



2007 Mid-Cycle Review:
Office of Research and Development's
Endocrine Disruptors Research Program
By the
Endocrine Disruptors Subcommittee of the
Board of Scientific Counselors
September 18, 2007

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Every four to five years research programs in Environmental Protection Agency's (EPA) Office of Research and Development (ORD) undergo an independent review of their science. The Subcommittee on Endocrine Disrupting Chemicals of ORD's Board of Scientific Counselors reviewed the Endocrine Disruptors Research Program on December 13-15, 2004, in Research Triangle Park, NC (insert website of BOSC report). Overall, the Subcommittee found that the Endocrine Disruptors Research Program was of high scientific quality and of direct relevance to legislation that the Environmental Protection Agency (EPA) administers and that it serves the Program Offices well. The goals and scientific questions of the Research Program were deemed to be appropriate. In addition, the Program was found to be nationally and internationally recognized as a multi-disciplinary set of research areas for both human health and wildlife that cuts across the risk assessment/risk management paradigm. The Subcommittee offered a number of observations and recommendations, which have been used to guide the Program during the annual planning cycle and revision of the Multi-Year Plan (MYP) for Endocrine Disruptors. A written response to these observations and recommendations was provided and a time-line for actions to be taken or planned was discussed in a briefing to the Executive Committee of the BOSC on September 13, 2005.

The following is a narrative that identifies the observations and recommendations made by the Subcommittee, provides ORD's 2005 response for planned actions, and an update on the progress made to address the recommendations. Several recommendations were repeated in multiple sections. In such cases, overlapping recommendations have been combined and discussed in the order in which they first appeared in the review document. Peer review comments or recommendations are shown below in *italics*. ORD comments, where needed for clarification, are in plain format, the actions underway or planned in 2005 are in **bold-faced type**, and progress since 2005, in addressing the recommendations, is in **red type**.

EXECUTIVE SUMMARY

Overarching Conclusions and Recommendations

1. The EDCs program is a combination of "problem-driven" and core research and has stood the test of time; however, progress reviews are encumbered to some extent, by the difficulty in defining the scope of activities considered to be part of endocrine disruptor research. There are a large number of toxic mechanisms that could be categorized as endocrine disruptors; therefore, EPA should clarify what is and what is not covered by the EDC program whenever the program is reviewed. Confusion regarding the classification and scope of endocrine disruptors is common in many assessments of these compounds (p. 6, 7, 30, 41).

Comment: ORD recognizes this issue. The Research Plan and MYP for Endocrine Disruptors Disruptors used the definition developed through an internationally convened workshop (Kavlock et al., EHP 104 (Suppl 4): 715-740, 1996). Since then the World Health Organization has developed another definition (WHO, 2002) which will be considered in the updating of the MYP.

But the crux of this issue appears to be a request to better clarify what the scope of the ORD EDCs Research Program is. As evident from the Program Review, the emphasis of ORD's Program has been 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, retinoids, and ecdysoids. The emphasis on a systems approach to the EDCs' issue and the realization that the central nervous system, liver, and other mechanisms are all critical components of any endocrine system, may compel us to look at a broader research scope. The challenge in the MYP update will be to balance clarifying the scope but leaving the door open to address critical gaps in endocrine mechanisms known or suspected to be involved in adverse effects in humans and wildlife that may not be addressed through other ORD or outside Research Programs.

2005 Proposed Action: ORD's Research Plan for Endocrine Disruptors and the MYP for Endocrine Disruptors reference the definition that is used to guide this Research Program. The guiding definition, what research is included, and how this relates with other ORD research will be further clarified in the next update of the MYP and in future Program Reviews

Progress: The updated draft MYP has adopted the definition of endocrine disruptors that the WHO published in 2002: *"An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations."* Furthermore, the draft MYP clarifies that the emphasis of EPA's research program is on impacts on the estrogen, androgen, and thyroid systems. It also points out that there are additional research efforts ongoing, to a smaller extent, on other mechanisms, including through steroidogenesis, aromatase, and the hypothalamic-pituitary-gonadal and hypothalamic-pituitary-thyroid axes.

2. The following are recommendations that would allow the Program Director to negotiate for needed research expertise from a position of strength and enhance the laboratories that participate in EDC program research: (1) hire additional personnel to share the workload of the participating laboratories; (2) elevate the position of the EDC Program Director to the level of the Laboratory/Center Directors; and (3) provide the EDC Program Director budget authority. (p. 7-8, 14).

Comment: ORD recognizes the priority of the EDCs Research Program in providing the support needed for the Agency to carry out its mandates. The National Program Director (NPD) for Pesticides and Toxics, who now oversees the EDCs Research Program, will make the best use of the available resources to implement a scientifically sound and highly relevant Research Program. (1) Agency resource limitations, including the ceiling on the number of personnel (Full Time Equivalents - FTEs), make hiring additional personnel unlikely at this time. Other options for supplementing the scientists in the EDCs Research Program will be explored. (2) In May 2005, ORD announced the establishment of NPDs for eight Research Programs. One of those was for

Pesticides and Toxics research, which includes the EDCs Program. There was much discussion among ORD's senior leadership regarding the relationship of the NPDs to the Laboratory/Center Directors. (3) "Budget authority" resides with the Senior Budget Officer (SBO) in ORD.

2005 Proposed Action: (1) The EDCs Research Program will be bringing on board several new postdoctoral fellows to fill critical gaps and help advance the Research Program. The NPD will encourage the Laboratories/Centers to pursue other Fellows (e.g., AAAS, ASPH) and will work with ORD's Office of Resources and Management Administration to explore the possibility of using other vehicles (e.g., recent graduate contract) to supplement the number of scientists in this Research Program. (2) The NPDs report individually to the Assistant Administrator (as do the Laboratory/Center/Office Directors) through the Deputy Assistant Administrators and work together as a group to assist in planning and implementing ORD's Research Programs. They are awarded senior level stature and make recommendations to the Laboratory/Center Directors, the Assistant Administrator, and Deputy Assistant Administrators (i.e., collectively the Executive Council) regarding research priorities. The details regarding the relationship of the NPDs, to the Executive Council, Science Council, and other Agency groups are being delineated in a document under development. (3) "Budget authority" resides with the SBO in ORD. The Executive Council decided that budget advice and recommendations will be sought from the NPDs who would be responsible for working closely with the Laboratory/Center/Office Directors

Progress: (1) Because of Agency resource limitations, including the ceiling on the number of personnel (Full Time Equivalents – FTEs), ORD has had to rely on other innovative mechanisms to supplement and complement the scientists in the EDCs Research Program. One approach has been to use existing vehicles to bring on board postdoctoral fellows, recently graduated students, student interns, and other fellows (i.e., through the American Schools of Public Health, American Association for the Advancement of Science) which do not count against our personnel ceiling. The EDCs Research Program has brought on board at least 7 postdoctoral research fellows to fill critical gaps and help advance the Research Program. The National Exposure Research Laboratory (NERL) in Cincinnati has had two molecular biology postdocs since before the last BOSC review. One has been involved with developing molecular biology tools to measure stressor-induced changes in aromatase gene expression levels in field studies, including the Potomac River studies in partnership with USGS. The other fellow developed molecular indicators of exposure to estrogenic compounds for a number of non-laboratory model species related to investigating the presence of and effects from EDCs within the Mainstem Ohio River. This latter fellow has been hired recently by NERL-Ci to conduct molecular biological studies to develop indicators of exposure to EDCs and pesticide stressors using genomic methods with small fish models. In 2006, NERL brought on board another postdoc to develop analytical chemistry methods for EDCs and pharmaceuticals. Also, an Oak Ridge Institute for Science and Education postdoc trainee is being brought to NERL-Ci in September 2007 to develop molecular indicators of exposure to EDCs, pesticides, and nanomaterials using genomic approaches with invertebrate species. ORD's National Health and Environmental Effects Laboratory (NHEERL) has been able to enhance the productivity of the EDCs MYP by adding one permanent staff and a total of 24

temporary employees – a combination of postdoctoral fellows (2 in the Human Health Research Program with an EDCs emphasis) and student interns. Furthermore, the National Program Director has had an ASPH Fellow assisting in coordinating research planning activities.

Another major mechanism whereby ORD enhances the work within a MYP is by teaming and leveraging capability across Research Programs. Most technical and science staff have their FTE split between more than one MYP. This enables a better coordination across plans and increases the potential magnitude of the impact of the research. An example is the characterization of low-dose effects of thyroid disrupting chemicals. The staff working on this project are also integrated into the Human Health MYP under Long Term Goal 1 where the program is enhanced by examining the human relevance of the various modes of action that result in thyroid disruption. The research to support the Agency's Endocrine Disruption Screening Program (EDSP) under Long Term Goal 3 of the EDCs MYP is enhanced by the development of a short-term *in vivo* medaka assay that can be used to characterize linkages between reproductive endpoints and diagnostic biomarkers for endocrine disrupting chemicals. The work and the FTEs for the research to develop the medaka assay are aligned under the Safe Pesticides/Safe Products (SP2) MYP. By targeted hiring of staff and trainees and leveraging work across the MYPs where goals are complementary we have been able to increase not only the capacity to do EDCs related work but also the capabilities available for increased impact.

Furthermore, ORD has been very proactive in enhancing its capabilities in the new high-density data array approaches including transcriptional profiling, proteomics, and metabolomics. Proteomic core capabilities have been developed in the ecology and human health laboratories, including making strategic hires, enabling proteomic approaches to become integral to the research program as endpoints for fish, rodent, and human studies. A toxicogenomics expert and a systems biologist have been hired by NHEERL who are taking the lead in coordinating application and interpretation of gene array data in risk assessment based research. NERL has developed a metabolomics core capability which is rapidly becoming integrated into a number of cross-ORD projects.

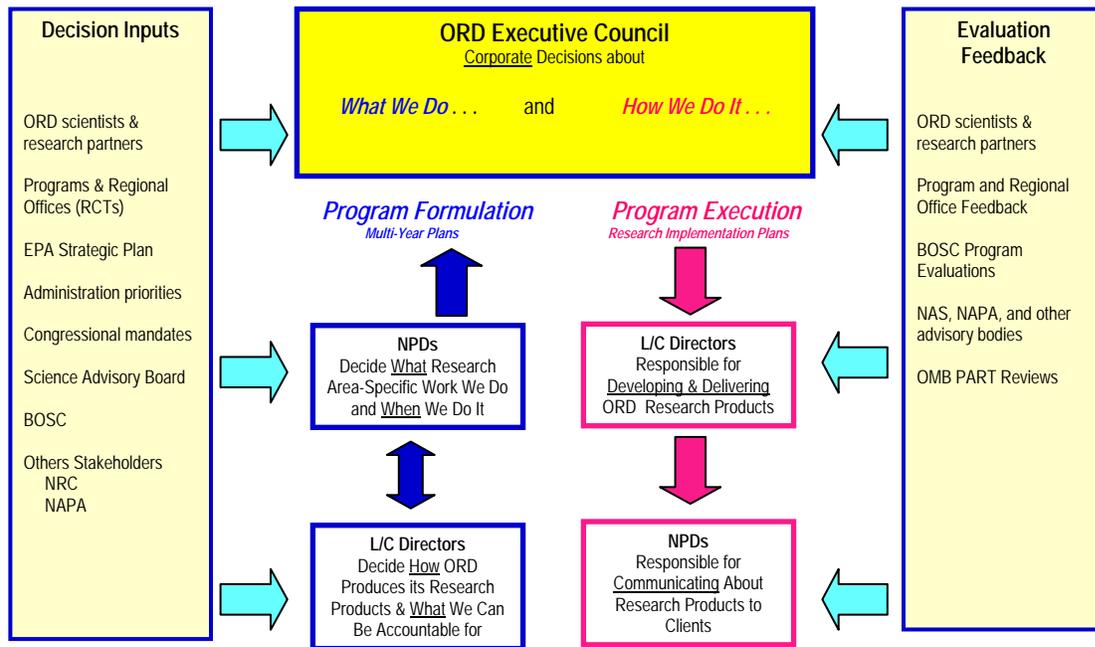
(2 & 3) The NPDs report individually to the Assistant Administrator (as do the Laboratory/Center/Office Directors) through the Deputy Assistant Administrators and work together as a group to assist in planning and implementing ORD's Research Programs. They are awarded senior level stature and make recommendations to the Laboratory/Center Directors, the Assistant Administrator, and Deputy Assistant Administrators [i.e., collectively the Executive Council (EC)] regarding research priorities. The details regarding the relationship of the NPDs, to the Executive Council, Science Council, and other Agency groups are being delineated in the draft document "Roles and Responsibilities of ORD's Senior Research Managers" that will undergo final review by the EC in September 2007. ORD has adopted a matrix-management structure to plan, budget, implement, and evaluate its Research Programs. The purpose of the document is to provide a better understanding of the relative roles and responsibilities of ORD's senior research managers related to these functions. For example, some of the responsibilities described include:

“The EC is the primary decision-making body for major planning and program/management decisions in ORD.”

“The NPDs are responsible for developing a strategic view of what research needs to be done in consultation with ORD’s stakeholders. These stakeholders include the ORD Laboratories, Centers, and Offices; the EPA Program and Regional Offices, external advisory groups such as the National Academy of Sciences, Science Advisory Board, and Board of Scientific Counselors, and others. In this respect, the NPDs work closely with Research Coordination Teams (RCTs), variably comprised of representatives from ORD organizations and from the Program and Regional Office.”

“Lab/Center Management determine how priority research is to be conducted and identify accountable ORD products.”

The following diagram depicts the interrelationships among the various senior ORD managers.



Following the ORD senior managers’ review of the roles and responsibilities document, the BOSC can be provided with more details concerning NPD responsibilities within ORD related to decision making authority including budget authority. For now, budget authority still resides

with the SBO in ORD. The Executive Council decided that budget advice and recommendations will be sought from the NPDs who would be responsible for working closely with the Laboratory/Center/Office Directors.

STRENGTHS AND CHALLENGES OF LONG-TERM GOAL 1:

Provide a better understanding of science underlying the effects, exposure, assessment, and management of endocrine disruptors

Intramural scientific expertise for the areas of human and aquatic species (i.e., fish, invertebrates, and amphibians) is very good; however, this is not the case in the area of wildlife toxicology. Consequently, much of the experimental research and expertise resides in the STAR grant recipients. It is advantageous to utilize the expertise of these scientists from outside the Agency; however, more expertise in the area of wildlife toxicology within the Agency may be required to fully attain the program's goals. Application of the wildlife models that are being developed may require Agency personnel to meet the exact needs of regulatory concern. Also, the evaluations of EDCs on wildlife within a risk assessment paradigm, including evaluation of uncertainties, almost certainly would require full-time EPA personnel. It is not clear from the review of information presented to this subcommittee that adequate personnel exist to address wildlife concerns of EDCs.

3. To meet the program goals and fulfill the exact needs of regulatory concern the BOSC recommends that the EDC program dedicate full-time EPA personnel to work in this [wildlife toxicity] area (p. 9, 22).

Comment: ORD had a wildlife toxicology program which ended in 1994. Currently, in general, ORD has a very limited program in the area of wildlife toxicity since other Agencies have greater depth and breadth there. Given our limited resources, ORD has determined where EPA's research can have its greatest impact and has carved out our niche to address 3 areas: 1) developing mechanistically based approaches for extrapolating toxicological data across wildlife species, media, and individual level response endpoints, 2) developing approaches for predicting population level responses to stressors, and 3) developing approaches for evaluating the relative risks from chemical and non-chemical stressors on spatially structured wildlife populations across large areas or regions. Further detail is given in the National Health Effects Research Laboratory's Wildlife Research Strategy (<http://www.epa.gov/nheerl/publications/>). Some of this research, which is conducted under the Safe Pesticides/Safe Products (SP2) MYP is looking at endocrine disruptors. Furthermore, there are wildlife toxicologists in EPA's Office of Prevention, Pesticides and Toxic Substances who are responsible for reviewing data on pesticides and other toxic chemicals for Agency decisionmaking.

2005 Proposed Action: As noted in the BOSC review, ORD has complemented its strengths in the areas of aquatic and human health related toxicity by engaging academic wildlife toxicologists through the extramural STAR program and will continue to do so, where appropriate. It is anticipated that some of the new post doctoral fellows that will be sought will be in the area of aquatic or invertebrate toxicology which will strengthen our ecological portfolio but will not move into other wildlife research. We will increase our efforts to collaborate across federal agencies to leverage the talent of their wildlife toxicologists. The EDCs Research Program is working with a contractor to have a synthesis document developed that is integrating the published results from ORD's extramural STAR and intramural programs on the impacts of EDCs on wildlife, including aquatic, amphibian, invertebrate, avian, reptile species.

Progress: As noted under the "progress" reported for response 2, some of the postdoctoral fellows that are working on EDCs' issues have backgrounds in the area of wildlife. Some are working on these issues under the EDCs Research Program and others are working in other ORD Research Programs. One of the great strengths of the ORD research program is its ability to leverage across MYPs to address important scientific and risk assessment issues from many perspectives at once. Within the SP2 MYP there are a number of projects that are developing mode of action based predictive screens and predictive models. Many of the modes of action include endocrine disruption and the ecosystem models focus on reproduction and development as the primary endpoint. The Assessment Tools for Evaluation of Risk (ASTER) which is a QSAR based approach that ties mode of action to effects is aligned under the SP2 MYP. The initial training set for this effort were estrogenic agents and the primary effect of reproductive failure in fish populations. SP2 research has also led to the development of an invertebrate model to screen for endocrine activity. Through a separate cross-laboratory interdisciplinary network of EPA (NHEERL, Duluth; NERL, Cincinnati, Athens; NCCT, RTP), an EPA grantee (University of Florida), and non-EPA partners (e.g., Joint Genome Institute of the Department of Energy), a systems-based approach is being used to define toxicity pathways for model chemicals with well-defined modes of action within the hypothalamic-pituitary-gonadal axis utilizing three small fish species, the Japanese medaka, zebrafish, and fathead minnow, as a basis for the development of techniques for extrapolation of toxicological effects across endpoints, species and chemicals. As noted, this latter effort is done in collaboration with the National Center for Computational Toxicology (NCCT). A postdoctoral fellow and an NCCT scientist developed a computational model of the steroid synthesis pathway in the fathead minnow ovary in the "Small Fish Project" and which is now being extended to the H295R cell system in conjunction with collaborators from Mitsubishi (Tokyo, Japan) and the University of Saskatoon. These are but a few examples of a number of *in silico*, *in vitro*, and *in vivo* approaches for wildlife that are being leveraged across research programs and are developing tools to predict the potential for adverse effects based on specific modes of action where the initial approach has been to use model endocrine active compounds.

Furthermore, there has been increased collaboration through the activities of the Interagency

Working Group. A workshop was held in February 2007 that brought together over 100 scientists from 14 federal agencies to share information on their activities as related to impacts of endocrine disruptors on ecosystems and the environment. Break out groups identified a number of areas where scientists from across the agencies could better coordinate their efforts with a focus on exposure and wildlife issues.

Scientists from USGS, USDA, and FDA, from EPA's Office of Water, from our intramural Laboratories and Centers, and the seven new grantees attended a workshop organized by the National Center for Environmental Research (NCER) to kick-off the integrated research effort on concentrated animal feeding operations (CAFOs) in August 2007. Intramural scientists will be collaborating with the grantees and with scientists from the other agencies to determine the impact of hormones from CAFOs on wildlife and the environment and to develop approaches to mitigate exposures.

Another example of collaborations is the seven year involvement of NERL scientists with a team of Canadian scientists to conduct an experimental whole lake study assessing the impact of an endocrine disruptor on the sustainability of a wild fish population. The study involved the addition of a synthetic estrogen (17 α ethinyl estradiol), that is used in birth control pills, to a natural lake located in the Experimental Lakes Area in northwestern Ontario, Canada. NERL's role in this study was to participate in the experimental design of the concentrations used for dosing and to evaluate vitellogenin gene expression (*Vtg* mRNA) in indigenous fathead minnows and pearl dace collected from a reference lake and a continuously dosed lake and in laboratory cultured fathead minnows exposed to water and sediments from the dosed lake and reference lakes.

The EDCs Research Program has also developed a draft synthesis document, through the help of a contractor; the chapter on "Exposure and Effects in Wildlife" is devoted to integration of the published results from ORD's extramural STAR and intramural programs on understanding exposure and effects of endocrine disruptors in wildlife. Topics include progress on identifying sources of EDCs in the environment such as wastewater treatment plants, animal feeding operations, and pulp and paper mills. Key EPA research accomplishment on the effects of EDC in wildlife species such as birds, frogs, fish, reptiles, and invertebrates are described. EPA research on species comparisons and differential sensitivity, and effects on wild populations are described (see more details under progress described for response 4).

4. Because of the complexities in extrapolating among the many species in the environment that may be affected by endocrine disruptors, it will be important for ORD to continue to collaborate with other federal, academic, nongovernmental (NGO), and industry partners to characterize better the range of variability among species (p. 9, 22).

Comment: ORD appreciates the fact that the BOSC has recognized its long history of research on species-to-species extrapolation. In fact, this topic is of paramount importance in several of ORD's MYPs. Improving our understanding regarding extrapolation across species is one of the ten questions that the EDCs Research Program is aimed at addressing. We are doing this through both our intramural and extramural programs. By pursuing research on mechanisms of action, the EDCs Research Program will provide significant information that is important in improving our ability to extrapolate (e.g., homology at the mechanistic level). ORD agrees that establishing collaborations and partnerships with other Federal and non-Federal research organizations ultimately strengthen ORD's overall Research Programs.

2005 Proposed Action: Our intramural Research Program will continue to evaluate potential interspecies differences and similarities among the various cellular mechanisms of action of current interest in the EDCs Research Program. Currently, these efforts include identifying the androgen receptor and estrogen receptor of different species, examining the response of these receptors to the same compounds using *in vitro* methods, as well as conducting a comparison of the *in vivo* response in several species. This work is being conducted in conjunction with investigators at two universities and a chemical company in Germany. In addition, our scientists will continue to collaborate with scientists from other organizations. This will be done on a scientist-to-scientist basis as well as under the auspices of the IWG.

Progress: Species extrapolation is an important component of all effects-related research and is much more than just extrapolation from traditional laboratory animals to humans. The value of many of the *in vitro* and higher-throughput whole animal screening and testing systems is due to the conservation of molecular endocrine physiology across phyla. Some of this research continues in the EDCs Research Program through 2013. For example, both the frog metamorphosis assay and the effects of thyroid disruption in rats are relevant for screening for potential human risk. The frog and the *in vivo* fish reproduction models are extrapolated to other aquatic species. The neurologic effects work associated with thyroid disruption will describe the human relevance of the data and the *in vitro* estrogen disruption correlated with the *in vivo* fish reproduction work will be extrapolated across aquatic species for prediction of population effects in fish. Other 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. ORD is also developing androgen receptor and estrogen receptor 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. The results from all of the aforementioned efforts 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.

There are complementary activities related to cross-species extrapolation ongoing through other ORD research programs. Currently, as an example, the Web-based Interspecies Correlation Estimation (<http://www.epa.gov/ceampubl/fchain/webice>) (SP2 MYP) can be used for extrapolating across terrestrial mammals and avian species and will soon include an aquatic species module. Web-ICE is currently focused on LD50 or LC50 data but this approach could include other toxicology data including endocrine disruption in the future. NCCT and the National Center for Environmental Assessment (NCEA) are working with the International Life Sciences Institute on the production of databases for parameters for use in physiologically based pharmacokinetic models. These databases would allow modelers to utilize peer-accepted values for the physiological parameters for rodents and humans, with inclusion of life-stage elements. One specific effort involves a physiologic model of the prostate under various scenarios of androgen stimulation or deprivation. The steroidogenic model mentioned in the progress for response 3, is also being used to aid in the extrapolation across species (fathead to human cell lines *in vitro*). The NCCT is conducting high throughput screening assays utilizing cells and/or receptors from multiple species to understand nuclear receptor activation across species, but this is more directed at PPAR than the estrogen/androgens/thyroid systems at the moment.

5. Studies have been reported regarding the use of predictive tools that can be used to prioritize the focus of specific treatment technologies and compounds. These findings could be integrated into EPA's EDC program (p. 9, 22).

Comment: One of the objectives of EPA's Computational Toxicology (Comp Tox) Research Program is to develop and apply predictive tools to prioritize chemicals for testing. An early proof of concept using EDCs as the chemicals of interest was implemented in our in-house laboratories, as well as through the STAR program. The directions of the Comp Tox Program were under development and the program itself was in its infancy when the current EDCs MYP was being developed. Regardless, the MYP for EDCs identifies a number of these tools as Annual Performance Measures.

Comp Tox work is progressing. For example: 1) There is on-going work to develop a hypothalamic-pituitary-thyroid (HPT) model providing a rational framework to organize and interpret toxicological data from the molecular to the organismal levels that will serve as a basis for development of predictive tools related to thyroid toxicity. This will enhance the Agency's use of quantitative structure activity relationships (QSARs), thus, providing a basis for sorting chemicals by mode of action (MOA). 2) Work on using a combination of genomics, proteomics, metabolomics, computational modeling, and whole animal endpoints to identify new molecular biomarkers of exposure to EDCs representing several MOAs is also progressing. These markers

are then linked to biomarkers of effects that are relevant for both diagnostic and predictive risk assessments using small fish models.

2005 Proposed Action: The NPD and the EDCs and SP2 planning teams will work with the Director of the National Center for Computational Toxicology (NCCT) to ensure greater linkages among the EDCs, Comp Tox, and SP2 Research Programs. These will be better characterized in the updated MYPs.

Progress: The Comp Tox research program has made incredible progress since 2005. This progress is being monitored periodically by a separate Subcommittee of the BOSC (<http://www.epa.gov/osp/bosc/subcomm-ctox.htm>). A recent activity that merits noting is the development of the ToxCast program (<http://www.epa.gov/comptox/toxcast/>). The ToxCast program, now in Phase I of development (see <http://www.epa.gov/comptox/toxcast>) is profiling +300 well-characterized toxicants in +400 *in vitro* HTPS endpoints in an attempt to develop bioactivity profiles that are predictive of apical outcomes and key modes of action (similar to what has been recently recommended by the NAS in their “21st Century Toxicology Vision” effort. Included in the assays are a number of nuclear receptors for the steroid hormone family (both binding and reporter gene assays). These data would be provided to the Office of Prevention, Pesticides, and Toxic Substances’ (OPPTS) Office of Science Coordination and Policy (OSCP) which is implementing the Agency’s Endocrine Disruptors Screening Program for evaluation as a prioritization tool in subsequent efforts. Results of Phase I are expected to be released by mid 2008.

In addition, in 2002 a request for applications was released through the Comp Tox STAR program to solicit research proposals that would lead to the development of HTP screening systems for identifying chemicals with estrogen, androgen, or thyroid hormone activities. The goal was to develop an extramural portfolio of research on the development of HTP screening systems to assist in prioritization of chemicals for further screening and testing of their potential as endocrine disruptors that would complement ORD’s internal efforts. Four STAR awards were made in 2003 for the development and application of models using a bioluminescent yeast reporter system, an invertebrate, a zebrafish embryo gene expression system, and the Japanese medaka fish. These grants are close to completion. The research is expected to contribute to the development of HTP screening systems to assist in prioritization of chemicals for further screening and testing of their potential as endocrine disruptors. The methods generated from these studies will provide for multiple platforms for the HTP screening of potential endocrine disrupting chemicals, in some cases even allowing for the remote, near real-time monitoring of potential endocrine disrupting chemicals in the environment.

Furthermore, the SP2 research program which was reviewed by another Subcommittee of the BOSC earlier this year (<http://www.epa.gov/osp/bosc/subcomm-sp2.htm>) has a number of relevant activities related to the development of predictive screening approaches that are ongoing:

1) **Developing toxicity pathway-based QSARs for prioritization within large chemical lists.** It incorporates QSAR-based hypothesis generation, strategic chemical selection for hypothesis testing, *in vitro* assay optimization and targeted testing, and QSAR evaluation and improvement for mechanistic classifications for Office of Pesticide Programs (OPP) pesticidal inerts and antimicrobials, chemicals for which data are lacking and predictions welcomed. 2) **Providing access to peer-reviewed literature and MOA-based QSAR models (ASsessment Tools for Evaluation of Risk).** The updating and improvements to the ASTER system will facilitate the identification of structural analogs and associated toxicity information to estimate potential hazard of untested chemicals or chemicals with limited toxic effects information. 3) **Simulating metabolism to enhance effects modeling.** Metabolic transformation types shown to enhance estrogenicity, but that currently are underrepresented in an existing simulator, are being studied using chemicals selected from priority OPP's lists. The ultimate goal is to demonstrate an approach for predicting chemical potential for metabolic activation. 4) **Using NMR-based metabolomics** to: a) understand and link the exposure and effects of EDCs within the HPG axis of small fish models. This includes linking responses from genomics, proteomics and metabolomics; b) define markers of exposure and to better understand the cumulative risk from triazole pesticides. Research will differentiate responses from triazole pesticides that exhibit different MOA, assess the impact of metabolism of the parent triazole pesticide on the observed outcome and determine the extent of conservation of triazole metabolism behavior and metabolomic profiles across various species (e.g., fish and rats); c) identify markers of exposure for important perfluorinated chemicals, which are endocrine disruptors, to gain a better understanding of toxic MOA and investigate the occurrence of markers of exposure from fish collected in contaminated field sites; d) develop a HTP metabolomics approach that involves exposing cell cultures to potentially toxic chemicals in order to screen large inventories of chemicals rapidly for potential adverse outcomes. Chemicals found to greatly alter the metabolite profile of cell cultures (relative to the control case) would be candidates for more extensive testing. 5) **Applying toxicity-pathway-specific protein expression models for chemical screening and prioritization.** Surface Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry is used to examine protein expression profiling as a means to screen chemicals for their MOA. *In vitro* exposure of fish hepatocytes and short-term *in vivo* minnow exposures are used to link diagnostic expression profiles between tissue level and whole organism assays, and across multiple fish species. As proof of concept, research efforts are initially focused on developing diagnostic models with established estrogenic and androgenic chemicals.

The Director of NCCT and the NPD for EDCs and SP2 Research Programs confer frequently to share progress on and identify potential leveraging of their programs' efforts. The 2006 SP2 MYP and the draft EDCs MYP have better characterized the linkages with one another and with the Comp Tox Research Program than in previous iterations.

In its early years, the EDCs Research Program had a small effort in the area of developing predictive tools for risk management approaches. However, given the significant reduction in resources in the area of risk management, that effort no longer exists.

6. The model and framework for development of critical information on EDCs for risk assessment is well established and progress is being made. Efforts now should focus on the development of risk assessment paradigms for EDCs and application of the research findings (p. 21).

Comment: EPA's position is that current approaches for risk assessment under specific endpoints (e.g., developmental, reproductive, cancer) are appropriate for use in evaluating endocrine disrupting chemicals. There are some relevant activities that are discussed in greater detail in responses to other recommendations that apply here as well, for example, the efforts in developing data and approaches to incorporate 'omics information into Agency decisions and consideration of the MOA approach.

2005 Proposed Action: EPA will continue to monitor research results that may affect current risk assessment practices, as they get published. If, and when, the Agency determines that risk assessment approaches need modification, they would convene a cross-Agency committee (as has been done with the development of other risk assessment guidelines) to deliberate and develop guidelines. In keeping with the guideline development process, there will be opportunity for public involvement, through workshops and solicitation of public comments.

Progress: EPA is conducting research to address several areas of importance related to whether the current approaches for risk assessments for endocrine disruptors of using the Agency endpoint-specific guidelines need modification. One important issue in risk assessment is to determine risk when exposed to a mixture of contaminants. Under the Food Quality Protection Act the EPA is directed to develop a cumulative assessment of risk when possible. Cumulative risk assessment is based on an identified similarity of the mode of toxic action across a group or class of chemicals. EPA's research addressing the risk assessment approach for cumulative risk for endocrine disruptors has focused on thyroid and androgen disruption. Mixtures of polyaromatic hydrocarbons that alter metabolism and result in decrements in thyroid function have been examined in rats as a model system for describing a mathematical approach to address the effects of mixtures. These studies have shown that the effect response model suggests an additive effect from the mixture. Additional studies are underway to examine the consequence of exposure to mixtures of phthalates either alone or in combination with other antiandrogens that act through common and different modes of action when administered during critical developmental states of life. A workshop on the topic of cumulative risk that would bring together scientists who are conducting research, developing cumulative risk assessments, or who have raised hypotheses as to how these assessments should be conducted is in its early stages of development. The goals of the workshop will be to determine the state of the science and identify targeted research needs.

Another area important to risk assessment where EPA's research program has focused is on the

characterization of dose-response in the low dose or environmentally relevant range. An RFA was issued in 2004 under which three grants were awarded. Two of the awardees are working through cooperative agreements with intramural ORD scientists looking at low dose impacts on the thyroid system. The other grantee is examining the hypothesis that *in utero* exposure to low doses of cadmium impacts estrogen-like effects in the HPG axis and consequently alters the onset of puberty, predisposes to obesity, and accelerates the development of the mammary gland in female offspring. Other intramural 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 identification of low dose effects and characterization of the shapes of the dose response curves.

In addition, there has been activity in the area of developing assessments for certain EDCs. A number of Integrated Risk Information System (IRIS) hazard assessments have been or will be conducted by NCEA for EDCs. Endocrine screening data and data from new technologies are being incorporated into the risk assessment paradigm. An example of this is the IRIS assessment for dibutyl phthalate (DBP), which utilizes toxicogenomics research findings to establish a mode of action for male developmental reproductive effects, thereby influencing the selection of critical endpoints for risk assessment. The DBP assessment is being utilized as a case study addressing a more general application of toxicogenomics data in risk assessment (for more details see progress reported under response 9).

STRENGTHS AND CHALLENGES OF LONG-TERM GOAL 2:

Determine the extent of the impact of endocrine disruptors on humans, wildlife, and the environment

Although priorities for the chemicals studied have been appropriate, EPA should continue to improve its interactions with other agencies that have a strong interest in EDCs (i.e., CDC, NIEHS, National Toxicology Program [NTP], National Institute for Occupational Safety and Health [NIOSH], and FDA) to identify new sources of environmental and human exposures to EDCs. Moreover, EPA should mine data made available from the OECD High Production Volume (HPV) Program and work with FDA to investigate the role of pharmaceuticals in the environment as a source of endocrine disruptors.

7. EPA should continue to improve its interactions with other agencies that have a strong interest in EDCs to identify new sources of environmental and human exposures, including investigating the role of pharmaceuticals as sources of EDCs. EPA should mine data made available from the HPV Program (p. 11, 30).

Comment: The NPD chairs the IWG on Endocrine Disruptors and will use this opportunity to

strengthen our relationships with the other Agencies. EPA is engaged in the topic of pharmaceuticals in the environment in a number of ways. There is a small research effort under the Water Quality Research Program (WQRP). ORD is also participating on a steering committee for an International Workgroup on "Pharmaceuticals in the Environment" sponsored by Society of Environmental Chemistry and Toxicology (SETAC). This workgroup is composed of members from academia, government and industry and is focused on pharmaceuticals impacts and potential risks in the environment. In addition to the intramural research, a solicitation through STAR resulted in the award of six grants. Finally, under the auspices of the Office of Science and Technology Policy, an interagency task group on Pharmaceuticals and Personal Care Products (PPCP) was established in late Fall, 2004. This workgroup is co-chaired by ORD and FDA and reports to the Toxics and Risk Subcommittee under the Committee of the Environment and Natural Resources. The PPCP task group is working closely with the chair of the IWG on EDCs. The charge to the PPCP task group is to develop a strategic Framework document that would identify: 1) work currently underway across the Federal government; 2) areas of common interest to explore collaboration; and 3) data gaps which would be prioritized relative to greatest impact. It is unclear as to what extent data from the HPV program will shed some light onto the effects of these chemicals on the endocrine system but the opportunity to evaluate will be pursued. Knowledge regarding the potential sources of these chemicals will provide insights regarding potential exposures and may assist in the determination of screening priorities.

2005 Proposed Action: The NPD chairs the IWG on Endocrine Disruptors and will use this opportunity to strengthen our relationships with the other Agencies. Efforts are already underway to organize a multi-Agency sponsored workshop related to sources of exposure. The IWG decided to focus this workshop on looking at the impact of two exposures, wastewater treatment plants and concentrated animal feeding operations (CAFOs), on ecosystems. The NPD will continue to work closely with the co-chairs of the interagency PPCP task group and look for opportunities for joint efforts. The NPD will work with the NPD for WQRP to explore leveraging PPCP activities. In updating the EDCs MYP, the planning team will consider linkages with the WQRP's PPCP efforts. The NPD will work with OPPTS to make the data not only from HPV but also from VCCEP, available to ORD for data mining and research hypothesis generation.

Progress: As noted above, there are two interagency working groups, one on endocrine disruptors and one on pharmaceuticals in the environment (PiE). The purpose of both of these IWGs is to provide a forum for identifying ongoing research, define data gaps, and identify areas in which the Federal government could collaborate to address such data gaps. The EDCs' IWG had a workshop earlier this year that focused on impacts of EDCs on ecosystems and the environment. The EDCs' IWG includes interest on all agents that interfere with the endocrine system. The PiE IWG is examining the current body of knowledge for both human and veterinary pharmaceuticals in the environment, including antibiotics and the implications for antibiotic resistance. The PiE IWG will develop research strategies for human and veterinary pharmaceuticals in the environment and for antibiotic resistance by December 2007. 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. Once the research needs are identified, decisions will be made not only within EPA but within the other representative Agencies as well as to how these needs will be addressed. Within EPA, there are several research programs that could consider addressing the identified needs, e.g., EDCs, SP2, Water Quality, Human Health, and it will be determined through close collaboration across these programs what will be addressed and how.

Linked to this activity is the fact that several years ago, the EDCs Research Program determined that it would develop a cross-Laboratory/Center multidisciplinary effort (first described in the 2003 EDCs MYP) that would characterize the impact of hormones released from CAFOs into the environment. Intramural research began in several divisions of the effects, risk management and exposure laboratories a few years ago. An RFA was issued in 2006 and seven awards were recently made. At least three of the awards will be in the form of cooperative agreements to facilitate close collaboration with intramural scientists. Although some of the hormones released into the environment are from natural steroids from the animals, some are the result of excretion of endocrine-active pharmaceutical/veterinary agents.

EPA's risk management research program has developed analytic methods and is applying them to test for natural and synthetic steroid hormones in wastewater effluents, biosolids, drinking water, as well as CAFOs.

In general, the EDCs Research Program has expanded its interactions with other organizations in conducting its research. For example, research in the area of wastewater treatment plants has been conducted in collaboration with: 1) EPA's 10 Regional Offices, who provided ORD with samples of WWTP effluents from across the regions, 2) the Office of Water with whom we are conducting a study of influents and effluents at nine WWTPs, looking at a several hundred chemicals, some of which are known or suspected EDC, and looking at the different types of treatment technology and their effectiveness in reducing exposures and endocrine activity, 3) the city of Chicago, using one of their WWTPs to conduct our research on effluents and biosolids, 4) the Ohio River Sanitation Coalition, using our vitellogenin expression assay in fathead minnow as a tool to evaluate impact of effluents downstream from WWTPs, and 5) the Global Water Research Coalition where we are applying one of the methods we developed for use as a screening assay for individual chemicals and optimizing it to determine how effective a tool it is to detect endocrine activity in the complex mixtures of WWTP effluents collected from around the world in a multinational integrated study.

EPA has a number of activities regarding the mining of data but these are not being done under the EDCs Research Program. For example, EPA is working with the Organization for Economic Cooperation and Development (OECD) on their Molecular Screening Initiative. While not EDC-restricted, much of the international activity in genomic applications has been using EDCs. The ToxCast concept is now emerging as a relatively common path forward in this effort, and an international consortium is slowly taking shape. The NCCT is developing structure indexed databases (DSSTox and ToxRef) that are linking information in the public domain and in the

OPP's DERs (Detailed Evaluation Records) for the active ingredients in pesticides (covering subchronic, chronic, multi-generational, and prenatal toxicity studies). ToxRef will provide, for the first time, a relational database of the effects of pesticides in key toxicology studies. It will be the gold standard upon which the bioactivity signatures of ToxCast Phase I will be generated. NCCT is also in discussions with OPPT to bringing the toxicology data from HPVIS (High Production Volume Information System in ACToR (the Aggregated Computational Toxicology Resource) being developed by a senior bioinformaticist in the NCCT (see related progress in response 12 below). OPPTS just announced the release of 28 draft screening-level hazard characterization documents on 101 High Production Volume Challenge. The characterizations are based on screening-level hazard data that was submitted by the U.S. chemical industry through the HPV Challenge Program or otherwise available to us through prior reporting or submission

8. It was not feasible for scientists conducting epidemiologic studies to attend the face-to-face program review meeting. Even though the subcommittee members were provided information from the Tulane conference and one of the subcommittee members attended the conference, the BOSC recommends that subsequent reviews of this program include poster presentations by each of the scientists funded by this interagency program (p. 11, 30).

Comment: A symposium for the epidemiology grantees had been planned eight months prior to knowing that a Program Review of the EDCs research would be planned. The symposium took place just 6 weeks before the Program Review. It was decided that rather than have grantees come to the review that we would invite the Reviewers to the symposium, provide the results of the symposium to the Reviewers, and have a single poster at the Program Review that integrated the status and progress that each grantee was making.

2005 Proposed Action: The grantees will be brought together next summer for another grantees progress review. In the future the NPD and the planning team will avoid scheduling progress reviews of grantees near the time of anticipated Program Reviews. For the next Program Review, an invitation to the epidemiology grantees will be extended requesting their participation.

Progress: In 2001, EPA partnered with NIEHS, NIOSH, and CDC to fund 12 grants to study the human health impact of endocrine disruptors. EPA awarded 5 research grants to support 5-year epidemiological studies. In 2004, EPA sponsored a joint session in conjunction with the e.hormone conference at which all 12 of the recipients presented progress on the projects (http://es.dpa.gov/ncer/publications/workshop/pdf/2004_endocrine_disruptors_proceedings1.pdf). In 2006, EPA sponsored an endocrine disruptors progress review workshop at which all 5 of the EPA-sponsored epidemiology grantees presented their progress (<http://es.epa.gov/ncer/publications/workshop/endocrineworkshp71306.html>). Due to the complexity of research projects of this type, variable success in recruitment, delays in urine and blood sample analysis (backlog at CDC), and several administrative delays, the grants have been

extended with no additional funding. All of the grants have submitted up-to-date progress reports in the past year. Several of the projects have produced significant publications, as well as frequent presentations at scientific meetings and are expected to result in reliable data. Final reports on all epidemiology grants are expected in FY2009. An invitation will be extended to the investigators requesting their participation at the next BOSC review.

9. It will be important for EPA to take the leadership role in the application of the “omics” technologies to address many of the science questions critical for evaluating environmental and human health effects of EDCs. This will require a strong commitment to a systems biology approach and computational toxicology as well as effective interactions with those generating much of the basic data (p. 11, 30, 36).

Comment: ORD has initiated a Research Program in the area of Computational Toxicology which is using EDCs for its proof of concept pilot. The program has three objectives: 1) improve linkages across the source to outcome paradigm, 2) develop predictive tools, and 3) improve quantitative risk assessments and is being implemented intramurally and extramurally through STAR. Through the Comp Tox program ORD is collaborating with many other federal agencies (e.g., NIEHS, DOE), private companies (e.g., Gene Logic, Iconix, IBM), and universities. Some of this research is being conducted through the SP2 program, e.g., development of systems biology models for fish and amphibian. One project in particular is addressing the question, “Can existing toxicogenomics data improve Environmental Protection Agency (EPA) chemical health risk assessments?” To address this question, a case study will be performed in which toxicogenomics data for dibutyl phthalate, a chemical with endocrine activity, will be incorporated qualitatively in the hazard characterization step of a recent or ongoing EPA chemical health risk assessment. Integrating toxicogenomics data into an assessment case study will identify areas that may be impacted by toxicogenomics data, and contribute to the development of criteria and approaches for incorporating toxicogenomics data in assessments.

Other research is being conducted through the EDCs Research Program. For example, ‘omics tools are being used to develop and evaluate semi-high throughput approaches for screening chemicals for endocrine activity. One of these has been so successful already that OPPTS is including it among the battery of assays under consideration for EDSP. Data from similar efforts are also feeding into improving QSAR models for prioritization of chemicals for testing. ORD’s in-house capability has been complemented through the STAR extramural program by issuing three Requests for Applications in the areas of high throughput screens, systems biology approaches (using the HPG and HPT axes), and Environmental Bioinformatics Research Centers (EBRCs).

EDCs researchers participate in a cross-Agency Genomics Task Force that is lead by the Office of the Science Advisor. Subgroups of this Task Force are developing guidance on data submission, data quality, data management, data analysis, data storage and training. The goal is to begin to position the regulatory side of the Agency to handle the types of data that are likely to become

part of submissions of toxicological dossiers. For example a Genomics Action Plan has been developed that is addressing incorporation of genomics information in activities such as prioritization and monitoring, both of which are relevant to the EDCs Research Program. Other links to the EDCs program are the application of genomics data in identification of stressor modes of action, identification and assessment of impacts on susceptible subpopulations and life stages, and improving mixtures assessments.

Action: EPA is positioning itself as a leader in the ‘omics field through research and policy developments. EDCs scientists will continue to play a critical role in both of these areas. The Director of NCCT and the NPD will continue to hold frequent meetings to coordinate activities. They will work with the planning team to ensure that the linkages among the Comp Tox, EDCs, and SP2 programs are captured in the updated MYPs.

Progress: As noted above, the Agency’s Office of the Science Advisor has played a leadership role in this area, bringing together the talents from across the EPA organizations (<http://www.epa.gov/osa/spc/genomicsguidance.htm>). They continue to organize and hold training sessions for scientists and managers. ORD has been active in the incorporation of high-density data approaches in ecological and human health risk assessment based research. Both metabonomic and proteomic approaches are being used to investigate the effects of estrogenic compounds on the fathead minnow, zebrafish, and medaka. Transcriptional profiling is being applied to understand the molecular underpinning of reproductive effects in fish and rodents. Combined transcriptomics and metabolomics are currently being used to further characterize the alterations in the male reproductive tract after exposure to a series of fungicides. These approaches are an integral part of multiple research activities across the Human Health, Drinking Water, and SP2 Research Programs, in addition to the EDCs Research Program. In addition, the NCCT is having ArrayTrack installed that could serve as a repository for genomic data, whether from the intramural program or from the regulated community as part of any data submission.

One effort, more closely tied to EDCs and mentioned briefly above, is the case study developed for DBP. In order to explore the use of new toxicogenomics technologies in risk assessment, a case study, using DBP, has been developed in NCEA. This project is funded in part through the Comp Tox Research Program, is collaborating with expert scientists from one of its STAR-funded Environmental Bioinformatics Research Centers, and addresses needs of risk assessors across the Agency, who are already reviewing toxicogenomics data for consideration in their assessments. The peer reviewed report from this project is designated as a measure for completion in FY 2009 under the Human Health Risk Assessment Research Program. The results of the case study review have been utilized to illustrate the use of toxicogenomics in risk assessment in a June 2007 Risk Assessment Forum training course and in several national and international symposia on the topic. Upcoming milestones include the following:

- Sep 2007 – Completion of internal review draft document
- Dec 2007 – One-day colloquium
- Jan 2008 – Completion of external review draft

- Mar 2008 – External Panel peer review meeting
- Document finalization and publication.

10. *EPA should continue to investigate the common ground between ecological and human health because the Agency is in a unique position to do so (p. 11, 30).*

Comment: The NPD and the planning team agree that the EDCs program is in a unique position to develop approaches that integrate human health and ecological assessments. The assessment portion of our Research Program has been relatively small. However, a case study was developed using mode of action (MOA) information across animal species and humans determining the relationship between MOA and species relatedness (i.e., evolutionary relationships). A case study assessing the utility of this approach was performed for Bisphenol A (BPA), a chemical shown to have endocrine disrupting activity. For the tested vertebrate species, the data support a relationship between species relatedness and the estrogen agonist MOA. Thus, the cross-species MOA approach holds promise for predicting the MOA among untested species for toxic agents. Such predictions could be useful for applying MOA information in an integrated ecological and human health risk assessment as well as for screening and toxicity testing prioritization of chemicals such as the EDSP since the program is concerned with protecting human and wildlife health. The STAR extramural program was used earlier this year to solicit proposal on methods to characterize exposures to mixtures which would be of relevance to human and ecological health. Currently, several intramural laboratory projects are looking at impacts of exposures on aquatic species and rodent models used for human health.

2005 Proposed Action: The NPD and the planning team will take on the challenge to develop approaches that integrate human health and ecological assessments. We will consider improving the integration of projects that will contribute to evaluate human and ecological health (e.g., using the MOA approach). A pilot for this consideration may be centered around CAFOs, where we will be increasing our intramural efforts and issuing a STAR solicitation.

Progress: There has been a moderate degree of activity to address this recommendation. The case study described in the 2005 response has been published. The research on cross-species extrapolation looking at ER and AR and on aromatase in the fish and rat (mentioned in progress under response 4) both will provide valuable information regarding the bridge between ecological and human health. The STAR program awarded five grants in 2006 for the development of innovative approaches for measuring the concentrations and activities of EDCs in environmental and biological media. Results from these grants will serve as another bridge by developing a suite of methods for EDCs exposure analysis that can be used for environmental or human monitoring studies. At the IWG workshop in February 2007 there was some discussion of conducting an integrated multidisciplinary study that would look at both ecological and human health impacts at a site-specific community. However, there have not been any further discussions. In our proposed actions in 2005, we suggested that a pilot to look at the common ground between

ecological and human health may be conducted in relation to the CAFOs research project. However, most of this effort is focusing on characterizing the occurrence of endocrine activity, its impacts on ecological health, and the development of remediation approaches. It may be possible to add a component, in the future, depending on resources, which could evaluate potential impacts on human health in communities where CAFOs flourish.

STRENGTHS AND CHALLENGES OF LONG-TERM GOAL 3:
Support EPA's screening and testing program

11. ORD is beginning to develop core competencies in genomics and quantitative structure activity relation (QSAR) methods, both of which hold promise in endocrine disruptor identification. Because these areas are so data intensive, it will be important for ORD to train or hire experts in bioinformatics to work with the life sciences experts already on staff (p. 8, 24, 36)

2005 Proposed Action: ORD is addressing the need for increasing our competency in bioinformatics in two ways. The first is with new hires. The National Exposure Research Laboratory already has hired two bioinformaticians. The NCCT has issued job announcements for an additional two intramural bioinformaticians. Second, we have issued a solicitation through STAR for an Environmental Bioinformatics Research Center. The award for the EBRC will be made in the form of a cooperative agreement so that there will be strong interactions between extramural intramural scientists.

Progress: A lot of progress has been made in this area since 2005. ORD has been very proactive in enhancing its capabilities in the new high-density data array approaches including transcriptional profiling, proteomics, and metabolomics. NHEERL has established a genomics core which has the capability of performing DNA arrays on samples from multiple species including human, mouse, and rat. As the ecology research-relevant species come on line, they too will be included in the armamentarium. Proteomic core capabilities have been developed in the ecology and human health laboratories, including strategic hires, enabling proteomic approaches to become integral to the research program as endpoints for fish, rodent, and human studies. NHEERL has hired a toxicogenomics expert and a systems biologist who are taking the lead in coordinating application and interpretation of gene array data in risk assessment based research. NERL has developed a metabolomics core capability which is rapidly becoming integrated into a number of cross-ORD projects. They have also hired a bioinformatician.

In the past 12 months, NCCT has hired three senior level scientists to work in the areas of bioinformatics and computational systems biology. One scientist is developing the ACToR system, which will be a master warehouse of chemical, biological and toxicological data (it will include the contents of DSSTox, ToxRef (the active pesticide toxicology database, and ToxCast HTS data); he is also coordinating the research interaction of the STAR Environmental Bioinformatic Research Centers (further detailed below). Another one is leading the "Virtual

Liver” project whose long term goal is a computational model of hepatic function and toxicity that will be useful in understanding modes of action and in extrapolation across dose, life-stage and species. The third just joined EPA last week and will be focusing on development toxicology from a computational viewpoint. NCCT also has 5 postdoctoral fellows working on various computational models.

In 2005, the National Center for Environmental Research (NCER), as part of the STAR Computational Toxicology Research Program funded The Carolina Environmental Bioinformatics Research Center at the University of North Carolina at Chapel Hill and The New Jersey Research Center for Environmental Bioinformatics and Computational Toxicology, a collaborative effort between the University of Medicine and Dentistry of New Jersey, Princeton University, and Rutgers University. Both Centers are funded through 2010. Both awards were made in the form of cooperative agreements so that there would be close collaboration with ORD scientists, especially from NCCT.

The Carolina Center brings together multiple investigators and disciplines, combining expertise in biostatistics, computational biology, cheminformatics and computer science to advance the field of computational toxicology. The Center is developing novel analytic and computational methods, creating efficient user-friendly tools to disseminate the methods to the wider community and is applying the computational methods to data from molecular toxicology and other studies.

The NJ Center comprises computational scientists, with diverse backgrounds in bioinformatics, cheminformatics and enviroinformatics. The team is addressing multiple elements of the toxicant “source-to-outcome” sequence as well as developing cheminformatics tools for toxicant characterization.

In February 2007, NCER issued the RFA “Computational Toxicology Centers: Development of Predictive Environmental and Biomedical Computer-Based Simulations and Models.” NCER intends to grant two awards, each with a maximum duration of 4 years. The goal is for the centers to translate wet chemistry/biology to *in silico* predictive simulation. Funding for the new Centers should begin in 2008.

The major challenge that ORD faced with regard to the screening and testing program is handing off its research to the program offices so that validation and implementation can occur in a timely way. It should be noted, however, that much of the delay in validation and regulatory acceptance is because this process takes place largely outside the Agency (p. 12).

12. The transfer of protocols to contract laboratories has been problematic. This has led to a substantial commitment by EPA staff to refine and troubleshoot assays, and it has had a negative effect on other core research activities that are the responsibility of ORD staff. The BOSC recommends that there be a mechanism in place to ensure the timely transfer of protocols to OPPTS (p. 35).

Comment: The problem has been with the type of contracts and the contractor to whom OPPTS transfers the protocols once ORD has transferred them to OPPTS. As a result, OPPTS is seriously behind in their validation effort. Furthermore, OPPTS has had to rely on ORD's EDCs scientists to help analyze data developed through the contractor. This results in ORD staff being diverted from their planned experiments to serve as consultants to OPPTS.

2005 Proposed Action: OPPTS senior management and the NPD will be meeting with NHEERL senior management to emphasize OPPTS' priorities and time lines and to reach agreement on how to meet their expectations.

Progress: Significant progress has been made in completing the research needed to develop and standardize assays for the Agency's EDSP. The Tier 1 assays are in various stages of validation by OPPTS for use in implementation of EDSP. All of the Tier 1 assays will be validated and peer reviewed by the time EDSP is implemented in 2008. OPPTS has published its plan for peer review (http://www.epa.gov/scipoly/oscpendo/pubs/assayvalidation/peerreview_process.htm). Remaining research is finalizing the fish and the frog life cycle assays for implementation in Tier 2. These assays will be handed off to OPPTS for validation in 2010. ORD scientists, throughout this process, continue to serve as valuable resources to OPPTS as the assays are validated, under peer review, and ultimately implemented. Research ongoing under Long Term Goal 1 will help provide OPPTS with the foundation it needs to interpret the data as they are submitted to the Agency. ORD scientists will serve as consultants at that time as well.

ORD has a large number of research accomplishment within NHEERL that have contributed significantly to a basic understanding of the toxic responses to estrogens, anti-androgens (RTD), and thyroid toxicants (within ETD), which in turn has led directly to the development of improved methods for endocrine disruptor detection

13. This research is diffuse and is occurring in multiple divisions within NHEERL and many of the accomplishments in these areas have been difficult to capture in the list of APGs. The BOSC recommends that EPA try to summarize this research and its relevance to EDC identification in subsequent reports and revisions of the MYP (p.35).

Comment: The NHEERL Implementation Plan for Endocrine Disruptors identifies the directions of NHEERL's research and provides integrated summaries of the projects' efforts as of fall 2004. The Implementation Plan was included in the background material that was sent to the EDC Subcommittee peer reviewers prior to the Program Review and was cited in the charge questions for review.

2005 Proposed Action: The EDCs planning team is beginning to update the MYP. It will

develop a more coherent way in which to summarize the accomplishments to date and characterize their impact and cite linkages to other relevant documents, such as the NHEERL Implementation Plan, the National Risk Management Research Laboratory's Risk Management Evaluation for Endocrine Disruptors, the Comp Tox Implementation Plan, and the MYPs of other Research Programs, e.g., SP2, Human Health, WQRP. The research is also being summarized in three topical synthesis documents (Effects in Wildlife, Effects on Development, Screening & Testing) that will compile and integrate the intramural and STAR extramural research accomplishments.

Progress: Since the research program has been ongoing for well over a decade, it has been a challenge to portray the significant accomplishments in a coherent way. We are using multiple approaches to communicate our results.

We have developed a draft synthesis document that summarizes and integrates the intramural and extramural accomplishments has been prepared and it helps to address the BOSC's recommendation that research should be summarized more often in reports that emphasize accomplishments and relevance to meeting program goals. The draft report is composed of an overview and series of individual chapters on specific topics where a substantial body of research has been completed. The report will be used for external scientific reviews of the program, communications, and future research planning. It is being supplied to the BOSC Subcommittee.

The report, "EPA Achievements in Endocrine Disruptors Research: Key Accomplishments from 1996-2007" describes the accomplishments of the first ten years of the integrated Endocrine Disruptors Research Program. The projected audiences for the report are external reviewers, existing and potential partners, and ORD research planners. The report contains a chapter overview of the Research Program and three chapters of key accomplishments in the areas of highest impact - Wildlife, Early Development, Screening and Testing. The overview describes the program's goals, history, benefits, and performance. Each chapter provides highlights of research results in relation to progress made in understanding potential risks to human health and the environment. Some important areas covered in the report are estrogenic, androgenic, and thyroid effects of EDCs as well as progress in understanding dose-response relationships, modes of action, species differences, and cumulative risk. In each chapter, the major impacts and outcomes of peer-reviewed research are summarized. EPA is currently working on the third draft of the report with contractors at ICF, Inc. The final report will be available as an Adobe Acrobat PDF on CD and posted on the EPA web site. A full bibliography of peer-reviewed publications resulting from the research will be distributed with the report. The final report is expected to be completed in fall of 2007.

Screening and Testing Chapter: The report chapter on "Advances in Screening and Testing" describes the relationship between the ORD endocrine disruptors research program and the regulatory program based in OSCP. The contributions that ORD has made to the Screening and

Testing Program include developing and participating in the validation process of the assays, preparing training materials, performing demonstration and supplementary studies, reviewing and interpreting data, drafting test guidelines, and serving as technical experts on peer review panels. Specific contributions to priority setting, Tier 1, and Tier 2 assays are described.

Early Development Chapter: The report chapter on “Understanding Outcomes of Early Developmental and Childhood Exposure” integrates a wealth of EPA intramural and STAR research on this topic. The chapter summarizes key accomplishments that EPA has made to research on effects of EDCs on fertility, male and female reproduction, growth, and development of endocrine systems. The research spans research on mechanisms of toxicity in *in vitro* systems to epidemiological studies in human populations. Estrogen, androgen, and thyroid system toxicity are covered.

Wildlife Chapter: The report includes a chapter “Exposure and Effects in Wildlife” summarizing EPA contributions to understanding exposure and effects of endocrine disruptors in wildlife. Topics include progress on identifying sources of EDCs in the environment such as wastewater treatment plants, animal feeding operations, and pulp and paper mills. Key EPA research accomplishment on the effects of EDC in wildlife species such as birds, frogs, fish, and invertebrates are described. EPA research on species comparisons and differential sensitivity, and effects on wild populations are described.

In the draft updated MYP significant accomplishments have been listed in a separate Appendix and aligned under annual performance goals in an effort to bring them together in context. The listing is still in a preliminary draft stage and we recognize that it needs additional attention before finalization.

We have also prepared a bibliography that lists, by Long Term Goal, over 500 peer reviewed publications as products of the program. The bibliography was given to a contractor to conduct a bibliometric analysis to provide us with an indication as to how effective our products are in being cited by other researchers and the degree to which we are having an impact and publishing in high impact journals. Both the bibliography and the bibliometric analysis were supplied to the BOSC Subcommittee.

Finally a website where our results can be communicated broadly is under development. We are in the process of developing fact sheets for targeted elements of the research program. We will also post documents such as the MYP and progress reports, and provide linkages to other Agency websites that have information on EDCs.