely have adverse physiological impacts on all species, and the question will be asked if the current *in vitro* and *in vivo* assays in the EDSP are adequate to detect these changes; and (2) because the regulation of the aromatase gene expression varies between target tissues within a species, as well as between species, there may be a need for additional methods to evaluate EDCs for potential effects on aromatase activity.

Data from these studies will be useful (1) for understanding whether a change in aromatase activity represents a common toxic pathway for the action of EDCs; (2) for understanding the extent to which EDCs disrupt tissue-specific aromatase activity in each species; and (3) for comparing the relative sensitivities of reproductive and developmental processes in two different vertebrate species to perturbation by aromatase-modulating EDCs. Additionally, since an *in vitro* placental aromatase is being considered as an assay for the Tier 1 Screening Battery of EDSP, data from this theme could be used as a basis of comparison of the *in vitro* assay compared to data obtained from *in vivo* studies.

APG -Provide OPPTS, OW, the Regions and other organizations with new exposure assessment and risk management tools to characterize and reduce exposure to EDCs – FY 2013

1.4.1 Develop and Evaluate Methods for Characterizing Environmental EDC Exposures

Research Goals and Approaches: Research is developing and/or refining chemical and molecular methods for selected EDCs and endocrine-active pharmaceuticals that can be used to qualitatively and/or quantitatively measure indicators of exposure in real-world aquatic environments. The high quality data produced using these exposure methods will be used to characterize selected sources, the fate of the chemicals once released in the environment, and for developing tools for reconstructing exposures and apportioning exposure sources.

ORD is developing chemical and molecular indicators of exposure on the highest priority pharmaceuticals identified in the bioinformatics report "An Informatic Approach to Estimating Ecological Risks Posed by Pharmaceutical Use." Chemical methods are being developed and controlled laboratory exposures conducted to identify molecular markers of exposure. Chemical methods are also being developed to characterize EDCs and endocrine-active pharmaceuticals in water, sediment, and tissues. Current pilot field

measurement projects designed to evaluate EDC exposure methods under real-world environmental conditions (CAFOs, wastewater treatment plants, experimental stream facility exposures, Ohio River, South Branch and mainstream of the Potomac). Future pilot studies are being planned for validating the new/refined methods.

This research is complementary to efforts conducted through the STAR program (see theme **2.2.2** for details)

Impact and Outcomes: Research will provide:
1) A suite of validated sampling, analytical and molecular methods will be provided to State, Regional, and Program Office scientists for their use in characterizing real-world EDCs exposures (including some pharmaceuticals) in aquatic ecosystems and apportioning these exposures to their major sources.
2) A systems approach will be developed to characterize and better understand how selected non-chemical stressors influence exposures to individual and mixtures of multiple EDCs in aquatic environments.
3) Innovative bioinformatics approaches will be developed for prioritizing future EDCs exposure research activities.

1.4.2 Removal of EDCs by Drinking Water Treatment Processes

Research Goals and Approaches: Research is providing information on the ability of conventional and advanced drinking water treatment processes to remove EDCs from source waters and subsequently reduce human exposure to EDCs.

A number of the chemicals identified as potential EDCs may be present in surface or ground waters used as drinking water sources due to their introduction from domestic and industrial sewage treatment systems and wet-weather runoff. Many of these compounds have already been shown to be present in surface waters in the U.S., leading to a growing concern over the possible presence of EDCs in drinking waters. Although there has not yet been a determination of risks posed by EDCs in finished waters, it is prudent to explore if strategies already employed to manage other drinking water risks can also manage risks associated with EDCs.

This research project has three components. The first is the development of appropriate analytical methods to identify and quantify the EDCs being evaluated in the project. Analytical methods for steroid hormones and for a group of alkylphenolic compounds have been developed. The second component is the application of a reporter gene assay(s), such as the MVLN assay, to evaluate the presence of estrogenic activity in water samples. The MVLN assay complements the analytical work, by detecting the presence of compounds with estrogenic activity, including those that may be missed analytically and can be used to estimate the level of estrogenic compounds present in water samples before and after treatment.

The third part of the study is to evaluate the removal of EDCs by various unit processes used in conventional and advanced drinking water treatment. Bench-scale experiments (jar tests) have been conducted to evaluate the ability of coagulation, alone and in combination with PAC or chlorine, to remove three estrogens from Ohio River water. Additional analytical and bioassay work is planned to characterize the chlorination by-products of the estrogens.

Full-scale drinking water treatment plant unit operations and integrated plant treatment will be characterized with respect to EDC removal and will include distribution when possible. To conduct this research, additional analytical instrumentation and improvements to current analytical methods will be required. Work in this research area will be integrated, or allow for, similar work being conducted by others in the

field, such as USGS and academia. Every effort will be made to leverage against existing research in this area and to avoid unnecessary redundancy.

Impact and Outcomes: This research is providing information on the ability of various drinking water treatment technologies to remove EDCs that may be present in source waters. The initial phase of this work, the determination of the treatability of reproductive hormones, was conducted at the request of, and partially funded by the Office of Ground Water and Drinking Water (OGWDW). The results of this research will be used by OGWDW, the states and municipalities, and drinking water utilities for selecting optimal EDC removal processes and subsequently reducing human exposures to EDCs.

1.4.3 Wastewater Treatment/Biosolids

Research Goals and Approaches: Research is determining to what extent biosolids are a contributing source of EDCs into the environment and characterizing their fate once land-applied. Research is also evaluating existing risk management strategies for the disposal of biosolids and beginning to evaluate alternative risk management options.

In the US, over 3 million dry tons of treated sewage sludge (or biosolids) are applied on agricultural lands. Recently, questions have arisen about the risks of this practice with concern that current practices are not sufficiently protective of pathogen and chemical exposures. Biosolids may contain significant quantities of steroid hormones, alkyl phenols, and other endocrine disrupting chemicals. Through land application, these chemicals may be introduced into the environment where they may impact surface and ground water, soil, and sediments. To evaluate these questions, the concentrations of pathogens, other microbes, nutrients, and EDCs are being measured before and after biosolids application to land. The persistence, transport, and fate of these analytes are being studied and when possible, kinetic rates are being calculated to characterize the system. This research is conducted in conjunction with ORD's Water Quality Research on biosolids and has laboratory and field components.

Impact and Outcomes: The regulations which govern the land application of biosolids were promulgated in 1993. Since that time, methods for treating and handling biosolids have evolved. In 2002, the National Research Council (NRC) advised that the regulations should be updated, including a determination if additional chemicals should be regulated. Based in part on this recommendation, EPA will evaluate current regulations and revise them as needed. Research on EDC persistence, transport, and fate following land application of biosolids will facilitate science-based decisions in these regulations.

1.4.4 Natural and Synthetic EDCs from Wastewater Treatment– Source Characterization, Environmental Fate, and Risk Management

Research Goals and Approaches: Research is developing tools to determine: 1) the fate of EDCs in conventional wastewater treatment plants; 2) if typical wastewater plant design and operational strategies maximize removal of EDCs; and 3) the treatment capability of on-site wastewater treatment systems for EDCs.

ORD's risk management research on EDCs is determining the efficacy of existing risk management approaches to minimize exposure to suspected EDCs and developing new risk management tools where needed.

Reports in the literature demonstrate that wildlife in surface water near wastewater treatment outfalls can show effects consistent with exposure to estrogenic compounds. Other reports show that certain suspected

EDCs, especially reproductive hormones and estrogenic alkylphenols are commonly present in wastewater treatment effluents and associated surface waters. These compounds are removed to varying degrees during WW treatment and are therefore, commonly found in the effluents following wastewater treatment.

The research on evaluating both existing and innovative risk management strategies incorporates bench, pilot, and field scale investigations. Research is evaluating the fate of estrogenic and androgenic hormones and characterizing their biodegradation rates under redox conditions typically found in WWTPs.

At the pilot scale, two pilot plants have been constructed and are operated to simulate a municipal WWTP. The plants are fed a simulated wastewater with constant dosing of EDCs to allow a mass balance analysis of the plant and the individual unit processes. Research has also been initiated at the full plant scale. A collaborative project among several ORD laboratories, Region 5, and a regional wastewater utility is evaluating the digesters' efficacy to study alkylphenols, hormones, and bisphenol A.

A second focus of this research is determining techniques to optimize existing management strategies or develop alternative management strategies. Once unit operations and technology performance are understood, engineering solutions can be developed to reduce the EDCs discharge. Additional research is being developed in the areas of on-site WWT technologies. These technologies include septic systems, constructed wetlands, and other on-site technologies.

Impact and Outcomes: The results of this research will be used to help WWT operators understand the capability of their plants to remove EDCs, how process variables influence performance, and how to improve the operation of their plants to minimize effluent levels of EDCs. In the future, if EPA concludes that EDCs in effluents must be regulated, the Office of Water will require performance information on conventional and innovative treatment to make regulatory determinations.

1.4.5 Developing Tools to Characterize CAFOs as Sources of Environmental Estrogens

Research Goals and Approaches: Research is developing tools to: 1) determine concentrations of estrogens and estrogen conjugates in lagoon effluents used for land application of manure from different types of CAFOs; 2) evaluate whether land application of CAFO manure represents a significant route for introduction of estrogens into ground waters; 3) assess long-term fate of estrogens in ground water negatively impacted by land application of CAFO manure; and 4) ascertain whether additional risk management steps are required.

Some of the most potent EDCs include both natural and synthetic estrogens, which are known to be used or naturally produced by the three major categories of CAFOs - cattle, poultry and swine. ORD researchers have found that swine lagoon effluent can contain high levels of estrogen conjugates that are expected to be much more soluble and mobile than the free estrogens. Very few studies address estrogen conjugates, and hence the overall risk of CAFO contributions of estrogens to the environment may be underestimated.

Three projects are contributing to this research theme: 1) CAFO Lagoon Survey, 2) EPA Region 4 RARE Project, and 3) ORD Integrated Research Effort on CAFO Hormones. Additional projects may be identified later that can also contribute.

- CAFO Lagoon Survey – Through the Water Quality Research Program, several different types of CAFO lagoons at multiple locations are being sampled to provide a comprehensive characterization of chemical and microbial parameters. Estrogen conjugates are being included into that analytical suite. Target operations include a dairy, two wet poultry operations, three types of swine operations, and

possibly a beef feedlot. Emphasis is placed on obtaining samples that are representative of the lagoon effluent used for land application for CAFO manure, and these data will be used to assess the total estrogen load being released into the environment from these practices.

- EPA Region 4 RARE Project – A Regional Applied Research Effort (RARE) project is underway to evaluate the use of stable isotopes for source tracking of nitrate in ground waters impacted from dairy and poultry CAFOs. Estrogens and estrogen conjugates in those ground water samples found to be impacted from CAFO operations are being analyzed. Six to ten sites in Region 4 are being selected for study. These data will be used to help determine the potential for ground water impact form CAFO-derived estrogens.
- ORD Integrated Research Effort on CAFO Hormones – Researchers from this theme are contributing to the cross-ORD project by focusing on estrogens, estrogen conjugates, and ground water impacts. See theme **2.2.1**.

Impact and Outcomes: ORD's research is developing the tools necessary to assess the risk that CAFOs pose for the release of hormones into the environment. This research is intended to provide sufficient information for EPA to decide:

- is risk too great based on environmental data?
- do more sites need to be studied?
- can we make recommendations for land management strategies based on what we have?
- can we use these tools to assess effectiveness of new land management strategies?

The ultimate impact of this research will be to determine whether CAFOs provide sufficient risk so as to require regulation or management to minimize release of hormones.

Long Term Goal 2:
Determination of the extent of the impact of endocrine disruptors on humans, wildlife and the environment to better inform the federal and scientific communities

APG - Provide OPPTS, OW, the Regions and other organizations with data to determine the potential for adverse developmental/reproductive effects in human populations following exposures to EDCs – FY 2011

2.1.1 Endocrine Disruptors: Epidemiologic Approaches

Research Goals and Approaches: Research is supporting epidemiologic studies on the impact of endocrine disruptors on human development and reproduction. In 2000, EPA, the NIOSH, NIEHS, and NCI announced a joint program to support research on exposure to endocrine disruptors and adverse health effects in humans, with a focus on epidemiologic approaches. Effects of interest included reduced fertility or altered reproductive function, pregnancy outcomes and developmental abnormalities of offspring of exposed women, hormonally mediated cancers among offspring exposed *in utero*, and endocrine related malignancies. Study designs that clearly differentiated exposure categories and used quantitative exposure assessment methodologies were of special interest.

Collectively, twelve awards totaling almost $19 million were provided for studies to be conducted over a period of three years. Given the nature of this complex research, a number of the researchers requested and received no-cost extensions. The epidemiology studies are investigating effects on reproduction and development and other health outcomes among exposed subjects or their offspring. Chemicals under study include dioxin compounds, polychlorinated and polybrominated biphenyls, heptachlor, DDT/DDE, and other polyhalogenated persistent pollutants, perfluorooctyl compounds, and phthalates. Investigative teams are using a variety of study designs, and methods to measure and quantify exposure and to identify susceptibility, including biomarkers and the evaluation of gene-environment interaction. All of the studies will develop a quantitative estimate of risk of health effects associated with exposure.

EPA funded the following five studies, all of which are still ongoing:

Endocrine Disrupting Chemicals and Thyroid Outcomes
Henry Anderson, Wisconsin Department of Health and Family Services, STAR Grant No. R830254
Follow-up of a cohort of frequent and infrequent consumers of sport fish contaminated by polybrominated diphenyl ethers (PBDEs), DDT, and PCBs from the Great Lakes. Recruitment is ongoing in Wisconsin, Michigan, Illinois, and Ohio for assessment, by questionnaire and in serum samples, of dietary and occupational exposure to PBDE, PCBs and other environmental contaminants. Exposure measures are examined in relation to serum thyroid parameters and reproductive hormone levels.

Latent Effects of Gestational Exposure to Heptachlor
Dean B. Baker, MD, MPH, University of California, STAR Grant No. R829439
Follow-up study of offspring of women living on the Hawaiian island of Oahu during 1981-1982 who drank cow's milk contaminated with heptachlor epoxide and a nonexposed comparison group. The relation of exposure, based on birthplace and modeled estimates of gestational heptachlor epoxide exposure, with biological indicators of reproductive and immune function will be evaluated. Recruitment and data collection are continuing.

Dioxins, Male Pubertal Development and Testis Function
Russ Hauser, MD, MPH, ScD, Harvard School of Public Health, STAR Grant No. R829437
Prospective cohort study of boys with prenatal and postnatal exposure to dioxins in a region of Chapaevsk, Russia with environmental contamination by area chemical plants. Researchers will evaluate the association of dioxin and dioxin-like compounds in serum with growth and the timing of pubertal development. Recruitment and data collection are ongoing. In a pilot study the investigators characterized the physical growth and sexual maturation in 2579 boys, ages 10 through 16.99 years residing in Chapaevsk, Russia in order to establish region specific reference data. Chapaevsk boys are thinner than both U.S. and Russian boys, and have a later onset of puberty and attainment of sexual maturity than boys from other countries (JPEM **16**, 169-178 (2003)). The pilot study also found that serum dioxin levels are higher in these boys compared to children living in Europe, Japan, or the United States. Predictors of serum dioxin concentration were evaluated. Serum dioxin concentrations are most highly predicted by age, consumption of local non-chicken meat and local fish.

Persistent Organic Pollutants and Endometriosis Risk
Victoria Holt, PhD, Fred Hutchinson Cancer Research Center, STAR Grant No. R829438
Case-control study among members of a large health maintenance organization evaluating modification by polymorphisms in estrogen metabolism genes (GSTM1 & COMT) and metabolic enzymes (CYP1A1 & 1A2) of the effect of chemical exposure on risk of endometriosis. Preliminary analyses indicate that self-reported exposure to herbicides and fungicides is associated with a 65% and more than two-fold increase in endometriosis risk, respectively. Genetic polymorphisms do not appear to modify these risks. Investigators are evaluating serum levels of several organochlorine compounds, as well as a methoxychlor metabolite in urine, in relation to endometriosis risk in this cohort.

Phthalates in Pregnant Women and Children
Shanna H. Swan, PhD, University of Missouri-Columbia (now at University of Rochester), STAR Grant No. R829436
Phthalate metabolite concentrations in urine of mothers during and after pregnancy and their babies enrolled in a pregnancy cohort study in four U.S. cities. Recruitment and data collection is ongoing. Early analyses of about half of the cohort indicate that phthalate levels are detected above the limit of detection in at least 50 % of samples. Phthalate levels in mothers' urine vary by geographic region and are associated with poor semen quality in the fathers. Preliminary analyses indicate that phthalate levels are inversely associated with anogenital distance (AGD) in males, a finding consistent with what is seen in phthalate-exposed rodent models. AGD is a sensitive indicator of masculinization in rodents. This study showed that AGD was shorter in baby boys whose mothers had higher concentrations of four commonly used phthalates in their prenatal urine (EHP 113:8, 2005, pp. 1056-1061).

Impact and Outcomes: This interagency research effort represents the largest portfolio of coordinated federal funding looking at the impact of endocrine disruptors on humans. It is producing a body of work that will contribute significantly to the state of scientific understanding about human exposure and health responses to suspected endocrine disrupting chemicals found in the environment. Policymakers and scientists will use the results of these studies to identify questions for future research, to inform risk assessments, and to inform decisions designed to protect public health.

APG - Provide OPPTS, OW, the Regions and other organizations with information from the application of cross-disciplinary tools to characterize the occurrence and potential biological effects from and develop management approaches for EDCs in complex environmental media – FY 2012

2.2.1 Assessment of the Occurrence and Potential Risks of EDCs in Discharges from Concentrated Animal Feeding Operations

Research Goals and Approaches: Research is an integrated and cooperative ORD effort involving scientists from the three ORD National Laboratories, and extramural researchers funded through the NCER STAR grants program. The overall goal of the project is to characterize the magnitude and extent of the impact of estrogenic and androgenic hormones in waste from CAFOs and determine the impact of current CAFO waste management strategies on the fate and effects of hormones.

EPA investigators and STAR researchers are collaborating to:

- Develop robust *in vitro* and analytical methods to identify and quantify compounds responsible for endocrine (e.g., androgenic, estrogenic) activity of complex CAFO discharges.
- Identify ecologically-relevant biomarkers, in aquatic species (primarily fish), of exposure to estrogenic/androgenic CAFO discharges through use of state-of-the-art genomic approaches
- Evaluate the environmental fate, transport and metabolism of CAFO-derived EDCs relative to occurrence in surface and ground waters.
- Assess possible ecological impacts of EDCs from CAFOs using a combination of laboratory and field studies.
- Evaluate capability of existing risk management technologies for CAFOs to reduce exposure to EDCs.
- Characterize the magnitude and extent of the impact of hormones released by CAFOs and determine the impact of current CAFO waste management strategies on the fate and effects of hormones.

Specific tasks (by lab and center) include:

(1) Development of validated analytical methods for detecting a suite of natural and synthetic steroids with potential endocrine activity. (NRMRL, NHEERL)
(2) Identification and optimization of *in vitro* techniques designed to detect biological activity of complex mixtures containing both "known" (i.e., analytically-detected) and unknown endocrine-active substances. (NHEERL)
(3) Application of methods developed under (1) and (2) to evaluate the fate, transport and metabolism of biologically-active steroidal hormones found in different types of CAFOs. (NRMRL)
(4) Application of biologically-based fractionation (also called TIE or toxicity identification evaluation) techniques to aqueous samples associated with CAFOs to precisely identify the chemicals responsible for observed biological activity. This information is needed for the identification/development of appropriate remedial/control options for risk management. (NHEERL)
(5) Development and validation of biomarkers in aquatic animals indicative of exposure to CAFO discharges. These endpoints will focus on gene (transcriptome) or protein (proteome) expression, and will be developed through a combination of laboratory studies with known EDCs from CAFOs, and field studies with actual CAFOs. Ideal biomarkers would be indicative of (and diagnostic for) both exposure to endocrine active substances and adverse effects in exposed animals. This research will initially focus on fish but could expand to other species of concern. (NERL)
(6) Conduct controlled laboratory experiments with chemicals identified in (4), as well as field studies with caged or natural assemblages of fish to assess the ecological risk of CAFO-discharges to populations. Endpoints considered in studies with natural populations could reflect both apical responses (e.g., sex ratios, abnormal gonadal histopathology) and diagnostic biomarkers. (NERL, NHEERL)

(7) Conduct a limited monitoring study/survey of different types of CAFOs using techniques developed under (1), (2) and (5) to determine the scope of the issue and/or types of CAFOs that present the greatest unacceptable risk(s) associated with EDC exposure/effects. (NERL)
(8) Based on the results of (1)-(7), identify remediation/control strategies for managing the risk of EDCs, such as steroidal hormones, in CAFO wastes. (NRMRL)
(9) An RFA was developed in coordination with the intramural research efforts to build upon the strengths of the intramural research and expand the ability to achieve the overall goals of the integrated project. The RFA Fate and Effects of Hormones in Waste from Concentrated Animal Feeding Operations (CAFOs) was issued in 2006 and seven grants and cooperative agreements were awarded for research to characterize the magnitude and extent of the impact of hormones released by CAFOs and to determine the impact of current CAFO waste management strategies on the fate and effects of hormones. Projects will start in 2007 and go until 2010. (NCER)

- Environmental transport and fate of natural and synthetic steroid hormones that accompany discharges and the disposal of animal wastes from cattle, dairy, swine and poultry CAFOs and how different management practices may affect fate and transport. (*U. of Wisconsin*)
- Assessing the relative amount of hormones discharged from tile-drained agricultural fields under different manure and lagoon effluent application practices, hormone persistence in fields under these conditions, and the impacts of hormone loads on aquatic organisms. (*Purdue U.)*
- Fate and transport of exogenous and endogenous hormones in cattle manure within the feedlot and after application to crop land. (*U. Nebraska*)
- Evaluating and modeling occurrence, fate, and transport of synthetic and endogenous steroid hormones released by cattle and cows from CAFOs into ground water and surface water. (*UC-Berkeley*)
- Occurrence and fate, and biological effects of steroids from agricultural application of poultry litter and the effects of different agricultural practices on mitigating environmental steroid loads. (*U. Maryland*)
- Developing a total facility estrogen budget a swine farrowing CAFO and effects and transport of estrogens following field applications. (*Duke U*.)
- Levels of hormones in broiler waste from poultry CAFOs, the effect of waste storage on hormone concentrations, and how common land application practices affect the fate of hormones found in poultry waste in soil and surface run-off. (*U. Georgia*)

To achieve tasks (1)-(6), focused studies will be conducted at a small number of CAFOs with several key characteristics, including (a) ready access for sample collection and/or field work, (b) adjacent surface and ground waters that may be affected by discharges/leaching, and (c) extant populations of animals (fish) that might be impacted by endocrine-active materials. The intramural ORD effort will initially focus on beef and dairy CAFOs where (based on previous studies) androgenic and estrogenic EDCs, respectively, would be expected to occur. Some of the intramural research is leveraged with that of the Water Quality Research Program through which research on pathogens, nutrients, and antibiotics is conducted.

Impact and Outcomes: The results of this integrated project will contribute to site-specific risk assessments and development of risk management options for hormones in waste from CAFOs. Increased knowledge of risks associated with CAFOs as potential sources for hormones in surface and groundwater, waste, and soil will be used to support the current activities of the EPA OW and Regional Offices with respect to the regulation of CAFOs. Regulations are currently being developed to control nutrient pollution from CAFOs.

In addition, some of the materials we will focus on in these studies (synthetic steroids) are regulated as veterinary pharmaceuticals. Currently pharmaceuticals in the environment, both human and veterinary, are

considered an emerging topic of environmental concern by EPA and other Federal Agencies. This work will contribute directly to developing methods for, and better defining the occurrence of, pharmaceuticals used for livestock production.

Finally, in addition to steroidal EDCs, there are a number of other materials used for livestock production that are of potential environmental concern, such as pesticides and antibiotics. The types of studies/approaches used for this work could very well be expanded to consider potential risks of EDCs.

2.2.2 Develop and Evaluate Methods for Characterizing Environmental EDC Exposures

Research Goals and Approaches: Research is developing and evaluating a suite of methods to characterize environmental EDC exposures for use in environmental or human monitoring studies. This research is complementary to that conducted under theme **1.4.1** and is supported through the extramural STAR research program. A request for applications (RFA) was solicited and five awards were made in 2006 for research on Exposure Measurement Tools for Endocrine Disrupting Chemicals in Mixtures. One of the awards was made as a cooperative agreement with ORD. The STAR research is aimed at developing innovative approaches using a combination of novel detection methods and new technologies for measuring the concentrations and activities of mixtures of EDCs in environmental and biological media.

Rapid Detection of Trace Endocrine Disrupting Chemicals in Complex Mixtures: A Full- Spectrum Deconvolution Technique with a UV-Transparent Passive Concentrator, *University of Oklahoma*
Suspected EDCs have been detected in the environment as mixtures with numerous other compounds (pharmaceuticals, personal care products, detergents, natural organic matter) and at extremely low concentrations, on the order of ng/L to m g/L, making them difficult to quantify without extensive pre-concentration procedures. The investigators are developing a method for rapid monitoring and detection of EDCs at trace concentrations in natural waters and test the method using samples of river water collected from sites where a USGS survey has previously detected multiple EDCs.

Development of receptor- to population- level analytical tools for assessing endocrine disruptor exposure in wastewater-impacted estuarine systems,
University of South Carolina
Research is producing new tools for identifying and quantifying endocrine-active contaminants in complex environmental mixtures and for defining endocrine disruptor exposure in sensitive estuarine systems. The work is complementing and extending previous and current research on development of targeted, quantitative analytical methods and bioassays for specific classes of EDCs in environmental samples from wastewater-impacted estuaries.

Developing Rapid Assessment Tools to Evaluate the Biological Effects of Complex and Biologically Active Chemical Mixtures,*Saint Cloud State University*
Research is testing the hypothesis that mixtures of estrogenic chemicals will have adverse effects on the reproductive health of exposed aquatic organisms that cannot solely be accounted for by the summation of individual effects. The researchers are surveying relevant exposure concentrations for mixtures of EDCs that include alkylphenol polyethoxylates, estrogens, and pharmaceuticals in municipal waste water.

Integrated Microfluidic System for Bioluminescent Bioreporting, Separations, Vibrational Spectroscopy, and Microcantilever Transducer Evaluation of Endocrine Disrupting Chemicals, *University of Tennessee*
Research is: (1) testing an array of EDCs in environmentally /biologically relevant combinations and matrices for surface enhanced Raman spectroscopy (SERS) response characteristics to create a

quantitatively rigorous spectral library; (2) developing nanomechanical response signatures of EDCs on microcantilever arrays (mCA) treated with molecular recognition phases (MRPs); (3) integrating bioreporter yeast into living conditions suitable for predictable function on a microfluidic platform and optimizing those conditions with electrophoretic separation parameters for EDCs; and (4) validating a fully integrated microfluidic device that houses bioluminescent reporters of EDC presence, separations, and quantitative vibrational and nanomechanical detection of EDC-containing samples of known and unknown composition in "realistic" matrices.

Systems Approach to Assessing Cumulative Exposure to Endocrine Disrupting Chemicals, *North Carolina State University*
Research is developing tools that allow for the comprehensive assessment of exposure to and effects of EDCs in order to adequately evaluate risks associated with such chemicals. EDC exposure issues are particularly tenacious since EDC mixtures can elicit toxicity through a variety of mechanisms that cannot be discerned by analytical chemistry or reporter-gene approaches. The researchers are developing a gene-expression based, whole-organism approach to evaluating cumulative exposure to EDCs, using the water flea (Daphnia magna) as a sentinel of exposure and a systematic array of gene-expression based signals that will serve as a dosimeter of exposure to EDCs individually or in mixtures.

Impact and Outcomes: Results will improve the ability to characterize how and to what degree human and wildlife populations are exposed to EDCs. The outcomes of this research will be tools and methods that can be applied to monitoring and managing human and wildlife population exposures to EDCs. While other EDC research is greatly improving our understanding of the effects of EDCs in animals and humans, the exposure tools and methods being developed under this program will improve measurement of concentrations of EDCs and relative activities of mixtures and, ultimately, the relationship between exposure and effects.

2.2.3 In Vitro Screening of Environmental Samples for Estrogenic and Androgenic Activity: Effluents from CAFOs, Pulp Mills, and Wastewater Treatment Plants, and Combustion Byproducts

Research Goals and Approaches: Research is determining whether *in vitro* assays can be used to assess endocrine activity in samples of complex environmental media as a screen for potential effects on fish, wildlife, and human health from a broad array of sources (e.g., effluents from CAFOs, wastewater treatment, and pulp mill operations, combustion wastes). ORD is participating in several collaborative projects using *in vitro* transcriptional activation and binding assays to characterize the estrogenicity and androgenicity of these types of environmental samples. Some of these samples include CAFO (concentrated animal feedlot operation) and other animal effluents collected in the field, effluents from tertiary wastewater treatment, and industrial discharge, and from combustion byproducts from diesel fuel, wood and electrical burns. ORD is evaluating environmental samples for endocrine activities in order to: 1) identify the potency of the samples, 2) attempt to identify specific chemicals in samples responsible for the endocrine activity, and 3) try to determine the potential impacts of these chemicals on fish, wildlife and human health. Due to the complex composition of these discharges, the identification of chemicals responsible for hormonal activity cannot always be performed using chemical analyses alone. For this reason, chemical identification in most cases is guided by the cell-based bioassays that can pinpoint hormonally-active sample fractions.

Much of this research is accomplished with collaborators from other laboratories in ORD, with scientists from EPA's regional offices, academia, and international research coalitions and, in some cases, is complemented by analytical chemistry and fish studies (see theme **2.2.1**). This research builds upon and expands previous efforts (see Accomplishments Appendix). Other work on the estrogenicity of combustion

byproducts from electrical burns has been extended from solely *in vitro* to include short-term *in vivo* studies (i.e., uterotropic assay).

Impact and Outcomes: This research is exploring the utility and efficiency of applying assays that had been developed to screen individual chemicals for endocrine activity to detect activity in complex mixtures from environmental media. These assays are now being optimized for evaluation of a variety of effluents from CAFOs, sewage treatment plants, and pulp mills, and combustion byproducts. The result of this research will be of particular interest within EPA to OW, OAR, and the Regions. There is great interest in this research from the water industry who would like to have a "tool box" of assays that they could use to screen their effluents for hormonal activity both before and after treatment.

Long Term Goal 3:
OPPTS uses endocrine disruptor screening and testing assays developed by ORD to create validated methods that evaluate the potential for chemicals to cause endocrine mediated effects in order to reduce or prevent risks to humans and wildlife from exposure to endocrine disrupting chemicals

APG - Provide OPPTS with standardized protocols for testing chemicals for their potential endocrine mediated effects to meet FQPA requirements – FY 2011

3.1.1 Development of an Amphibian Growth and Reproduction Testing Protocol

Research Goals and Approaches: Research is developing and standardizing an amphibian growth and reproduction full life cycle test for validation and subsequent implementation in the Tier 2 of the Agency's EDSP and through the OECD harmonization of EDC testing.

This research builds upon the previous research that led to the development of the partial life cycle testing methods for amphibian species that evaluate early embryonic development and metamorphosis for use in Tier 1 screening (see Accomplishments Appendix and links to theme **1.3.4**) However, there are no standardized tests for evaluating chronic effects on development and maturation of the amphibian HPG. Like Xenopus laevis, X. tropicalis can be routinely cultured in the laboratory, ovulation can be induced using human chorionic gonadotropin, animal stocks are readily available from commercial breeders, and has the advantage of reaching sexual maturing in a third of the time it takes X. laevis.

Research is expanding our knowledge of the sexual maturation of X. tropicalis and establishing an approach for full life cycle testing. The intent is to develop methods allowing for continuous exposure starting shortly after fertilization proceeding through sexual maturity and induced spawning. Further, research will establish the relevant endpoints for evaluating xenobiotic effects on growth and reproduction. Since it is not clear whether a full life cycle test is warranted the sensitivity of a full life cycle protocol will be compared to several partial life cycle protocols.

Impact and Outcomes: This research is in response to the need identified by the Agency advisory committees on endocrine disruptors and adopted by OPPTS recommended that the Tier 2 battery include a full life cycle assay for amphibians to assess chemicals for their potential endocrine disruption on growth and development. Once ORD completes the development and standardization of the protocol, OPPTS will validate the protocol and implement it in its EDSP. Further this work should provide alternative partial life cycle testing methods which may be more practical to perform and less costly.

3.1.2 Development of a Multi-Generation Fish Assay Protocol

Note: This research project is in the ORD Safe Pesticides/Safe Products (SP2) Multi-year Plan. It is included here because it contains aspects applicable to EDC MYP goals. Specifically, the project will provide a protocol useful for Tier II endocrine fish multi-generational testing while addressing SP2 goals of assessing the effects of chemicals on fish populations using diagnostic measures made during multiple phases of testing.

Research Goals and Approaches: The goal of this portion of the SP2 research project (theme **2.1.3**) most relevant to EDC LTG3 is to a) develop a multi-generation testing protocol using the Japanese medaka to evaluate chemical effects on reproduction, development and growth; and b) to compare endpoints and effect

concentrations noted across generations with test results from short-term assays to determine if, and how, short-term test results can be used to predict effects across generations. This project involves the development of an internationally harmonized medaka multi-generation exposure protocol that can be used to evaluate population-relevant endpoints (i.e., fecundity, fertility reproductive behavior, phenotypic and genotypic sex of each generation). A number of molecular and histological endpoints diagnostic of chemical mode-of-action are included in the short and longer-term assay to serve as a basis of comparison across tests designs and effect endpoints as well as allowing extrapolation among species.

Impact and Outcomes: This research is designed to provide *in vivo* fish assays, shorter-term and multi-generation, that can be used to characterize linkages between reproductive endpoints and diagnostic biomarkers for endocrine disrupting chemicals. Additionally, the results of short- and long-term tests using medaka will be used to characterize the utility and limitations of short-term tests to predict multi-generation effects, as well as determining how population-relevant endpoints (i.e., fecundity, fertility reproductive behavior, phenotypic and genotypic sex of each generation) are best used in predicting population effects for classes of chemicals known to affect reproductive pathways. This research will result in improved methods for acquiring information describing the quantitative relationship between chemical concentrations and adverse biological effects, and will inform how this is translated into ecological and population-level impacts.

3.1.3 Scientific and Technical Support in the Development, Standardization and Validation of Protocols for the Implementation of Tier I Screening and Tier II Testing Batteries for the Agency's Endocrine Disruptors Research Program

Research Goals and Approaches: Research and scientific support has been ongoing, since the enactment of FQPA in 1996, to develop, standardize and validate a number of *in vitro* and *in vivo* mammalian and ecotoxicological assays for use in a Tier I Screening Battery designed to detect chemicals that alter the estrogen, androgen or thyroid systems in humans, fish and wildlife. Additionally, other multigenerational studies covering a variety of taxa are undergoing the development and validation process for use in a Tier II Testing Battery. The general stages of the validation process include: (1) protocol development; (2) demonstration that each assay achieves its purpose and is reliable, leading to a standardized protocol; (3) demonstration that the standardized protocols can be performed consistently at different laboratories with comparable results; (4) the evaluation of the protocols by an independent scientific peer review; and (5) the selection of the final assays for incorporation into the OECD/FIFRA/TSCA Test Guidelines and implementation into the EDSP.

The activities within ORD in support of the EDSP include not only the research that leads to the development of the protocols but providing scientific assistance and technical support to OSCP throughout the development, standardization and validation process by (1) identifying scientific and technical questions that should be addressed during the pre-validation process; (2) designing and conducting studies which address and resolve these issues; (3) evaluating data submitted by contract and other laboratories assisting with the validation process; and (4) providing senior scientists as consultants to OSCP and national and international advisory boards/committees that are actively involved in the validation process.

Impact and Outcomes: An important role of ORD scientists is to not only develop data and testing methods but also to support the regulatory programs by transferring the technology, interpreting the science, and assisting in the implementation of new testing methods by the latter. This scientific and technical support is critical for the Agency to meet the Congressional mandate to develop and implement an EDSP that can identify environmental estrogens and other hormonally active substances. The 1998 EDSTAC Final Report recommended *in vitro* and *in vivo* assays as part of the Tier I Screening Battery and more

complex multi-generation assays across five taxa for the Tier II Testing. The OSCP is currently coordinating efforts to standardize and validate the assays for potential use in the EDSP. ORD scientists are assisting OSCP in these efforts by providing the technical expertise required to meet the deadlines for validation of the assays and tests and to move forward with the development of new, innovative approaches for reducing animal use in the EDSP.

The overall impact of these activities has been demonstrated by the completion of the standardization and pre-validation studies for the Tier I assays, by the international interest in the use of the protocols and the transferring of this technology, and by the invitations to the ORD investigators to serve on national and international workgroups and committees to develop harmonized guidelines and methods for detecting EDCs. Similar activities are expected as the Tier II assays are being finalized. In addition ORD scientists have and will continue to serve as a resource (e.g., deliver presentations, prepare integrated summary reports, review contractor studies) for OSCP in their interactions with advisory committees.

APPENDIX V
Key ORD Investigators

Research Theme	Key ORD Investigators
1.1.1	S Laessig, K Crofton, M Gilbert, M DeVito, and grantees
1.1.2	LE Gray, V Wilson
1.2.1	M Gilbert, K. Crofton, M DeVito, J Royland
1.3.1	S Euling
1.3.2	L Mills, S Laws
1.3.3	P Hartig, V Wilson, LE Gray
1.3.4	G Klinefelter
1.3.5	J Tietge, S Degitz, Hornung, J Korte
1.4.1	D Bencic, M Kostich, D Lattier, R Flick, A Batt, J Lazorchak, S Laessig and grantees
1.4.2	K Schenck, N Dugan, D Williams, H Mash, T Speth
1.4.3	C Acheson, R Herrman, T Dahling, S Stoll
1.4.4	M Mills, E Kleiner, S Wright
1.4.5	S Hutchins, M Mills
2.1.1	B Glenn, S Laessig and grantees
2.2.1	G Ankley, J Lazorchak, M Mills, S Hutchins, V Wilson, LE Gray, S Laessig and grantees
2.2.2	V Wilson, P Hartig, LE Gray
3.1.1	S Degitz
3.1.2	R. Johnson
3.1.2	R. Cooper, E. Gray, S. Laws, T. Stoker, J. Goldman, G. Ankley, J. Tietge, S. Degitz, R. Johnson

APPENDIX VI
ACCOMPLISHMENTS

(Note this section is still under development. For now the separate document on "Accomplishments" is a more complete reflection of the achievements of the EDCs Research Program.)

APPENDIX VII
ACRONYMS

ACC	American Chemistry Council
APG	Annual Performance Goal
APM	Annual Performance Measure
ATSDR	Agency for Toxic Substances and Disease Registry
AWWARF	American Water Works Association Research Foundation
BBDR	Biologically Based Dose Response
CAFO	Concentrated Animal Feeding Operation
CDC	Centers for Disease Control and Prevention
CENR	Committee on Environment and Natural Resources
CT	Computational Toxicology
DBP	Dibutyl Phthalte
DDT	Dichlorodiphenyltrichloroethane
DES	Diethylstilbestrol
DOD	Department of Defense
DOE	Department of Energy
DOI	Department of Interior
ED	Endocrine Disruptor
EDCs	Endocrine Disrupting Chemicals
EDRP	Endocrine Disruptors Research Program
EDSP	Endocrine Disruptors Screening Program
EDSTAC	Endocrine Disruptors Screening and Testing Advisory Committee
EPA	Environmental Protection Agency
EU	European Union
FDA	Food and Drug Administration
FQPA	Food Quality Protection Act
G-8	Governments of the 8 leading nations
GEDRI	Global Endocrine Disruptors Research Inventory
GPRA	Government Performance and Results Act
GWRC	Global Water Research Coalition
HHRA	Human Health Risk Assessment
IPCS	International Programme on Chemical Substances
IRIS	Integrated Risk Information System
IWG	Interagency Working Group
JRC	Joint Research Centre
mCA	Microcantilever Arrays
MOA	Mechanism/Mode of Action
MRPs	Molecular Recognition Phases
MYP	Multi-Year Plan
NCEA	National Center for Environmental Assessment
NCER	National Center for Environmental Research
NCI	National Cancer Institute
NERL	National Exposure Research Laboratory

NHEERL	National Health and Environmental Effects Research Laboratory
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NOAA	National Oceanographic and Atmospheric Agency
NRC	National Research Council
NRMRL	National Risk Management Research Laboratory
NSF	National Science Foundation
NSTC	National Science and Technology Council
OECD	Organization for Economic Cooperation and Development
OPP	Office of Pesticide Programs
OPPT	Office of Pollution Prevention and Toxics
OPPTS	Office of Prevention, Pesticides and Toxic Substances
ORD	Office of Research and Development
OSCP	Office of Science Coordination and Policy
OSTP	Office of Science and Technology Policy
OW	Office of Water
PAGE	Poly Acrylamide Gel Electrophoresis
PAH	Polyaromatic Hydrocarbon
PBTK	Physiologically Based Toxicokinetic
PCBs	Polychlorinated Biphenyls
PCDFs	Polychlorinated Dibenzofurans
PHAH	Polyhalogenated Aromatic Hydrocarbon
PiE	Pharmaceuticals in the Environment
PPCPs	Pharmaceutical and Personal Care Products
QSAR	Quantitative Structure Activity Relationship
RARE	Regional Applied Research Effort
RFA	Request for Applications
RM	Risk Management
RME	Risk Management Evaluation
SDWAA	Safe Drinking Water Act Amendments
SERS	Surface Enhanced Raman Spectroscopy
STAR	Science to Achieve Results
S&T	Screening and Testing
TEF	Toxicity Equivalent Factor
TIE	Toxicity Identification Evaluation
USDA	US Department of Agriculture
USGS	US Geological Survey
WHO	World Health Organization
WQ	Water Quality
WWTP	Waste Water Treatment Plant