U.S. EPA Office of Research and Development Computational Toxicology Research Program Implementation Plan for Fiscal Years 2009 to 2012

Providing High-Throughput Decision Support Tools for Screening and Assessing Chemical Exposure, Hazard, and Risk

This document has been reviewed by the United States Environmental Protection Agency, Office of Research and Development (ORD) and approved for public release, but it does not necessarily constitute official Agency policy. This plan follows the first generation Computational Toxicology Research Program Implementation Plan for Fiscal Years 2005 to 2008, in providing a strategic overview of research for fiscal years 2009 to 2012. This plan was reviewed by ORD senior management and members of the Science Council, as well as by the Computational Toxicology Subcommittee of the ORD Board of Scientific Counselors on September 29 and 30, 2009, in Research Triangle Park, NC.

Table of Contents

List of Figures

List of Acronyms

ACToR	Aggregated Computational Toxicology Resource
BBDR	biologically based dose-response
BE	biomonitoring equivalent
BfR	Federal Institute for Risk Assessment
BOSC	Board of Scientific Counselors
CCL	Candidate Contaminant List
CDC	Centers for Disease Control and Prevention
CEBS	NIEHS Chemical Effects in Biological Systems
ChEBI	Chemical Entities of Biological Interest
CoP	communities of practice
CTISC	Computational Toxicology Implementation and Steering Committee
CTRP	Computational Toxicology Research Program
DNT	developmental neurotoxicity
DSSTox	Distributed Structure-Searchable Toxicity Database
ECOTOX	ECOTOXicology Database
EDC	endocrine-disrupting compound
EDSP	endocrine disruptor screening program
EMAGE	Edinburgh Mouse Atlas of Gene Expression
EPA	U.S. Environmental Protection Agency
ExpoCast™	Exposure Forecasting Project
ExpoCoP	Exposure Science Community of Practice
FDA	U.S. Food and Drug Administration
FTE	full-time equivalent
FTTW	Future of Toxicity Testing Workgroup
FY	fiscal year
HEASD	Human Exposure and Atmospheric Sciences Division
HEDS	Human Exposure Database System
HPV	High-Production Volume
HTS	high-throughput screening
ICCA-LRI	International Council of Chemical Association's - Long-Range Research Initiative
IRIS	Integrated Risk Information System
KB	knowledge base
MetaPath	a metabolism pathways expert system
MICA	Mechanistic Indicators of Childhood Asthma
MOA	mode or mechanism of action
MOU	memorandum of understanding
MTA	material transfer agreement
MYP	multiyear plan
NCCT	National Center for Computational Toxicology
NCEA	National Center for Environmental Assessment
NCER	National Center for Environmental Research
NCGC	NIH Chemical Genomics Center
NERL	National Exposure Research Laboratory
NHEERL	National Health and Environmental Effects Research Laboratory
NHGRI	NIH Chemical Genome Research Institute

NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NLM	National Library of Medicine
NPD	National Program Director
NRC	National Research Council
NRMRL	National Risk Management Research Laboratory
NTP	National Toxicology Program
OECD	Organization for Economic Cooperation and Development
OPP	Office of Pesticide Programs
OPPT	Office of Pesticides, Prevention, and Toxics
OPPTS	Office of Pesticides, Prevention, and Toxic Substances
ORD	Office of Research and Development
OSCP	Office of Science Coordination and Policy
OW	Office of Water
PBPK	physiologically based pharmacokinetic
PMRA	Pest Management Regulatory Agency
QA	quality assurance
QMP	quality management plan
QSAR	quantitative structure-activity relationship
RIVM	The National Institute for Public Health and the Environment, Netherlands
RTD	NHEERL Reproductive Toxicology Division
SAB	Science Advisory Board
SAP	Scientific Advisory Panel
SAR	structure-activity relationship
SHEDS	Stochastic Human Exposure and Dose Simulation
STAR	Science To Achieve Results
TIVS	Texas-Indiana Virtual STAR Center
ToxCast™	Toxicity Forecasting Project
ToxML	Toxicity Databases Data Management Leadscope
ToxRefDB	Toxicity Reference Database
UMDNJ	University of Medicine and Dentistry of New Jersey
UNC	University of North Carolina
v-Embryo™	Virtual Embryo Project
v-Liver™	Virtual Liver Project
v-Liver-KB	v-Liver Knowledgebase
VT	virtual tissue
WHO	World Health Organization

Executive Summary

This document lays out the fiscal year 2009 to 2012 objectives of the U.S. Environmental Protection Agency (EPA), Office of Research and Development (ORD) research program in computational toxicology. Computational toxicology is the application of mathematical and computer models to help assess chemical hazards and risks to human health and the environment. Supported by advances in informatics, high-throughput screening technologies, and systems biology, EPA is developing robust and flexible computational tools that can be applied to the thousands of chemicals in commerce and the contaminant mixtures found in America's air, water, and hazardous-waste sites. The ORD Computational Toxicology Research Program (CTRP) is composed of three main elements. The largest component is the National Center for Computational Toxicology (NCCT), which was established in 2005 to coordinate research on chemical screening and prioritization, informatics, and systems modeling. The second element consists of related activities in the National Health and Environmental Effects Research Laboratory (NHEERL) and the National Exposure Research Laboratory (NERL). The third and final component consists of academic centers working on various aspects of computational toxicology and funded by the EPA Science to Achieve Results (STAR) program. Together, these elements form the key components in the implementation of both the initial strategy, *A Framework for a Computational Toxicology Research Program* (US EPA, 2003), and the newly released *The U.S. Environmental Protection Agency's Strategic Plan for Evaluating the Toxicity of Chemicals* (US EPA, 2009). Key intramural projects of the CTRP include digitizing legacy toxicity testing information Toxicity Reference Database (ToxRefDB), predicting toxicity (ToxCast™) and exposure (ExpoCast™), and creating virtual liver (v-Liver™) and virtual embryo (v-Embryo™) systems models. EPA funded STAR centers also are providing bioinformatics, computational toxicology data and models, and developmental toxicity data and models. All CTRP projects participate in the Agency's formal quality assurance program and regularly undergo peer review. The models and underlying data are being made available publicly through the Aggregated Computational Toxicology Resource (ACToR), the Distributed Structure-Searchable Toxicity (DSSTox) Database Network, and other EPA websites. Thus, the CTRP is providing the foundation for advancing high-throughput toxicology and risk assessments and, thereby, closing critical data gaps for thousands of chemicals and helping EPA better assess and manage chemical risk.

The CTRP is evolving beyond the initial focus on hazard identification and chemical prioritization, as expressed in the new long-term goal of providing high-throughput decision support tools for assessing chemical exposure, hazard, and risk. There is an increasing emphasis on using high-throughput bioactivity profiling data in systems modeling to support quantitative risk assessments and greater involvement in developing complementary higher throughput exposure models. Discussions are well underway among NCCT, NHEERL, NERL, the National Risk Management Research Laboratory, the National Center for Environmental Assessment, and the National Center for Environmental Research, managers of the STAR program on how the CTRP will play a major role in future integrated ORD programs centered on looking at chemical hazards and risks from a life cycle viewpoint. This integrated approach will enable analysis of life stage susceptibility and understanding of the exposures, pathways, and key events by which chemicals exert their toxicity in developing systems (e.g., endocrine related pathways). The CTRP will be a critical component in next generation risk assessments utilizing quantitative high-throughput data and providing a much higher capacity for assessing chemical toxicity than is currently available.

This second generation CTRP implementation plan is highly consistent with the Agency's priority for improving the management of chemical and contaminant risks. In January 2009, a

memo sent to all EPA employees by Administrator Jackson listed managing chemical risks as one of five top priorities and stated:

> "More than 30 years after Congress enacted the Toxic Substances Control Act, it is clear that we are not doing an adequate job of assessing and managing risks of chemicals in consumer products, the workplace and the environment. It is now time to revise and strengthen EPA's chemicals management and risk assessment programs."

With contributions from across ORD, the CTRP will provide EPA program offices better decision analysis tools for hazard and exposure screening and assessment, which then can be used to better manage the risks of chemicals. The CTRP is acquiring an international reputation for leadership in the introduction of innovative high-throughput technologies and computational approaches for identifying toxicity pathways and characterizing response to environmental exposures. It is through this effort that problems will be addressed and solutions to EPA's chemicals management and risk assessment programs will be developed.

I. History of the CTRP and the NCCT

A. Defining the Mission of Computational Toxicology at EPA

> ***CTRP Mission Statement**: To integrate modern computing and information technologies with molecular biology to provide the Agency with decision support tools for high-throughput risk assessment.*

Computational toxicology applies mathematical and computer models and molecular biological and chemical approaches to explore both qualitative and quantitative relationships between chemical exposure and adverse health outcomes. Recent technological advances make it possible to develop molecular profiles using high-throughput and high-content methods that identify the impacts of environmental exposures on living organisms. With these tools, scientists can produce a more detailed understanding of the hazards and risks of a much larger number of chemicals. The integration of modern computing with molecular biology and chemistry is enabling scientists to better understand a chemical's progression through the environment to the target tissue within an organism and, ultimately, to the key steps that trigger an adverse health effect. Currently, risk estimates most often are based on gross outcomes of disease, such as occurrence of cancer, a neurological disorder, or a visible birth defect. The National Research Council (NRC) in its 2007 report, *Toxicity Testing in the 21st Century: A Vision and a Strategy*, called for a concerted effort to move toxicology from a primarily descriptive science to a more predictive one by utilizing largely human based in vitro studies to understand the biological pathways by which chemically induced diseases occur. The Environmental Protection Agency's Computational Toxicology Research Plan is working to aid this transformation by evaluating the key molecular changes occurring in the function of critical human toxicity pathways within cells, tissues, individuals, and populations. The key will be connecting these changes quantitatively and systemically to the types of adverse health effects that have been the traditional basis of EPA risk assessments and to use this understanding to reduce the current uncertainties in the extrapolation of effects across dose, species, and chemicals. The Office of Research and Development CTRP is composed of three main elements. The largest component is the National Center for Computational Toxicology, which was established in 2005 to coordinate research on chemical screening and prioritization, informatics, and systems modeling. The second element consists of related activities in the National Health and Environmental Effects Research Laboratory (NHEERL) and National Exposure Research Laboratory (NERL) of ORD. The third and final component consists of academic centers working on various aspects of computational toxicology and funded by the EPA Science to Achieve Results (STAR) Program.

The rapid and ongoing success of the CTRP is impacting hazard and exposure identification and helping to close data gaps, identify toxicity pathways, suggest modes of action, and make for more efficient utilization of precious resources on the highest priority chemicals. Besides these initial outcomes from the higher throughput approach of the CTRP, informatics and modeling efforts will provide more in-depth and quantitative molecular understanding of how biological systems respond to environmental chemicals. These knowledgebases and in silico tools will reduce or quantify uncertainties relating to biological susceptibility, species differences, and dose response as part of a faster and more intelligent targeted testing paradigm in support of quantitative risk assessments.

B. Timeline of CTRP Development

In fiscal year 2002, Congress ordered a redirection of $4 million from available EPA funds,

> "…for the research, development and validation of non-animal alternative chemical screening and prioritization methods, such as rapid, non-animal screens and Quantitative Structure Activity Relationships (QSAR), for potential inclusion in EPA's current and future relevant chemical evaluation programs."

To fulfill this directive, EPA embarked on development of a research program that (1) was consistent with the Congressional mandate, (2) complemented and leveraged related ongoing Agency sponsored efforts to consider alternative test methods, (3) further advanced the research to support the Agency's mission, and (4) would not duplicate the mission and programs in this area conducted by other agencies (see Figure 1 for a timeline of CTRP development).

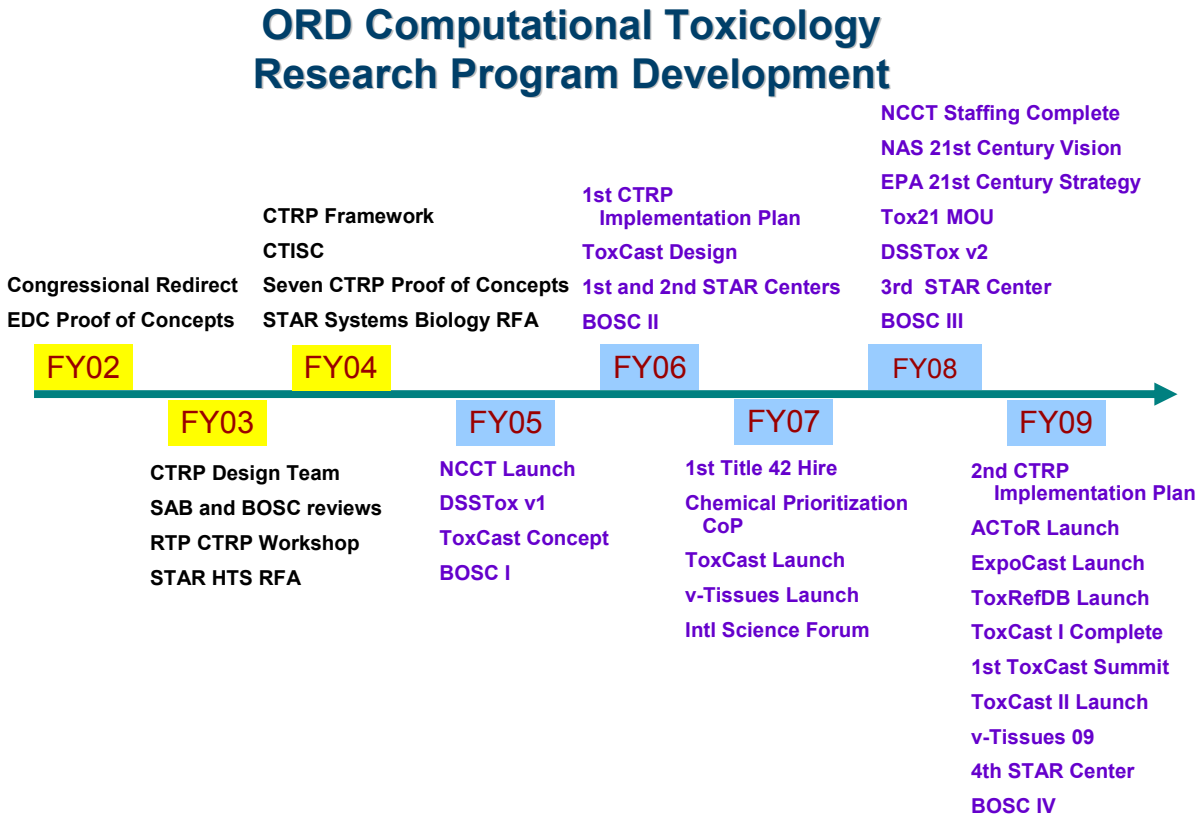

Figure 1

Thus the CTRP was initiated to target these goals and, in the process, significantly advance toxicology and risk assessment as currently practiced by the Agency and the broader environmental sciences community. In FY2002 to 2003 pilot projects were funded to demonstrate computational toxicology could be adapted to the study of endocrine disruptors. Early successes of these efforts included refinement of estrogen receptor ligand binding data for development of quantitative structure-activity models, evaluation of EPA-developed cell lines for

detecting estrogen and androgen activities from various species and the development of an alternative test method for evaluating effects on steroidogenesis in H295R cells (see EDSP Assay Status).

With increasing attention to and expectations for the CTRP in FY2003, ORD developed *A Framework for a Computational Toxicology Research Program*, which was published in FY2004 and provided strategic direction for the program. This document was the product of a cross-ORD design team of scientists and was endorsed by the Science Advisory Board (SAB). ORD hosted a workshop in Research Triangle Park, NC, in late FY2003 to introduce the CTRP framework (Kavlock et al., 2003) from which the three objectives for EPA computational toxicology were translated into the three initial long-term goals (LTGs) for the program:

1) risk assessors use improved methods and tools to better understand and describe the linkages of the source-to-outcome paradigm,
2) EPA program offices use advanced hazard characterization tools to prioritize and screen chemicals for toxicological evaluation, and
3) EPA assessors and regulators use new and improved methods and models based on the latest science for enhanced dose-response assessment and quantitative risk assessment.

With issuance of the CTRP framework, ORD began the process of implementing a more formalized program. A cross-Agency working group, the Computational Toxicology Implementation Steering Committee (CTISC) was formed in FY2004 to oversee the selection and funding of projects across ORD. Seven cross-ORD projects were initiated as result of CTISC action, and these "new start" projects became a critical component of the first generation CTRP implementation plan. Greater detail on the accomplishments of these seven projects is provided in Section I.F.2.

In October 2004, then EPA Science Advisor and Assistant Administrator for ORD, Dr. Paul Gilman, announced the formation of the NCCT, which began official functions in February 2005. The announcement states:

> "The Center will advance the science needed to more quickly and efficiently evaluate the potential risk of chemicals to human health and the environment. The Center will coordinate and implement EPA's research on computational toxicology to provide tools to conduct more rapid risk assessments and improve the identification of chemicals for testing that may be of greatest risk."

NCCT quickly became the hub for ORD CTRP research. NCCT formed key partnerships with the other laboratories and centers within ORD, which formed the second critical element. Partnerships with NHEERL, NERL, the National Risk Management Research Laboratory, and the National Center for Environmental Assessment helped in the execution of not only the seven cross-ORD "new start" projects awarded by the CTISC in 2004, but also in several original NCCT-led projects, including the Distributed Structure-Searchable Toxicity Database (DSSTox), Toxicity Forecasting Project (ToxCast™),Toxicity Reference Database (ToxRefDB), and the virtual tissues projects looking at liver and embryo. Greater detail on the accomplishments and future plans for these and other NCCT projects is provided in Section I.F.1.

The third critical component of the CTRP is extramural partners and research, much of which is supported by NCER through the STAR program. In FY2003 and 2004, two separate STAR Requests for Applications (RFA) that funded projects in high-throughput screening and systems biology were issued. In FY2006, two STAR academic centers to support the advancement of bioinformatics in environmental health were funded, with a third center for computational

toxicology funded in FY2008. An award for a fourth center, which will focus on pathways and models of developmental toxicity, was made in late FY2009. Additional information on the STAR centers, their accomplishments, and future plans is provided in Section I.F.3.

In *ORD's Computational Toxicology Research Program Implementation Plan for FY2006-2008*, these various research efforts supporting the three LTGs identified in the CTRP framework were grouped into five research tracks: (1) Development of Data for Advanced Biological Models; (2) Information Technologies Development and Application; (3) Prioritization Method Development and Application; (4) Providing Tools and System Models for Extrapolation Across Dose, Life Stage, and Species; and (5) Advanced Computational Toxicology Approaches to Improve Cumulative Risk Predictions. Within these five tracks, all of the NCCT, ORD intramural, and STAR funded extramural project plans and interconnections were defined in this first-generation implementation plan. The next generation of the CTRP implementation plan carries forward many of these same research components into FY2009 to 2012.

As noted in the U.S. EPA Strategic Plan for Evaluating the Toxicity of Chemicals (see Section I.E.2), many environmental statutes require that EPA consider both human and ecological health risks, and although the initial emphasis of the CTRP has been on improvements in human health assessments to provide the critical mass and resources necessary to be successful, the program also, over time, must address ecological concerns as well. As we look to the future, we see opportunities to leverage existing efforts such as the Tox21 project (see Section II.D.3) and research on alternative species (e.g., the zebrafish projects underway at NHEERL and at the newest STAR Center) to help transition the CTRP to a more balanced human and ecological assessment program.

C. Resources for the CTRP

Funding of the CTRP has been relatively stable over the past several years, and this has enabled the program to develop consistent with the strategic plan. In FY2009, the program was funded at ~$15 million and 32 FTEs. Approximately 50% of the resources are allocated to NCCT, 25% to the STAR program, and the remainder to NHEERL and NERL. The majority of the FTEs (~22) are located in NCCT. Figure 2 displays the history of the budget through the President's FY2010 request, which would provide an increase in funding, initially to support Phase II of ToxCast™, but then broader aspects of the program in the out years.

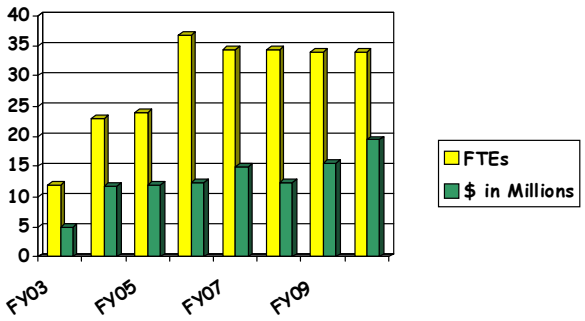

Figure 2

NCCT is organized into three primary functional groups: (1) Chemical Prioritization, (2) Systems Modeling, and (3) Informatics, with a small group of administrative support personnel (Figure 3). In addition to the permanent Federal staff of 23, there are a number of postdoctoral fellows, predoctoral, student contractors, and Senior Environmental Enrollees a grantee Organization) who support various aspects of the program. In 2006 and 2007 NCCT successfully recruited three senior-level Title 42 scientists in bioinformatics and systems biology. These hires have proven critical for NCCT to establish core research projects in predictive and systems toxicology

and the necessary informatics and computational infrastructure. More recently, in 2008, two research positions were filled with scientists coming through the NCCT postdoctoral program. Full Curriculum Vitas of the staff are provided at the NCCT website. The NCCT currently is recruiting a public affairs specialist to improve both internal and external communications.

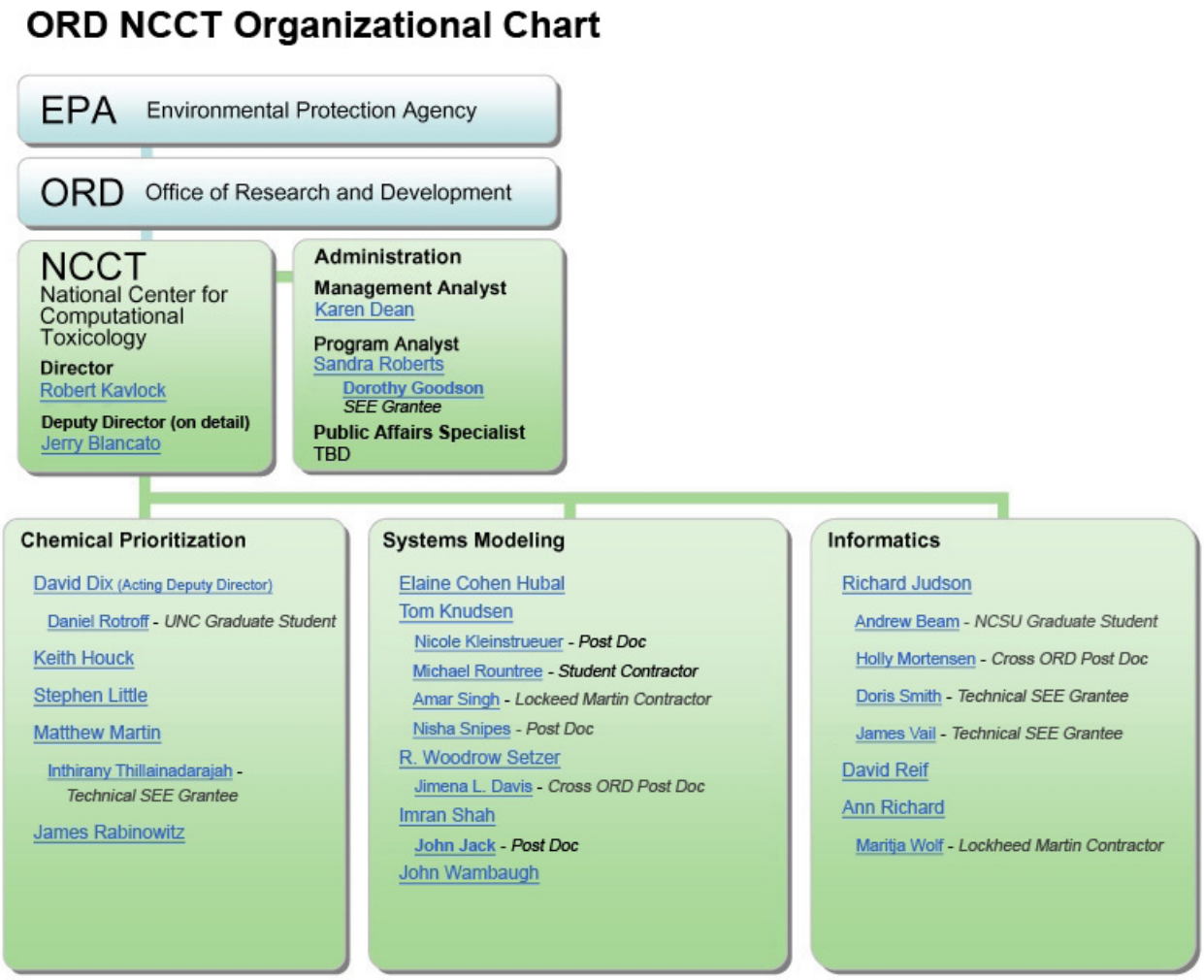

Figure 3

D. BOSC Reviews of the CTRP

To help guide the CTRP, ORD established a standing subcommittee of the ORD Board of Scientific Counselors to provide review and advice to NCCT and the CTRP. To view all prior BOSC reviews and ORD responses, please visit NCCT BOSC Reviews.

The BOSC first met in April 2005 to review the organization of NCCT, initial plans for implementation, and progress of the early CTRP work. The panel commented very favorably on the NCCT's early progress and the steps, processes, and etc. outlined to achieve its goals. The composition of staff, plans for future hiring, establishment of working partnerships, and the Center's strategic plan especially were highlighted. Several recommendations were made for consideration. Two main recommendations were to (1) develop a formal implementation plan for

the future, and (2) to develop communities of practices (CoPs) within the EPA, which could serve as a networking function for interested scientists. ORD's Computational Toxicology Research Program Implementation Plan (FY 2006 – 2008 was completed in April 2006, in time for the next BOSC review. Two CoPs have been organized and are discussed later in this document. A few other minor suggestions also were made, which were addressed in the formal ORD response to the review.

On June 19 and 20, 2006, a second BOSC site visit was held to assess and evaluate NCCT progress in executing the first implementation plan and incorporating prior BOSC recommendations. In this review, it was noted that, in the 16 months of its existence, "NCCT has made substantial progress in (1) establishing goals and priorities; (2) making connections within and outside EPA to leverage staff's considerable modeling experience; (3) expanding its capabilities in informatics; and (4) significant contributions to research and decision making throughout the Agency." The BOSC also noted that "many of the recommendations made by the BOSC during its first review have been acted on by NCCT." This review occurred just before NCCT hired its first two scientists under ORD's new Title 42 authority, which brought needed experience in informatics and systems biology to address several of the key recommendations of the BOSC from this second review.

The third review by the BOSC occurred on December 17 and 18, 2007. In this review, the BOSC noted that NCCT continued to make substantial progress in setting priorities and goals and specifically acknowledged the "increased capabilities in bioinformatics through the funding of two STAR centers and in informatics and systems biology through staff hires; expansion of its technical approaches to other programs within the Agency; and formation of the Tox21 collaboration with the National Institute of Environmental Health Sciences (NIEHS) and the National Human Genome Research Institute (NHGRI) as a complement to its ToxCast™ project. They went on to recommend that (1) client offices participate in future reviews to ensure all parties understand how NCCT's efforts can address the most relevant needs of the Agency; (2) interactions with risk assessors in the Agency be enhanced, particularly related to how ToxCast™ might be utilized by them; (3) complementary efforts in exposure prioritization be undertaken; (4) the directions and milestones be detailed and applications of the virtual tissues to risk assessment be clarified; and, (5) finally, a more precise definition of the database for compilation and rigorous quantitative analysis of the ToxCast™ data. Overall, the review considered the goals of the Center to be well described, very ambitious and innovative, and important for the future of research at EPA.

The next BOSC review is scheduled for September 29 and 30, 2009, and will focus on the products from the first CTRP implementation plan and the future directions outlined in this second generation plan.

E. NRC Report and EPA's Strategic Plan for 21st Century Toxicology

Two significant activities have increased the visibility and importance of EPA's computational toxicology research efforts over the past 2 years. The first is an NRC report, commissioned by EPA that presented a vision of how toxicological evaluations should be conducted in the future. The second activity, motivated by the NRC report, was development of an EPA strategic plan to transform how the Agency addresses chemical toxicity.

1. NRC Report on Toxicity Testing in the 21st Century

In 2007, the NRC released a report titled *Toxicity Testing in the Twenty First Century: A Vision and Strategy*, which outlines a long-term vision for developing novel approaches to chemical toxicity characterization and prediction. This vision addresses several concerns about the current best practice methods for toxicity characterization that rely heavily on extensive animal testing. These concerns are the desire to reduce the number of animals used in testing, to reduce the overall cost and time required to characterize each chemical, and to increase the level of mechanistic understanding of chemical toxicity.

The NRC report outlines an approach for toxicity determination in which each chemical first would be characterized for a number of properties related to environmental distribution, exposure risk, physico-chemical properties, and metabolism. These activities fall under the heading “chemical characterization,” and (with the exception of metabolism) exclude bioactivity in the target organism. The second stage is “toxicity pathway characterization,” in which a series of cell-based and non-cell-based (in vitro) tests would be used to indicate which (if any) “toxicity pathways” are activated by the test chemical. A major challenge posed by this approach is that few such toxicity pathways currently are understood, and assays to probe these pathways therefore, generally are lacking. Next, for a subset of chemicals, “targeted testing” would be carried out to refine our understanding of the effects of triggering specific toxicity pathways. Targeted testing might involve additional in vitro assays or a limited amount of in vivo animal testing. A final phase is “dose response and extrapolation modeling,” which would use new and existing data and models to perform low-dose extrapolation, toxicokinetics, and exposure estimation. All phases would have significant computer modeling components. The end result of these studies would be a determination of the potential toxic effects (including mode and mechanism of action) of a compound, as well as estimates of dose-response behavior.

There is a relatively simple argument as to why an in vitro-based approach should be able to predict whole animal or human toxicity. The effect of a chemical ultimately is caused by direct or indirect molecular interactions of the chemical with one or more cellular components. These interactions can be receptor or enzyme binding, disruption of a lipid membrane, localized production of free radicals, or non specific dephosphorylation. Nonetheless, if two chemicals have the same biological interactions and have the same distribution and kinetics within an organism, then the two chemicals should present the same bioactivity profiles and potential toxic effects. This concept highlights a major benefit of the in vitro, mechanism-based approach; it provides a way to extrapolate from one chemical to the next based on a set of relatively inexpensive and quick biochemical or cellular assays. Achieving this vision, however, will take many years because of a number of circumstances that are described in the NRC report. It is noteworthy that the NRC report did not downplay how difficult the task of developing this new approach to toxicity testing would be, proposing a timeline of 20 years, with annual investments on the order of $100 million to fully achieve this vision. However, for initial chemical screening and prioritization, a generalized set of assays for key biological targets and pathways can be implemented successfully in a much shorter and less costly fashion.

2. EPA Strategic Plan for Evaluating the Toxicity of Chemicals

In response to the release of the NRC report, EPA established an intraagency workgroup, the Future of Toxicity Testing Workgroup (FTTW), under the auspices of the Science Policy Council. The FTTW includes representatives from across the Agency, including the regions and program offices. It produced *The U.S. Environmental Protection Agency’s Strategic Plan for Evaluating the Toxicity of Chemicals*, which serves as a blueprint to ensure a leadership role for

EPA in pursuing the directions and recommendations presented in the 2007 NRC report. The strategy presents the Agency's vision of how to incorporate a new scientific paradigm and new tools into toxicity testing and risk assessment practices with ever-decreasing reliance on traditional approaches. The overall goal of the strategy is to provide the tools and approaches necessary to move from a near exclusive use of animal tests for predicting human health effects to a process that relies more heavily on in vitro assays, especially those using human cell lines.

The program envisioned in this strategy builds on the traditionally major components of the risk assessment process (i.e., hazard identification, dose response, exposure assessment, risk characterization) by overlaying a toxicity pathways approach on the source→fate→transport→exposure→outcome continuum. Specific components include the three integrative and interactive focal areas described below:

(1) **Toxicity Pathway-Based Chemical Screening and Prioritization.** This research will focus on identifying toxicity pathways and deploying in vitro assays to characterize the ability of chemicals to perturb those pathways, and further development and implementation of the ToxCast™ concept to establish the predictive relationship of the new assays for identifying adverse outcomes in humans or ecological populations.

(2) **Toxicity Pathway-Based Risk Assessment.** This element will focus on reducing key risk assessment uncertainties currently associated with the extrapolation of data from animal studies to humans, from high doses to relevant human exposures, and to different population susceptibilities (e.g., children). The program will achieve these ends by developing knowledgebases of toxicity pathways, toxicological responses, and information on biological networks; constructing dynamic computational models of tissue biology that link diverse data together to understand the progression of events from exposure to effect; and demonstrating that this new vision of toxicity testing adequately predicts human risk using case studies.

(3) **Institutional Transition.** Implementing major changes in toxicity testing of environmental chemicals and incorporating new types of toxicity data into risk assessment will require significant institutional changes in terms of how EPA transitions to the use of new types of data and models; how EPA deploys resources necessary to implement the new toxicity testing paradigm, such as hiring scientists with particular scientific expertise and training existing scientific staff; and how EPA educates stakeholders and the public.

Although the workgroup identified a range of partners in this effort, and some planning on the relative role of these partners has been developed, the specific areas of work to be conducted and funded by EPA versus these partners, needs to be further assessed. Decisions on these relative roles will have a significant impact on EPA resources required to implement the vision. Regardless, the CTRP will play a central role in all three strategic goals from identifying and conducting high-throughput screening on chemical libraries of interest, to developing systems-level models of biology for application in risk assessment, to training program offices in the understanding and use of the new technologies.

F. Significant Accomplishments of the CTRP: FY2006 to 2008

1. Accomplishments of the NCCT

With the CTRP being in existence for nearly 6 years, and the NCCT for more than 4 of those, a number of projects contained within the CTRP are now delivering important products, information, and accomplishments to regulatory offices within the Agency, toxicologists in the international community, and the regulated chemical industry. Details of such accomplishments are excerpted below, and greater detail is available in the descriptions of the 9 NCCT-led projects provided in Appendix IV.A., with additional information on the annual milestones and projected impacts of the projects provided in summary format in Appendix IV.C.

A number of projects on informatics, chemical prioritization, and systems-level models of biology are beginning to provide the foundation for high-throughput, decisionmaking tools that EPA program offices can apply to chemical hazard screening and risk assessment. Descriptions of these projects are presented below:

- **ToxRefDB.** A relational, electronic toxicity reference database (ToxRefDB) was developed in partnership with the Office of Pesticide Programs (OPP), that contains results of more than 30 years and $2 billion worth of rat and mouse chronic, rat multigenerational, and rat and rabbit developmental studies for more than 400 chemicals (*Martin et al. 2009a* ; *Martin et al. 2009b*; *Knudsen et al. 2009*). This relational database is enabling the Agency, for the first time, to readily discern patterns of toxicity in the assays and to assess the value of the design of the assays in assessing toxicity. It also will be invaluable in the interpretation of the HTS data derived in the ToxCast™ Program. This database will be expanded to include developmental neurotoxicity assays, and potentially, results from the Endocrine Disruptor Screening Program, thus affording a one-stop shop for animal bioassay data.
- **ACToR.** The Aggregated Computational Toxicology Resource (ACToR) provides an Internet-based portal of information on chemical structure, bioassay, and toxicology data for environmental chemicals from 200 sources of public data for more than 500,000 chemicals and a central integrated public resource for all DSSTox, ToxCast™, and ToxRefDB data (*Judson et al. 2009*). ACToR was released to the public in the second quarter of FY2009 and will undergo periodic updates over the next several years as we add data and functionalities, and respond to user feedback.
- **DSSTox.** The Distributed Structure-Searchable Toxicity Database Network (DSSTox) was updated with several high-interest chemical inventories, including ToxCast™ Phase I chemicals, EPA high production volume chemicals, and the two primary public genomics inventories (GEO and ArrayExpress), an online structure-browser, and linkages and coordination with internal and outside resources such as ACToR and PubChem (Williams et al. 2009; Williams et al. 2009). DSSTox, in coordination with ACToR, is also responsible for all chemical information registration and review for ToxCast™ and Tox21 (see below for a more detailed explanation of Tox21) projects and is the source of high-quality chemical structures for the Organization for Economic Co-Operation and Development (OECD) QSAR Toolbox.
- **ToxCast™.** ToxCast™ is a major effort to evaluate the comprehensive use of HTS assays to provide biological fingerprints of activity that can be used to predict adverse outcomes in rodents and in humans (*Dix et al. 2007*). With the initiation of nine contracts in FY2007, the CTRP began to generate a myriad of molecular in-vitro based data for the ToxCast™ program. Data collection for more than 300 chemicals from over 500 assays in Phase I began in FY2007 and was completed in the second quarter of FY2009. An internal EPA workshop

regarding ToxCast™ was held in March 2008, and a public ToxCast Data Analysis Summit workshop was held in May 2009, with more than 30 national and international material transfer agreements (MTA) analysis partners participating. Analyses are focusing along three dimensions: (1) comparing bioactivity profiles across chemical classes; (2) correlating specific assays or pathways with toxic phenotypes; and (3) correlating phenotypic syndromes with bioactivity profiles. It is expected that analysis of the data will continue over several years, both by EPA and external groups, as the compendium of data represents a truly unique and innovative resource. A significant number of additional partners were brought into the program in FY2008 and FY2009 via MTAs. Additional contract awards are anticipated to augment the breadth of biological pathways contained in Phase II, which will launch in late FY2009. A critical feature of Phase II is the plan to include drugs that have failed in human clinical trials, thus providing human toxicity data on which to benchmark ToxCast™ bioactivity profiles. One major pharmaceutical company, Pfizer, already has agreed to collaborate in this regard, and the Health and Environmental Sciences Institute has adopted the concept as their Emerging Issues Proposal for 2009, promoting the opportunity for a much wider group of pharmaceutical companies to contribute failed drugs and clinical data. Thus, Phase II of ToxCast™ will be generating HTS data on 700 additional chemicals, some with animal toxicity information, clinical data, and additional data on human disease, susceptibility, and variability that will contribute to the goal of expanding, verifying, and translating in vitro bioactivity into predictions of potential toxicity.

- **Tox21.** To garner complementary expertise across the federal government to transform the field of toxicology to a more predictive science, EPA signed a memorandum of understanding (MOU) with the National Toxicology Program (NTP)/National Institute of Environmental Health Sciences (NIEHS) and the National Institutes of Health Chemical Genomics Center (NCGC)/ National Institutes of Health Chemical Genome Research Institute (NHGRI) in February 2008 (*Collins et al. 2008*). This Tox21 consortium now has four active working groups identifying chemicals, assays, informatic analyses, and targeted testing as plans proceed to have nearly 10,000 chemicals under study at the NCGC by the third quarter of FY2010 (*Kavlock et al. 2009*). Supported by interagency agreements between the NTP and EPA, this effort will conduct 50 or more HTS assays on this enlarged chemical library every year for the next several years.
- **Cumulative Risk.** Tools of computational toxicology for modeling exposure, effects and risk have been used in integrating data for the cumulative risk assessments of cholinesterase-inhibiting pesticides (organophosphates and carbamates), particularly for modeling and characterizing the confidence and uncertainties in the assessment. These methods have been used to set safe exposure limits for these chemicals in the field and home, and are also being used to help in the consideration of a cumulative risk assessment for the pyrethroid pesticides.
- **Communities of Practice (CoP).** Though not projects or tools in the typical sense, NCCT has formed several CoPs promoting the utilization of CTRP research in specific areas of computational toxicology. Each CoP has a charter and an open membership policy and is co-chaired by a member of NCCT. The CoPs operate via activities such as teleconferences, face-to-face meetings, team rooms, and workshops. By bringing together members from different parts of EPA, ORD, and the outside scientific, regulatory, and regulated community, CoPs help to promote the adoption of common practices and ontologies, guide development of common databases and software usage, aid in construction of training materials, provide recommendations on efficiencies of relevant operations, and act as a public outreach mechanism for ORD activities. To date, two CoPs have been established on Chemical Prioritization and Exposure Science. Both of these are large, active groups that meet monthly and have brought a wealth of ideas, interest, and international collaboration to various CTRP projects.

- **International Science Forum on Computational Toxicology.** In 2007, the CTRP hosted EPA's annual Science Forum. This was the first EPA Science Forum held outside of Washington, DC, and featured an international overview of the state of the science on computational toxicology (*Kavlock et al. 2008*). More than 300 people attended this forum on advances in computer sciences, molecular biology and chemistry, and systems models that can be used to increase the efficiency and the effectiveness by which the hazards and risks of environmental chemicals are determined. The forum surveyed the state of the art in many areas of computational toxicology and identified key areas important to move the field forward: proof-of-concept studies demonstrating the additional predictive power gained; more researchers comfortable generating and working with high-throughput data and using it in computational modeling; and regulatory authorities willing to embrace new approaches as they gain scientific acceptance. Ideas from this forum and efforts bridging from it were helpful in EPA's development of *The U.S. Environmental Protection Agency's Strategic Plan for Evaluating the Toxicity of Chemicals*.

2. Accomplishments by the CTRP ORD Intramural Partners

The seven cross-ORD "new start" research projects initiated by the CTISC in FY2004 advanced the field of computational toxicology and were important components in the first generation CTRP implementation plan and the establishment of NCCT. Details on the organization, goals, and cross-ORD participants for these projects were provided in the FY2006-2008 CTRP Implementation Plan, and a comprehensive listing of publications from these projects is included in Appendix IV.C. Descriptions of major accomplishments are provided below.

(1) Linkage of Exposure and Effects Using Genomics, Proteomics, and Metabolomics in Small Fish Models. This project used a combination of whole organism end points; genomic, proteomic, and metabonomic approaches and computational modeling to (a) identify new molecular biomarkers of exposure to endocrine-disrupting compounds (EDCs) representing several modes and mechanisms of action (MOA) and (b) link those biomarkers to effects that are relevant for both diagnostic and predictive risk assessments using small fish models (Villeneuve et al. 2007; Ankley et al. 2009). Data from the project have provided the basis for several important predictive modeling efforts. For example, the development of a graphical systems model focused on defining the HPG axis of small fish, which enables consideration of the interactive nature of a perturbed system at multiple levels of biological organization, ranging from changes in gene, protein and metabolite expression profiles to effects in cells/tissues that directly influence reproductive success. A second modeling effort involves development of a steady-state model for ovarian tissue to predict synthesis and release of testosterone and estradiol. Results from the model were compared successfully to data generated from the fathead minnow. Model-predicted concentrations of the two steroids over time corresponded well with both baseline (control) data and information from experiments in which estradiol synthesis was blocked by fadrozole. Modeling also has focused on the consequences of perturbations in the HPG axis relative to effects in individuals and populations. Here, a population model that employs a Leslie matrix in conjunction with the logistic equation (to account for density dependence) was used to translate laboratory toxicity information into prediction of population trajectories. In these analyses, changes in steroid or vitellogenin concentrations in female fish first were related to fecundity, and then, using this relationship, to population status in fish exposed to EDCs that inhibit production of vitellogenin most notably compounds that depress steroid synthesis (e.g., fadrozole, prochloraz, trenbolone). That analysis is unique in that it focuses on biochemical end points, female steroids, and vitellogenin, which reflect both toxic MOA of EDCs and have a functional relationship to reproductive success (formation of eggs). As

such, within the overall systems framework for the project, this computational model can serve as the basis via which genomic information can be linked quantitatively to responses in populations.

(2) **A Systems Approach to Characterizing and Predicting Thyroid Toxicity Using an Amphibian Model.** The main objective of this work was to develop a hypothalamic-pituitary-thyroid (HPT) model that is capable of integrating data from different levels of biological organization into a coherent system (Degitz et al. 2005). A simulation model has been developed to describe the thyroid axis of *X. laevis* tadpoles. Information pertaining to normal baseline HPT-axis development was collected to compare to the perturbed system. Thyroid and pituitary gland culture systems were developed to investigate the response of these components in isolation from the HPT-axis feedback mechanisms within the animal. Genes responsive to TSH were also measured in the thyroid gland and pituitary in vivo and in vitro in response to chemical exposure and TSH stimulation. Genes involved in the T4 synthesis pathway that robustly responded to TSH, such as NIS and thyroid peroxidase (TPO), were not changed when challenged with the chemical alone. Other thyroid-specific genes, such as thyroid transcription factor and thyroglobulin, were not TSH-responsive. Efforts were made to obtain TPO activity from *X. laevis,* but the small amount of tissue contributed to our inability to develop an assay. Therefore, porcine thyroid glands were used to isolate and measure TPO activity. Twenty-four chemicals have been tested in the in vitro assay for their capacity to inhibit TPO activity. Although the majority of the tested chemicals were negative, several were identified as TPO inhibitors. These positives were tested in the thyroid gland explant culture assays and the TH released into the culture media was measured by RIA. One of the test chemicals, a mercaptobenzothiazol, inhibited T4 release from the thyroid glands at concentrations that were not overtly toxic to the gland. Chemicals that test positive in the TPO inhibition assay and the *ex vivo* thyroid gland TH release assay will be tested in the abbreviated amphibian metamorphosis assay to determine activity in the HPT axis in vivo. This will begin to provide information on the predictive ability of the in vitro and ex vivo assays for identifying thyroid-disruptive chemicals.

(3) **Risk Assessment of the Inflammogenic and Mutagenic Effects of Diesel Exhaust Particulates: A Systems Biology Approach.** This project utilized a systems approach to developing and applying predictive computational models that quantitatively describe relationships between the composition of DEP and its genotoxic and inflammogenic potencies (Linak et al. 2007; Stevens et al. 2009). A significant accomplishment is derived from the development of a prototype ESP sampler, which has resulted in a significant improvement in DEP collection yield relative to conventional filter methods for particle capture that were in use previously. These analyses have produced an unprecedented physicochemical characterization of DEP, including XRF analysis of metal content and GC/MS analysis of organic species, as well as determinations of OC/EC, particle size and aerodynamic characteristics, etc. By design, this Phase generated inflammogenicand mutagenic DEPs of varying composition to address the central hypothesis regarding the inflammogenic and mutagenic potency of DEP. An extensive database has been generated and currently is being analyzed for publication and use in model development.

(4) **The Mechanistic Indicators of Childhood Asthma (MICA) Study: A Systems Biology Approach to Improve the Predictive Value of Biomarkers for Assessing Exposure, Effects, and Susceptibility in the Detroit Children's Health Study.** This computational toxicology effort integrated rodent and human research across the source-to-outcome continuum to link gene expression with clinical outcomes and biomarkers of exposure, early effect, and susceptibility for two broad classes of chemicals: (1) polycyclic aromatic hydrocarbons and (2) metals (Heidenfelder et al. 2009; Edwards et al, in press). In collaboration with Michigan State investigators, exposures of rodents to concentrated air particulates were completed using state-of-the-art mobile air exposure chambers. Genomic

analysis of rodent blood and lung tissues highlighted tissue-specific patterns of gene expression related to airborne exposures. For the children's study (whose protocols were approved by three separate internal review boards), a 20-page respiratory health questionnaire was mailed to the parents of 6,883 children aged 7 to 12 years recruited through the Henry Ford Health System. An indoor/outdoor MICA-Air component was added using an innovative participant-based air sampling approach that included measurements of nitrogen dioxide and selected volatile organic compounds. A subset (205) of these children had clinical examinations including measurements of pulmonary function and exhaled nitric oxide, collection of blood for both genetic and gene expression analysis, and nail and urine samples collected. Our analysis strategy represents a true "systems" approach in that each type of data is examined in the context of the broader MICA data set. This systems approach affords more robust conclusions, because the predictive value of biomarkers from particular data slices can be assessed for biological and statistical validity against the diverse set of supporting data.

(5) An Approach to Using Toxicogenomic Data in Risk Assessment: Dibutyl Phthalate Case Study. To address how genomic data may be used most effectively in risk assessment, this project was initiated with the goals of developing an approach to using toxicogenomic data in risk assessment and testing this approach in a case study. Recognizing that genomic data type (e.g., species, organ, design, method) varies, the approach included formulating questions that the toxicogenomic data may inform. Because microarray data is often informative of the MOA of a chemical, the approach included an assessment of the toxicogenomic dataset in conjunction with the toxicity dataset to relate the affected end points (identified in the toxicity dataset evaluation) to the pathways (identified in the toxicogenomic dataset evaluation) as a method for informing the MOA. Dibutyl phthalate (DBP) was selected for the case study, focusing on the male reproductive outcomes, because it has a relatively large and consistent genomic dataset, phenotypic anchoring of certain gene expression data for these outcomes, and an ongoing Integrated Risk Information System (IRIS) assessment. The case study team concluded (USEPA, 2009) that the "genomic dataset" should include all gene expression data (single gene, global gene expression, protein,and RNA) in the evaluation, as these data taken together provide a stronger basis for reproducibility of the global gene expression study findings. This evaluation found that the gene level findings from the DBP genomic studies (i.e., microarray, RT-PCR, protein expression) were highly consistent in both the identification of differentially expressed genes (DEGs) and their direction of effect. This project also identified research needs for toxicity and toxicogenomic studies for use in risk assessment. These include (1) parallel study design characteristics with toxicogenomic studies (i.e., dose, timing of exposure, organ/tissue evaluated) to obtain comparable toxicity and toxicogenomic studies to aid our understanding of the linkage between gene expression changes and phenotypic outcomes, (2) exposure time-course microarray data to develop a regulatory network model, (3) generate TK data in relevant study (time, dose, and tissue) and obtain relevant internal dose measure to derive best internal dose metric, and (4) multiple doses in microarray studies in parallel with phenotypic anchoring.

(6) Development of Microbial Metagenomic Markers for Environmental Monitoring and Risk Assessment. This project focused on the development of nonculture-based genomic methods for environmental monitoring and risk assessment. The research focused on the use of a microbial community genome (metagenome) approach to identify novel nucleic acid sequence markers for fecal contamination and source identification (Shanks et al. 2006; Santodomingo et al. 2007). The basic experimental design consisted of challenging genomic microbial community DNA from different fecal samples in genome subtraction studies to enrich for host specific microbial genes. Sequencing complete fecal metagenomes generated redundant information, making difficult the selection of host-specific genes. To

address this limitation, a novel approach called *genome fragment enrichment* was developed to select for DNA fragments present in a specific fecal microbial community and absent in other fecal communities. A patent disclosure was filed on this specific method. In each case, hundreds of enriched DNA fragments were sequenced and assigned a putative functional role. Although several assays were developed, these have to be further evaluated, particularly to determine the geographic stability of these methods, not only in the United States, but also in other parts of the world. To this end, we are working with researchers from different regions in the United States and from countries such as Canada, Brazil, Austria, Singapore, and Spain. Results of this research will overcome the current limitation of assessing the microbial water quality by measuring bacterial densities, which are both time consuming and do not provide information about the sources impacting a watershed. Such information is necessary to implement adequate pollution control and remediation practices.

(7) Simulating Metabolism of Xenobiotic Chemicals as a Predictor of Toxicity. The MetaPath research project is a collaboration with OPP scientists developing a capability for forecasting the metabolism of xenobiotic chemicals of EPA interest, to predict the most likely formed chemical metabolites, and to interface that information with toxic effect models, enabling prediction of parent chemical toxic potential and the identity of chemical metabolites of equal or greater toxicity than the parent chemical (Kenneke 2006). Key milestones include developing and expanding the in vivo and in vitro liver metabolism database, especially for chemicals and transformation reactions underrepresented in the current database; finalizing development of the searchable metabolism database and continuing to populate existing databases with additional metabolism data; enhancing the performance of the existing metabolic simulator by incorporating reliable metabolism data and expansion of relevant transformation reactions; and conducting in vitro experimentation to verify maps and metabolites forecasted by the metabolic simulator and evidence for enhanced estrogenicity.

3. Accomplishments by STAR Grantees in the CTRP

The STAR grants and centers have been a critical component of the CTRP from its inception. A brief summary of the accomplishments of the three existing STAR centers for this program is provided below. More information and fuller descriptions of these centers are available in Appendix IV.B.

NCER funded two STAR environmental bioinformatics centers as part of the CTRP in FY2006. The Research Center for Environmental Bioinformatics and Computational Toxicology at the University of Medicine and Dentistry of New Jersey (UMDNJ), Piscataway, NJ, and The Carolina Environmental Bioinformatics Research Center at the University of North Carolina (UNC), Chapel Hill, are operating as cooperative agreements and helping to facilitate the application of bioinformatics tools and approaches to environmental health issues supported by the CTRP.

To date, the UMDNJ center has made progress expanding the framework of the Food and Drug Administration ArrayTrack to ebTrack, an integrated bioinformatics system for environmental research and analysis enabling the integration, curation, management, first-level analysis, and interpretation of environmental and toxicological data from diverse sources. Other major accomplishments include the enhancement of Shape Signatures QSAR technology for chemical hazard identification, metabolic engineering tools for identifying important pathways within the overall hepatocyte metabolism, and computational procedures for quantifying the structure of

molecular bionetworks via the S-space Network Identification Protocol and the Closed-Loop Identification Protocol.

Major accomplishments of The Carolina Center for Environmental Bioinformatics include the development and refinement of a mouse model of variation in genetic susceptibility relevant to human populations, pathway modeling in genomic analysis, and new methods in QSAR modeling relevant to toxicity. This work complements other work in the CTRP, utilizing the unique strengths of the STAR center in genetics, toxicology, and statistical modeling. An early outcome of this work included dissection of the genetic regulation of liver gene expression. In addition, the center has refined expression quantitative trait locus (eQTL) analysis procedures. These methods serve the larger goal of elucidating the underlying mechanisms of toxicity. The center also has developed high-quality methods for testing biological pathway involvement in toxicogenomics studies, and a novel, hierarchical, two-step approach to model chemical structure for in vitro/in vivo toxicity data.

In FY2008, NCER funded a third center, through a cooperative agreement, The Carolina Center for Computational Toxicology at UNC in Chapel Hill. This center is applying high-performance computing techniques and resources to in silico multiscale modeling applications at the cellular, organ, and system-wide levels. In its first year, the center began to implement and design advanced mathematical approaches to modeling biological systems and biological-chemical interactions represented in ToxCast^{TM}, as well as other datasets.

Another high priority for EPA is to understand the molecular and cellular processes that, when perturbed, result in developmental toxicity. With a project start date of November 2009, NCER responded to this need by funding the Texas-Indiana Virtual STAR Center: Data-Generating in vitro and in silico Models of Developmental Toxicity in Embryonic Stem Cells and Zebrafish at the University of Houston, the Texas A&M Institute for Genomic Medicine, and Indiana University. This center will bridge the interface of in vitro data generation and in silico model development to answer critical biological questions related to toxicity pathways important to human development. This research should result in an improved predictive capacity for estimating outcomes or risks associated with developmental exposure to environmental toxicants.

G. Retrospective Summary of the CTRP and NCCT

The first 5 years of the CTRP and NCCT have seen a great deal of progress and accomplishment. These accomplishments have come from across ORD, the STAR grantees and, increasingly, from NCCT, as the center becomes fully staffed and projects mature. As the plan from the first implementation comes to an end, the CTRP is poised to carry out the vision of the NRC report on *Toxicity Testing in the Twenty First Century: A Vision and Strategy*, and *The U.S. Environmental Protection Agency's Strategic Plan for Evaluating the Toxicity of Chemicals.* This CTRP implementation plan for FY2009 to 2012 will go on to explain revisions to the program and specific projects that will be management priorities into the future.

II. Revision of the CTRP for FY2009 to 2012

A. Maturation of the Program

The accomplishments of the CTRP noted above have enabled the program to evolve beyond the early focus on hazard identification and chemical prioritization into broader areas of risk assessment. In doing so, a number of research activities are becoming increasingly intertwined. For example, results of the ToxCast™ program are providing data for models being developed in the virtual tissue programs, and, in turn, the virtual tissue programs are guiding targeted testing needs for other parts of the program. As a result, a blending of activities across the 3 LTGs in the first implementation plan has happened. Although the 3 LTGs served us well in the initial years of the program, we have reduced them in the current plan to a single goal, namely, *providing high-throughput decision support tools for screening and assessing chemical exposure, hazard and risk*. Following *The U.S. Environmental Protection Agency's Strategic Plan for Evaluating the Toxicity of Chemicals*, we will see increased emphasis on the use of new data types being generated in the CTRP in quantitative risk assessment and greater involvement in aspects of high-throughput exposure models and in analysis of results coming out of high-throughput bioactivity profiling. As noted in other places, discussions already are well underway among NCCT, NHEERL, NERL, and NCEA on the shape of such an expansion. This expansion will incorporate progress from ToxCast™ and ExpoCast™, as well as the v-Liver™ and v-Embryo™ projects, into informatics tools and databases that can be used for both research and regulatory applications. For example, in FY2011 we expect ToxCast™ will be winding down Phase II of its three-part development program, providing in vitro and in vivo toxicity data on a total of 1,000 compounds across hundreds of molecular targets and biological pathways. Over this same time, the Tox21 consortium will be building a chemical screening library of as many as 10,000 chemicals, conducting approximately one assay per week on this library, and providing additional in vitro bioactivity data. Within the ACToR database and Web site and in the analysis application ToxMiner, all of this bioactivity data will be merged with chemical information and the exposure data being curated from the ExpoCast™ project. At this stage in FY2011, steps will be taken to provide EPA's program offices decision analysis tools that incorporate hazard and exposure data for prioritization of further chemical testing. Prior to that time, the CTRP will be hosting periodic training courses on the new science and tools for program office and regional personnel. We envision this decision analysis tool being part of the ToxMiner application. In addition to statistically based predictive toxicology tools, ToxMiner will be able to reference the mapping of ToxCast™ assays to biological pathways curated from sources such as the Kyoto Encyclopedia of Genes and Genomes into a computable format for use in ToxMiner.

Depending on the precise pace of progress in ToxCast™ and ExpoCast™ and the development of screening and prioritization tools, additional resources could be freed up to support other components of the CTRP in FY2011 and FY2012. However, chemical prioritization will remain a priority area until it reaches the stated objectives. A natural extension of the chemical prioritization projects is using this data for systems modeling in the v-Liver ™ and v-Embryo ™ projects. It is these systems models that will help fully utilize the toxicity and exposure pathway information coming out of ToxCast™ and ExpoCast™ in next-generation, higher throughput risk assessments. In conjunction with Office for Prevention, Pesticides and Toxic Substances (OPPTS), we also have begun an examination of the feasibility of using HTS tools to evaluate the potential hazards of nanomaterials. This effort will begin with carbon nanotubes and nanomaterials being used in the OECD Working Party on Manufactured Nanomaterials and include materials submitted to the EPA via the program Premanufacturing Notification of the

Toxics Substances and Control Act. We expect the application of bioactivity profiling of nanomaterials to become an increasingly important component of the CTRP as we gain experience with their handling and screening results.

Relative to the STAR program, the number of funded centers will reach four or five (2 on environmental bioinformatics, 1 on computational toxicology, and 1 or 2 on virtual tissues) by FY2010 and consideration will need to be given to the second-generation centers as the first ones to be awarded begin to reach the conclusion of their funding cycle.

For historical purposes, the following is provided as transition information relative to the original 3 LTGs structure, with notation on the degree of emphasis the research activities that will be carried over into the new implementation plan. Much of the increased activity is predicated on continual and increasing support of the CTRP program, such as is being witnessed in the FY2010 budget process. The following section describes research areas of increased and decreased effort relative to the 3 FY2006 to FY2008 LTGs.

Long-Term Goal 1. *EPA risk assessors use improved methods and tools to better understand and describe linkages across the source-to-outcome paradigm.* Work was directed toward computational models and modeling systems that represented comprehensive descriptions of the underlying biology of adverse impacts caused by exposure to environmental agents. The whole-systems biology modeling approach was to develop a range of models, from those describing pharmacodynamic connections between exposure and effects to those describing complex endogenous pathways and the perturbations in such pathways resulting from environmental exposures. Also, ways to incorporate and use “omics” information in these models was explored. Finally, attempts were made at formulating models of common but complex disease processes that then are exacerbated by exposures to exogenous substances and stressors through the development of virtual organ models, the first being the virtual liver, and the second the virtual embryo.

- Increase efforts to develop virtual models of tissues (liver and embryo) that link across levels of biological organization from molecular-to cellular-to tissue-level responses
- Increase coordination of efforts across NCCT, NERL, NHEERL, and NCEA to ensure models of aspects of the source-to-outcome paradigm can be integrated and scaled to meet the increasing needs of chemical evaluations
- Decrease research efforts related to the validation and acceptance of PBPK models
- Decrease, after FY2009, linkage of exposure and effects using genomics, proteomics, and metabonomics in small fish models.

Long-Term Goal 2. *EPA program offices use advanced hazard characterization tools to prioritize and screen chemicals for toxicological evaluation.* Molecular biological tools were employed to develop fingerprints of biological activity of chemicals of concern to EPA. Computational models were applied to the fingerprints to derive associations with classical measures of toxicity derived from animal studies so that predictive models were developed, leading to more efficient testing paradigms and reduction in uncertainties in interspecies extrapolation. Proof-of-concept demonstration of ToxCast^{TM}, the forecasting tool, is scheduled to provide a number of EPA program offices with an extremely useful tool to improve the efficiency and effectiveness of hazard identification and risk assessment methodologies. There were also new and innovative ways developed to assimilate, evaluate, and use the myriad of data assorted with molecular and chemical information. Increasingly integrated chemical-biological effects databases are intended to spur new capabilities for data mining and chemical categorization in conjunction with HTS data. Development of an advanced computational

chemistry method also provided in silico means to predict complex interactions of environmental chemicals with biochemical receptors that then can lead to adverse effects.

- Increase support for Phase II of ToxCast™. The additional chemicals, which could total 1,000, will include a greater number of pesticides, pesticidal inerts, antimicrobials, industrial chemicals, water contaminants, and failed drug candidates. Phase II should be winding down sometime in FY2011, depending on the exact pace of progress in FY2009 and FY2010.
- Increase development of methods to analyze data and relate the results from the ToxCast™ studies to potential for hazard and risk from realistic human exposure levels
- Increase interactions between NCCT and NHEERL on identification of additional critical toxicity pathways to include in the chemical prioritization program
- Increase in conjunction with NERL, development of ExpoCast™, the exposure component of a chemical prioritization process
- Increase cross-governmental collaborations to employ quantitative HTS assays to predict human toxicity by engagement with relevant components of the NTP/NIEHS, NCGC/NHGRI, and FDA in the Tox21 program.

Long-Term Goal 3. *EPA risk assessors and regulators use new models based on the latest science to reduce uncertainties in dose-response assessment, cross-species extrapolation, and quantitative risk assessment.* The intent of this goal was to develop additional key modules for computational models of biological processes relevant to the induction of toxicity for high-priority environmental chemicals. These modules would help assess the interaction of exposure to environmental chemicals with other processes, such as underlying disease and concomitant intake of pharmacological agents. As a result, EPA will be less reliant on default assumptions for risk assessment and better able to accurately characterize the true uncertainty associated with risk predictions for various chemical classes (e.g., EDCs) under conditions more relevant to actual exposures and lifestyles

- Decrease chemical specific efforts vis a vis tools that have greater generic applicability
- Increase analysis of the resulting HTS data to (1) provide MOA information to specific risk assessments being conducted by EPA, (2) provide rationale for grouping of cumulative risk assessments based on toxicity pathways, (3) design a higher throughput risk assessment approach for chemicals based on exposure potential and perturbation of toxicity pathways; and (4) develop methods for analyzing and quantifying the uncertainty in dose-response model predictions

These modifications to levels of effort and emphases are consonant with distillation of the 3 LTGs from the FY2006 to 2008 implementation plan into a single goal-- *Providing High-Throughput Decision Support Tools for Screening and Assessing Chemical Exposure, Hazard and Risk*. This more efficiently will support the use of new data types being generated in the CTRP in quantitative risk assessments, as well as provide high-throughput hazard and exposure data and models for screening. Because the majority of the HTS data is being generated for human molecular targets and pathways, this will support a transition from the current dependence on animal-based toxicology. The combination of clinical data from pharmaceutical partners, expanding efforts to bring in human data on exposure, and multiscale systems models should make it possible to improve both the pace and quality of risk assessments.

B. CTRP Integration Across ORD Laboratories and Centers

Because of the relatively small size of NCCT and the ambitious nature of the CTRP mission, a key part of the process of advancing the science involves developing partnerships that are both within and external to ORD, to best leverage resources committed to the effort. Within ORD the majority of health, ecological, and risk assessment research is contained within a variety of Multi-Year Plans (MYPs) that incorporate efforts from multiple laboratories and centers and are coordinated by National Program Directors (NPDs). In the case of the CTRP, the Director of NCCT also leads the ORD Computational Toxicology Program. At present, there is an ongoing active dialogue between ORD laboratories and centers, and relevant NPDs regarding the future directions of the CTRP and other programs. To move beyond dialogue, NCCT has hosted or is hosting, rotational fellows from across ORD (NERL, NHEERL and NCEA to date) that spend 4 or more months working with NCCT scientists on CTRP projects.

In response to the Administrator's priorities, a pilot effort is being considered by ORD that addresses problems of broad national significance through highly integrated multidisciplinary research efforts. In the last several months, NCCT, NERL, NHEERL, NRMRL, and NCEA have been exploring opportunities for greater synergy in execution of the CTRP, and each laboratory and center is actively developing implementation plans for such an effort. This integration will include aspects of the Human Health Research Program MYP and the Safe Pesticides Safe Products MYP. The challenge will be to ensure these plans are adequately integrated and the outputs are suitably ambitious in nature. As EPA has just released its Strategic Plan for the Evaluation of the Toxicity of Chemicals (U.S. EPA, 2009), there is a window of opportunity to continue this momentum within the CTRP, while also expanding efforts to include critical aspects of exposure assessment (NERL), toxicity pathway coverage and targeted testing (NHEERL), life cycle analysis of chemical use (NRMRL), and quantitative risk assessment (NCEA). If successful, this integration of efforts across multiple laboratories, centers, and research programs could meld the numerous ongoing ORD research efforts in computational toxicology into a more functionally integrated multidisciplinary research program.

Figure 4 shows the stages in the source-to-outcome continuum on the horizontal axis, existing components of ORD research and development infrastructure needed to support risk assessment on the vertical axis, and products and tools for decision support on the far right. At present, many of these efforts are not integrated and, in some cases, not fully compatible; a major emphasis in the near future will be to bring more coordination and integration to these currently diverse efforts. To overcome these challenges, integrated cross-disciplinary teams involving exposure scientists, effects researchers, risk assessors, risk managers, and computational modelers will need to be further developed.

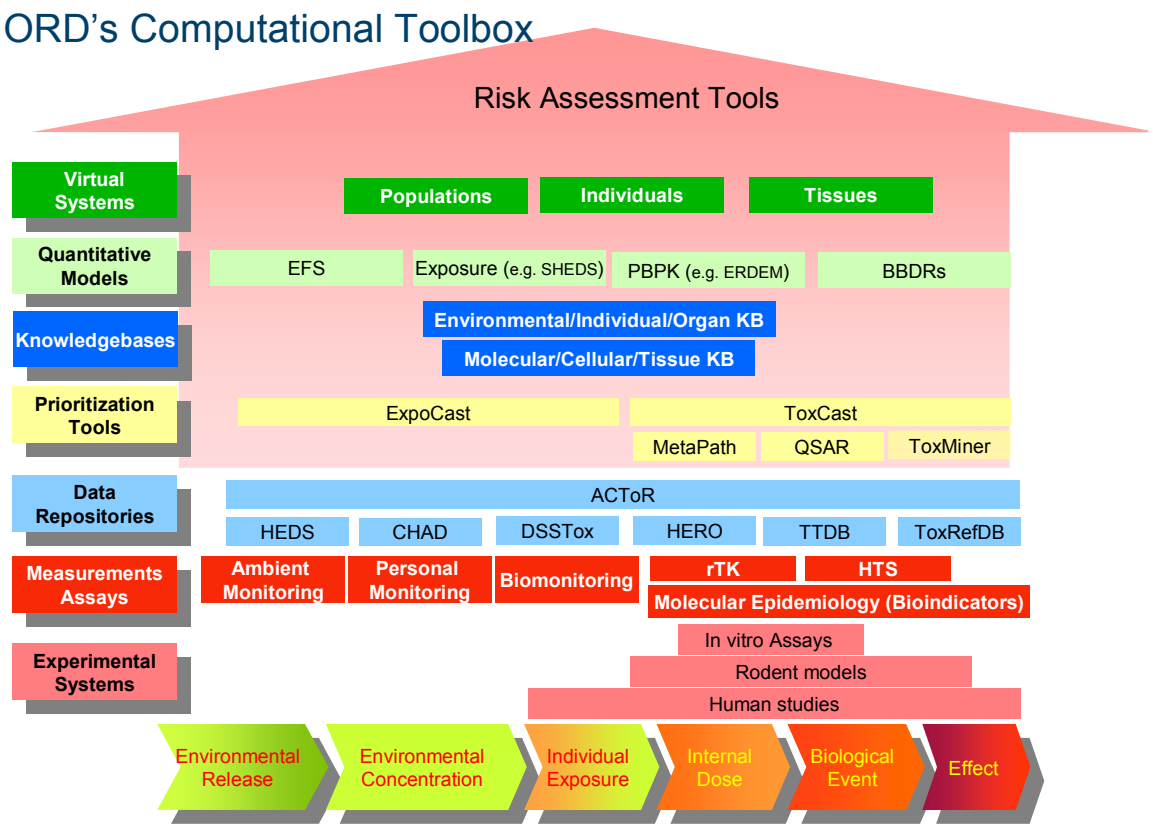

Figure 4

Understanding of complex, interrelated environmental stressors and potential impacts on human health has grown tremendously in recent years. Basic and clinical sciences, however, have significantly outpaced risk assessment science. Insights into health and disease exist, but it is unclear how to incorporate this information into risk assessment with current methodologies. Risk assessment will have to address a fundamental paradigm shift from a reliance on animal toxicology data derived primarily from rodent bioassays. The need for this shift is made more immediate by the challenges of applying new types of data stemming from advances in computational toxicology and the huge volume of data that will be generated from the European Union's Registration, Evaluation, and Authorization of Chemicals Program. High-throughput data will need to be translated into knowledge to support science-based decisions in risk assessment. The areas where this knowledge is expected to have an impact are defining toxicity pathways and informing risk assessors about interpretation of multiple MOAs for toxicity and providing insight into human variability in key pathways and human susceptibility. The impacts of this information will be qualitative and quantitative. For this type of information to be incorporated quantitatively, numerous challenges exist, not the least of which is extrapolation from in vitro test systems to in vivo human health outcomes. However, the challenges are not insurmountable if ORD, all of EPA, and key extramural partners work together in a coordinated, multidisciplinary fashion. The initial impacts of this new paradigm will probably be seen in cases of chemicals lacking significant datasets but for which toxicity predictions or rankings can be developed from HTS data. These results may be used to derive estimates of relevant potency to chemicals that have much larger databases and affect the same toxicity pathways. In addition, challenges in extrapolating from effective concentration in vitro will require additional considerations in the development of environmental exposure estimates. Examples of promising

CTRP efforts with antimicrobials and pesticidal inerts; EDSP compounds; and industrial chemicals, such as phthalates and perfluorinated chemicals; as well as with hepatocarcinogens and teratogens are all underway. Results from these studies have been presented and are being published in the peer-reviewed literature and incorporated into EPA databases and decision support software tools.

The CTRP will continue to provide critical research components, work on integrating these efforts across ORD, and facilitate the institutional transition necessary to ensure that these tools integrated by EPA programs and regions. This includes quantitative and mechanistic experimental data (e.g., ToxCast™) that are useful for chemical prioritization but also supports systems models that could be useful for quantitative risk assessments. Data generated from experimental systems can be used to define toxicity pathways and link them to adverse events via the MOA. This definition of adversity, versus toxicity pathways and key events will be used to identify the critical HTS tools for predicting disease outcomes in target populations. Future efforts also will need to focus on the translation of the computational and high-throughput methods into information that can be used in risk assessment. This is most apparent with respect to actual use in quantitative risk assessment (i.e., being able to use in vitro or computational methods to develop a point of departure for an IRIS assessment or other type of risk assessment) but is also relevant to prioritization (i.e. how does the relative potency information derived from the in vitro assays or models compare to in vivo potency) if information from screening assays is going to be used to drive further testing decisions. Key issues in developing the next generation of risk assessments include

- defining adversity—before these methods can be used in risk assessment, it will be vital to understand how “perturbations of toxicity pathways” relate to adverse effects that are of concern for human or ecological health; and
- development of decision methods and criteria for using high-throughput data in risk assessment.

The large interdisciplinary projects required to meet the goals of this program are dependent on well-integrated data repositories such as ACToR. These databases not only provide inputs for empirical models for prioritization and hazard identification but also access to well-structured data required for data mining needs to define and evaluate toxicity pathways and dose-response modeling needs. As hazard identification and risk characterization tools are developed, a gradual shift from reliance on empirical models supported by limited mechanistic information to incorporation of detailed MOA for predicting hazard can proceed. The first step in this process will be the development of prioritization tools out of ToxCast™, which incorporate toxicity pathway information to identify chemicals of most concern and to direct more detailed testing and modeling toward chemicals and toxicity pathways of highest importance. As computational models are developed that relate the perturbation of a toxicity pathway quantitatively to an adverse outcome (aided by development of virtual tissues), it should become possible to not only prioritize but also screen for chemicals where the evidence is convincing of either safety or toxicity. The toxicity pathways and MOAs for those chemicals where evidence is inconclusive would then become the subject of further experimental study. Eventually, quantitative prediction of risk from HTS data could be derived based on evaluation of toxicity pathway predictions from systems models of tissues and organs (e.g., v-Liver™ and v-Embryo™) and, perhaps, even simulated target populations.

A major step in higher throughput modeling and prediction of exposure will come from the CTRP ExpoCast™ project, a collaboration of NERL and NCCT focused on providing *Biologically Relevant Exposure Science for 21st Century Toxicity Testing*. The ExpoCast™ project is described in more detail in the project plans (see Appendix IV.A). It should be noted that

preexisting and other NERL research, databases, and models will be critical to the success of ExpoCast™ and the broader goals, of ORD's CTRP. As ExpoCast™, ToxCast™, and other modeling efforts succeed, it will be possible to prioritize testing and monitoring of large numbers of chemicals and other environmental contaminants. These prioritizations would be feasible because they would be based on hazard (H) and exposure (E) predictions of calculable and reasonable uncertainty that were derived solely from in silico and in vitro data (see Figure 5).

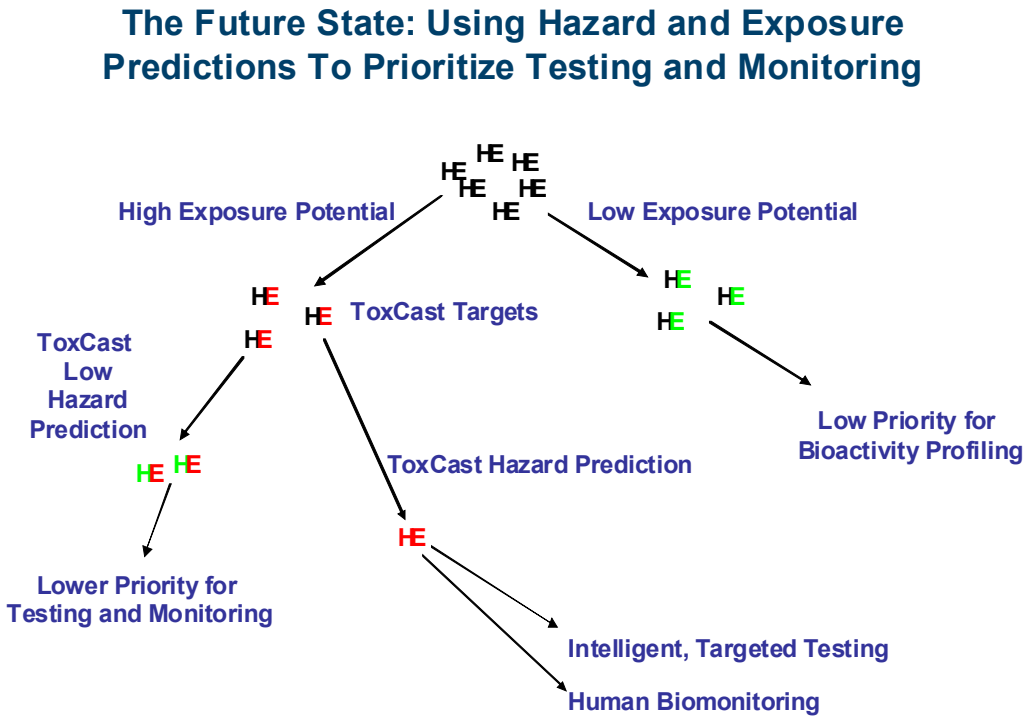

Figure 5

As suggested by the NRC report *"Toxicity Testing in the 21st Century: A Vision and a Strategy"* and *The U.S. Environmental Protection Agency's Strategic Plan for Evaluating the Toxicity of Chemicals*, the majority of current CTRP efforts are centered on advancing toxicity testing for assessing human health effects of environmental agents. However, under environmental legislative mandates (e.g., the Toxic Substances Control Act; the Federal Insecticide, Fungicide, and Rodenticide Act; and the Clean Water Act), most EPA programs regulate compounds to ensure both environmental and human health risks are properly managed. Statutory language and resulting policy typically require decisions for chemicals that encompass environmental and human health risks such that the CTRP eventually also will need to develop higher throughput and computational approaches for ecotoxicology and risk assessment. Notable progress has been made in the previous CTRP implementation plan and within other ORD research programs on the development and use of toxicity pathway models, toxicology knowledgebases (e.g., ECOTOX), and systems biology models (e.g., small fish model) in the field of environmental science. In follow-up to the current CTRP implementation plan for FY2009 to 2012, opportunities will exist to bring together relevant disciplines, data, and models across both human health and environmental risk assessment applications. As noted previously, we will be using existing resources and projects to leverage an increased attention to ecologically relevant

areas; however, given the challenges of developing a system for relevance to human health assessment, the near-term goals of the CTRP will have to remain largely focused on that area. It is expected that future versions of the CTRP and related implementation plans will accommodate further progress in ecotoxicology that can be incorporated and merged with the efforts relating to human health in this current CTRP plan.

C. Regional and Program Interactions

The CTRP is working closely with program offices, including the OPPTS, and the Office of Water (OW), to create informatics tools and databases of use toward the goals of both research and regulation. Within the past year, full-day sessions have been spent briefing senior management in these and other program and regional offices on the activities of the CTRP. This has resulted in shared participation, input, and authorship between program and CTRP staff in planning, research projects, scientific publications, and promulgation of regulatory decisions. Many examples of collaborations with program offices exist, including DSSTox, ACToR, ToxRefDB, and ToxCast™. Regional input has been more limited; briefings on the CTRP have been given to specific regions (e.g., 6 and 7), and to the regional risk assessors group. However, additional dialogue is needed as products begin to emerge from the program that could be of use to the regions, especially in relation to the Toxics Release Inventory and Superfund programs.

In moving forward over the next several years, the key points of engagement with the programs will be their use of the DSSTox, ToxRefDB, and ACToR databases as sources of chemical, toxicity and exposure information, and predictions and prioritizations based on results from ToxCast™, ExpoCast™, and the Virtual Tissues projects. More detailed descriptions of what these engagements will be are provided in the individual project descriptions in Appendix IV.A. Some specific examples include the continued co-development of ToxRefDB to include additional guideline test results, as well as to be positioned to record the output of the tier 1 endocrine disruptor screening batteries.

OPPTS-OPP. Driven by its needs to assess the effects of pesticidal inerts and antimicrobial agents, both of which suffer from limited data availability; OPP has been a strong proponent of this new approach to toxicity testing. OPP has defined a Strategic Direction for New Pesticide Testing and Assessment Approaches focused on developing and evaluating new technologies in molecular, cellular, and computational sciences to supplement or replace more traditional methods of toxicity testing and risk assessment. This integrated approach to testing and assessment is moving toward a new paradigm where *in vivo* (animal) testing is targeted to the most likely hazards of concern. As defined by OPP, the path forward will include close collaborations with the CTRP to predict chemical toxicity and exposure through application of efficient and effective screening tools including new in vitro assays that rapidly provide biological profiles of the toxicological potential of chemicals (i.e., ToxCast™). Exposure and biomonitoring data also will be critical to interpreting toxicity data and evaluating the effectiveness of the new testing and assessment paradigm. Over the next 5 years, OPP plans to enhance its integrated approach to testing and assessment to better determine what toxicity data are needed to further refine risk assessments for chemicals that do not have extensive toxicity information (e.g., inert ingredients, certain antimicrobial and biochemical pesticides, metabolites, and degradates of pesticide active ingredients). Over the next 10 to 15 years, as experience is gained and as understanding of toxicity pathways increases, an enhanced integrated testing and assessment approach will be implemented for all pesticides, including conventional agricultural pesticides. This approach will fully integrate hazard and exposure data, along with advanced systems modeling based on new in vitro data and an understanding of toxicity pathways to better predict

risks and to determine what additional data are necessary (i.e., virtual tissues). The key goals of integrated approaches to testing and assessment are to

- improve our ability to set priorities for what data to require,
- ensure that the data requirements are focused on the right issues, and
- efficiently reach the end result of effective risk assessment.

This approach would provide the ability to focus testing on pesticide chemicals and the effects that most likely could result in harm. As a result, testing would

- use fewer animals,
- take less time,
- be less expensive in data generation and review, and
- explore a broader range of potential adverse effects.

These goals and approach are wholly consistent with the goals and approach of the CTRP, and represent the close working relationship between OPP and ORD in developing these tools for integrated approaches to testing and assessment.

CTRP researchers are working with OPP and other parts of OPPTS, and with the OECD to explore and evaluate regulatory application of molecular screening assays in relation to chemical testing guidelines. The goals of this effort are to provide tools for (1) improving the understanding of mechanisms of toxicity; (2) identifying biomarkers of toxicity and exposure; (3) reducing uncertainty in grouping of chemicals for assessments, interspecies extrapolation, effects on susceptible populations, etc.; and (4) providing alternative methods for chemical screening, hazard identification, and characterization. Also, the CTRP and OPP are working with the OECD, on reducing and refining current animal testing guidelines using the ToxRefDB database and other tools (e.g., Extended One-Generation Reproductive Toxicity Test Guideline).

OPPTS-OPPT. For the Office of Pollution Prevention and Toxics (OPPT) there will be several key points of intersection with the CTRP over the next several years. These include using HTS technologies and bioactivity profiling to lend biologically based support to strengthen and potential revise of chemical categories currently in the new and existing chemicals programs, to evaluate the relative hazard of chemicals being evaluated by the Design for the Environment Program, for providing toxicity pathway data on specific groups of chemicals of high concern (e.g., the perflourinates), and for evaluation of the feasibility of HTS approaches to characterization of the bioactivity profiles of manufactured nanoparticles. On the exposure side, the CTRP expects to be engaged with exposure efforts within OPPT, such as the Inventory Update Rule, as procedures are developed for broad scale predictions of exposure potential across the life cycles of chemicals. CTRP researchers are working with OPPT and with the OECD to access and incorporate CTRP tools (e.g., DSSTox, ToxRefDB, ER QSAR models) into the OECD QSAR Application Toolbox. The toolbox is an international effort to provide tools for grouping chemicals, based on the understanding of mechanisms of toxicity, to extrapolate properties and effects from tested chemicals to untested chemicals.

OPPTS-OSCP. For more than a decade the Office of Science Coordination and Policy (OSCP) has led EPA's work to fulfill mandates contained within the Food Quality Protection Act of 1996 to identify chemicals that can interfere with the function of natural hormones (e.g., endocrine disruptors). This is an MOA that may result in significant adverse consequences in developing organisms (e.g., embryo, fetus, neonates, and children) should they be exposed to levels sufficient to cause perturbations of their endocrine systems. Animal models have shown that the levels that impact developing organisms can be much lower than those impacting adults, and that exposures during development can lead to adverse effects not seen until adulthood. The Agency has adopted a tier 1 screening battery that is designed to detect whether a chemical

substance interacts with the estrogen, androgen, or thyroid hormonal systems. Combinations of in vitro and in vivo assays are used to provide complementary measurements that detect the endocrine disrupting potential of a chemical. Given the rapid advances in computational and molecular sciences, discussions are underway with OPPTS on the next generation of tools that could be more efficiently applied to the large number of chemicals of potential concern for ability to disrupt the function of the endocrine system. Included in these discussions are the integrative potential of contributions from CTRP in HTS of chemicals and providing informatics and analysis solutions and tools; of the Human Health Research Program in conducting targeted follow-up testing and exposure analysis; of the Endocrine Disruptors Research Program in understanding MOAs, developing methods for assessing cumulative risks, and improving the ability to extrapolate results across species; of the Human Health Risk Assessment Program in assessing the contributions of multiple exposures (e.g., chemicals with common or different modes of endocrine action) across critical life stages; and of the Risk Management Research Program in providing information on chemical life cycles that pose the greatest potential exposure and risk and the development of tools for greener chemicals or processes and other mitigation strategies.

The CTRP has identified a large collection of nearly 10,000 chemicals that are of high priority for EPA program offices. Using available funding, a subset of 700 will be used in Phase II of ToxCast™ (cost per chemical of ~$20k). Going beyond the screening of these chemicals in the full suite of ToxCast™ assays (>500), ORD has the capacity under existing contracts to acquire the entire collection of chemicals and to screen them in a subset of ToxCast™ assays that cover a broad spectrum of endocrine-related activities. The cost of such HTS assays would be on the order of $1k to 4k per chemical, depending on precisely which subset of ToxCast™ assays were included. The use of HTS assays for receptor binding and transcriptional activation and QSARs for these end points to obtain empirical or predicted information on chemicals for which no data were available could provide an ordered list of which chemicals have the highest potential to interact with the estrogen, androgen, and thyroid receptors. Along with exposure information, this prioritized list then could be used to guide other effects research, exposure analyses, and assessment and management activities in the various parts of ORD. The results could be used to select chemicals for entry into subsequent phases of EPA's EDSP.

OW. For OW, the CTRP expects to be engaged with the assessment of the bioactivity of chemicals on the Candidate Contaminant List (CCL) and the derivation of subsequent CCL lists. In the course of identifying and assessing CCL compounds, the OW has to collect and analyze hazard and exposure data on thousands of the same compounds addressed in ACToR, DSSTox, and other CTRP databases. In future iterations of this process, the CTRP will be able to assist the OW with data curation and analysis and provide a wealth of new hazard and exposure data that is computable, as well as prioritization tools designed to use this data.

D. Priority Areas for CTRP Management

1. Toxicity Predictions and Chemical Prioritizations Incorporating Exposure

With the publication of the first predictive bioactivity signatures from Phase I and the initial proof of concept of the ToxCast™ program, we will have hazard predictions that can be incorporated into prioritizing chemicals for further screening and testing. Phase II, scheduled for launch in later 2009, will explore greater diversity of chemical structures and classes to evaluate the robustness of the signatures identified in Phase I. As indicated in Figure 5, having viable exposure predictions or estimations also will be critical to the envisioned prioritization scheme. ExpoCast™ will provide an overarching framework for the science required to characterize

biologically relevant exposure and can, thus, inform chemical prioritization by linking information on potential toxicity of environmental chemicals to real-world health outcomes. NCCT management will support continued collaborations with NERL and other critical partners within EPA and externally to improve accessibility to EPA human exposure data and create a consolidated EPA exposure database focused on measured concentrations in biological media. As NCCT pushes ahead into Phase II of ToxCast™, expanding the compounds in the screening program to include nanomaterials and chemicals with demonstrated human toxicities (e.g., failed pharmaceuticals), it will coordinate these efforts with ExpoCast™ to maximize utility of datasets to develop predictive models and decision support software for chemical prioritizations.

2. Strengthening Cross-ORD Collaborations

Given the broad nature of the challenges facing computational toxicology, the CTRP must engage collaborative partners across ORD to be successful. Cross-ORD collaborations have been a part of the CTRP from its inception and will continue to be a dominant feature of the program. Numerous collaborations from previous years will carry forward, including linkages at the management level, such as the MOUs with NHEERL and NERL to provide NCCT with administrative support functions for funds control, extramural management, quality assurance, and information management. Key research partnerships have developed between NCCT and the rest of ORD. In addition, NCCT continues to advise NCER on the formulation of ideas for new computational toxicology RFAs, providing suggestions for scientific peer reviewers, serving on relevancy reviews as appropriate and collaborating with the cooperative research partners of the STAR grants program. ORD's multiyear planning process provides another opportunity for linkage between the CTRP and related research efforts. Each of the MYPs is led by a National Program Director with support from staff of the laboratories and centers. NCCT participates actively in four of the MYP teams. Three contain similar research activities for screening and prioritizing chemicals, (i.e., Endocrine Disrupting Chemicals [EDCs; LTG III], Safe Pesticides/Safe Products [SP2; LTG I], and Drinking Water Research [DW; LTG II] whereas the fourth has a major focus on the incorporation of biologically based MOA information into quantitative risk assessment (the Human Health Research Strategy and the Human Health MYP; LTG II). Consideration also is being given to how these data and analyses can be incorporated into next-generation risk assessments, in association with ORD's Human Health Risk Assessment program and NCEA. NCCT management meets at least quarterly with the NPDs for these MYPs, in part to continue dialogue on sharing and coordination of resources among programs and ORD laboratories and centers beyond NCCT. Besides financial resources, NCCT is cultivating cross-ORD collaborations through shared postdoctoral fellows and students through NCCT rotational fellowships for ORD scientists. To date, scientists from NHEERL, NERL, and NCEA have participated in details of 4 months or longer in NCCT to work on collaborative research projects.

ORD is in the midst of a transformation to a system of integrated, multidisciplinary (IMD) research projects. The CTRP is actively engaged in planning and discussions on one such IMD proposal entitled "Decision Support Tools for Preventing, Reducing, and Managing Chemical Risks." This IMD project will address the tens of thousands of chemicals and millions of products that current regulatory decision tools do not have the ability to assess, in terms of impact on life-stage vulnerability, genetic susceptibility, disproportionate exposures, and cumulative risk. The project will incorporate some of the predictive, high-throughput tools for exposure and hazards being developed as part of the CTRP; scale them up; and, with attention to critical life stage impacts, create prioritization algorithms and next-generation risk assessments that highlight viable management options for prevention, mitigation, and risk reduction.

3. Tox21: A Federal Partnership Transforming Toxicology

The NRC report on *Toxicity Testing in the 21st Century* has significant implications for human health risk assessment, and, to accelerate progress in this area, two NIH institutes and EPA have entered into a formal collaboration known as Tox21 to identify mechanisms of chemically induced biological activity, prioritize chemicals for more extensive toxicological evaluation, and develop more predictive models of in vivo biological response. Consistent with the vision outlined by Krewski et al. in the NRC report, success in achieving these goals is expected to result in methods for toxicity testing that are more scientific and cost effective, as well as models for risk assessment that are more mechanistically based. As a consequence, a reduction or replacement of animals in regulatory testing is anticipated to occur in parallel with an increased ability to evaluate the large numbers of chemicals that currently lack adequate toxicological evaluation. Ultimately, Tox21 is expected to deliver biological activity profiles that are predictive of in vivo toxicities for the thousands of understudied substances of concern to regulatory authorities in the United States, as well as in many other countries.

The Tox21 collaboration is being coordinated through a 5-year MOU, which leverages the strengths of each organization. The MOU builds on the experimental toxicology expertise at the NTP, headquartered at the NIH/NIEHS; HTS technology of the NIH/NCGC, managed by the NHGRI; and the computational toxicology capabilities of EPA's NCCT. Each party brings complementary expertise to bear on the application of novel methodologies to evaluate large numbers of chemicals for their potential to interact with the myriad of biological processes relevant to toxicity. A central aspect of Tox21 is the unique capabilities of the NCGCs high-speed, automated screening robots to simultaneously test thousands of potentially toxic compounds in biochemical and cell-based HTS assays, and an ability to target this resource toward environmental health issues. As mentioned by Krewski et al., EPA's ToxCast™ program is an integral and critical component for achieving the Tox21 goals laid out in the MOU.

To support the goals of Tox21, four focus groups (1) Chemical Selection, (2) Biological Pathways/Assays, (3) Informatics, and (4) Targeted Testing have been established; these focus groups represent the different components of the NRC vision described by Krewski et al. The Chemical Selection group is coordinating the selection of chemicals for the Tox21 compound library to test at NCGC. A chemical library of nearly 2,400 chemicals selected by NTP and EPA is already under study at NCGC, and results from several dozen HTS assays are already available. In the near term, this library will be expanded to approximately 8,400 compounds, with an additional 1,400 compounds selected by NTP; 2,800 compounds selected by EPA and provided by the CTRP; and 2,800 clinically approved drugs selected by NCGC. Compound selection currently is based largely on the compound having a defined chemical structure and known purity, on the extent of its solubility and stability in dimethyl sulfoxide; (the preferred solvent for HTS assays conducted at the NCGC), and on the compound having low volatility. Implementing quality control procedures for ensuring identity, purity, and stability of all compounds in the library is an important responsibility of this group. A subset of the Tox21 chemical library will be included in Phase II of the ToxCast™ program, which will examine a broader suite of assays to evaluate the predictive power of bioactivity signatures derived in Phase I.

The Biological Pathways and Assays group is identifying critical cellular toxicity pathways for interrogation using biochemical- and cell-based high-throughput screens and prioritizing HTS assays for use at NCGC. Assays already performed at NCGC include those to assess (1) cytotoxicity and activation of caspases in a number of human and rodent cell types, (2) up-regulation of tumor suppressor p53, (3) agonist/antagonist activity for a number of nuclear receptors, and (4) differential cytotoxicity in several cell lines associated with an inability to

repair various classes of DNA damage. Other assays under consideration include those for a variety of physiologically important molecular pathways (e.g., cellular stress responses), as well, as methods for integrating human and rodent hepatic metabolic activation into reporter gene assays. Based on the results obtained, this group will construct test batteries useful for identifying hazard for humans and for prioritizing chemicals for further, more in-depth evaluation. As Tox21 progresses, it will offer an excellent opportunity to incorporate assays specifically relevant to the assessment of chemical hazard to wildlife. For example, as assays become available for the reactivity of nuclear hormone receptors (e.g., estrogen, androgen) from multiple species, we will have the ability to directly compare their responsiveness and test whether a particular species might be more sensitive to perturbations than others.

The Informatics Group is developing databases to store all Tox21-related data and evaluating the results obtained from testing conducted at NCGC and via ToxCast™ for predictive toxicity patterns. To encourage independent evaluations and analyses of the Tox21 test results, all data, including the comparative animal and human data where available, will be made accessible publicly via various databases, including EPA's Aggregated Computational Toxicology Resource (ACToR), NIEHS' Chemical Effects in Biological Systems (CEBS), and the National Center for Biotechnology Information's PubChem.

As HTS data on compounds with inadequate testing for toxicity become available via Tox21, there will be a need to test selected compounds in more comprehensive assays. The Targeted Testing group is developing strategies and capabilities for this purpose using assays that involve higher order testing systems (e.g., roundworms (*Caenorhabditis elegans*), zebrafish embryos, rodents).

In addition to the testing activities, the MOU promotes coordination and sponsorship of workshops, symposia, and seminars to educate the various stakeholder groups, including regulatory scientists and the public, with regard to Tox21-related activities.

4. Communicating Computational Toxicology

As the CTRP has matured, communication of progress has developed beyond the publication of peer-reviewed papers to include implementation of software and databases, Web sites and other applications. Given the importance of communicating and disseminating the products of the CTRP, recruitment of a public affairs/communications specialist for NCCT is currently underway to provide even greater results in this area.

a. EPA Program Office Training and Implementation of Computational Tools

The NCCT and CTRP partners from across ORD have given numerous seminars, held multiple 1- and 2 day workshops, and provided specific training and installation of computational tools for EPA program offices. These ad hoc approaches are now being formalized into an online menu of lectures and tutorials on a broad range of computational toxicology topics. Senior scientists from NCCT, NHEERL, and NERL are contributing to this resource, and a FY2009 NCCT recruitment of a communication specialist will accelerate development of this effort. In FY2010, computational toxicology training initially will focus on the tools ready for program office use, including DSSTox, ACToR, ToxRefDB, and the ToxMiner tool for analyzing ToxCast™ data.

b. Communities of Practice for Chemical Prioritization and Exposure Science

On the scientific level, the NCCT has initiated two CoPs in the areas of Chemical Prioritization and Exposure Science that are intended to unite practitioners in the designated fields. The concept of the CoPs was suggested by the BOSC in April 2005 and since has been adopted as a primary means of communication and integration of activities across ORD, EPA, and outside entities and stakeholders. These efforts will serve to enhance communication and coordination,

develop common standards, promote consistency, evaluate and provide guidance on best practices, recommend research priorities, and provide training to interested parties.

5. Developing Clients for Virtual Tissues

The virtual tissue projects are developing systems models of the liver and embryo that will make the data from CTRP databases and chemical prioritization projects more useful in quantitative risk assessments. Over the course of design and implementation of these systems models, interim milestones and deliverables will need to be developed and communicated to program offices. The process of communicating these products and taking feedback from the programs will serve to identify and establish longer term clients for these ambitious projects that are utilizing cutting-edge science that is very different from current regulatory practice.

The Virtual Liver (v-Liver™) project actively is engaging program office personnel to address challenges in MOA elucidation and quantitative dose-response prediction for chronic liver injury. In FY2009, chemicals that work through nuclear receptor pathways (e.g., CAR, PXR, PPARs) were chosen for a proof of concept. Information on these chemicals is being used to populate the v-Liver™ Knowledgebase (v-Liver-KB) and develop a liver simulator model. Program office staff from OPPTS and NCEA were consulted on the selection of these chemicals from key classes of pesticides and industrial chemicals. The first deliverable for risk assessors will be v-Liver-KB, which formally organizes information on normal hepatic functions and their perturbation by chemical stressors into pathophysiologic states. The v-Liver-KB will be deployed as an interactive Web-based and desktop tool to intuitively browse and query physiologic knowledge on chemicals. This then can be used to hypothesize and test putative MOAs and to link assay results from ToxCast™ and ToxRefDB with other evidence curated from the literature. This system will provide computable information on key events that transparently indicate the uncertainties and data gaps and that make inferences on MOA from experimental data. In addition, we will work closely with risk assessors to customize the system for specific requirements. Beta versions of the liver simulator will be applied to program office issues relating to key chemical classes; this will be an intensively collaborative process between CTRP scientists and OPPTS and NCEA staff. CTRP scientists working on the v-Liver™ project also will work with OPP staff on retrospective analyses of chronic and cancer in vivo test data, further introducing OPP to the v-Liver-KB and the simulator as appropriate.

Motivation for the Virtual Embryo (v-Embyo™) is the scientific need to understand mechanisms of toxicity and predict developmental defects from complex datasets. The research goal is to simulate embryonic tissues reacting to perturbation across chemical class, system, stage, genetic makeup, dose, and time. Data input is detailed knowledge of molecular embryology, as well as high-throughput data from in vitro models on signaling pathways and cellular phenotypes. Initial efforts have focused on retrospective analyses of regulatory data from reproductive and developmental toxicity tests in ToxRefDB. This work has been a collaborative effort with OPP and has fostered working relationships between the CTRP scientists and program office staff. Next steps are to identify appropriate developmental and reproductive toxicities that will support development of systems models and are also of regulatory interest to program offices. This will include the incorporation of high throughput data from morphogenetically competent in vitro assays, such as the Embryonic Stem Cell Test and Zebrafish Embryo Test through collaborations with NHEERL and outside partners. Virtual Embryo's first goals are to create a knowledgebase and simulation engine that enable in silico reconstruction of key developmental landmarks, which develop by regulating conserved signaling pathways and cellular processes and that are sensitive to environmental chemicals of scientific and regulatory interest to EPA program offices.

III. CTRP Project Summaries for FY2009 to 2012

A. Intramural Projects Coordinated by NCCT

There are 9 CTRP projects coordinated by investigators in NCCT that collectively comprise the core of the CTRP moving forward into FY2009 to 2012. Individual project plans for each of these are appended to this document (see Appendix IV.A). These projects span the source-to-outcome continuum of toxicology research (Figure 6), providing critical components for next-generation risk assessments.

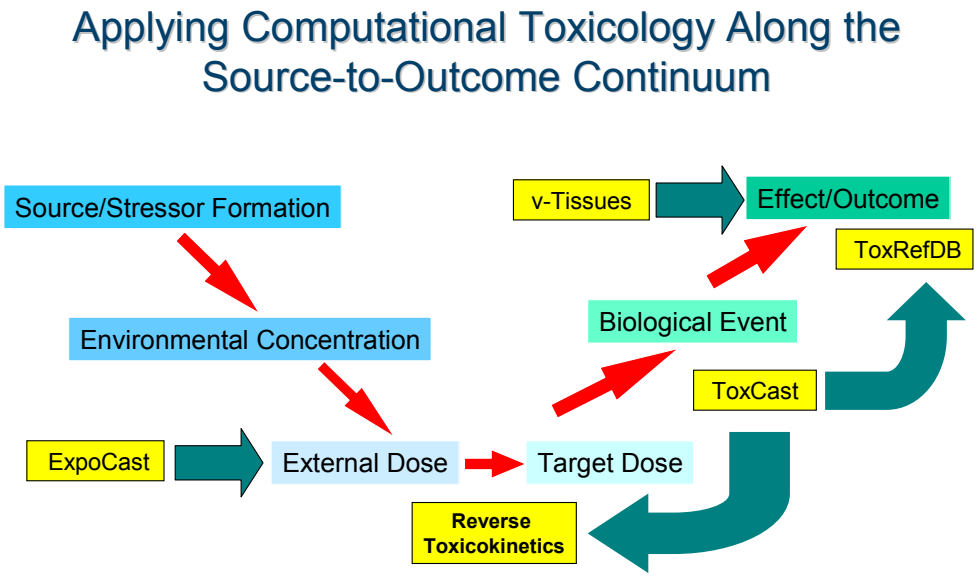

Figure 6

1. ACToR—Aggregated Computational Toxicology Resource

Lead/Principal Investigator: Richard Judson

ACToR is a Web-based informatics platform, organized at the top level by chemical and chemical structure, which is indexing, collecting, and organizing many types of data on environmental chemicals (*Judson et al., 2008*). Environmental chemicals are defined as those likely to be in the environment, including all chemicals regulated or tracked by EPA, as well as related chemicals, such as pharmaceuticals, that find their way into water sources. ACToR is indexing and linking to data from hundreds of sources, including the EPA; FDA; the Center for Disease Control and Prevention (CDC); NIH; academic groups; other governmental agencies (State and national); and international organizations, such as the World Health Organization (WHO). Information being indexed and gathered includes in vivo toxicity, in vitro bioassay data, use levels, exposure information, chemical structure, regulatory information, and other descriptive data. Planning for the project began in mid-FY2007; beta versions were available inside EPA since early FY2008; and a public version became available in December 2008.

ACToR consists of a back-end database and a front-end Web interface built on low-cost, publicly accessible applications and tools. Over the next 3 years, ACToR will expand to include more publicly available resources and data, including more information extracted from text reports and tabularized, and more information on chemical use and exposure. The latter effort will be coordinated with the efforts of ExpoCast™ and NERL to identify, index, and extract data from exposure-related resources of highest interest and importance to EPA programs. In planned upgrades to ACToR, the ability of users to perform flexible searches across different layers of data will be enhanced, and customized data downloads will be implemented. ACToR will serve as the primary vehicle to aggregate and publicly disseminate all published data associated with the ToxCast™, ToxRefDB, and Tox21 research projects. Additionally, the ToxMiner and NCCT Chemical Repository systems are being developed as part of ACToR. These are data repositories and data analysis engines for the ToxCast™/Tox21 projects.

2. DSSTox—Chemical Information Technologies in Support of Toxicology Modeling
Lead/Principal Investigator: Ann Richard

The DSSTox project has implemented high-quality data review procedures for standardized chemical structure annotation, created linkages connecting diverse toxicity resources within and outside of EPA, and published high-quality EPA chemical inventories and toxicity data files spanning more than 10,000 substances. The broad utility of DSSTox data files for cheminformatics and modeling applications provides significant opportunities to influence the course of predictive modeling strategies and to encourage wider engagement of toxicologists in toxicity data representation. The DSSTox project will continue efforts to expand chemical data file offerings into less well-represented areas of toxicology (immunotoxicology, toxicogenomics, etc.) and provide varied representations of summary toxicity endpoints. In addition, this research will explore new representations of chemical structure in relation to the biology (e.g., analog measures, chemical features, chemical classes) and new representations of biological end points in relation to modeling (e.g., quantitative end points in terms of potency, summarized or grouped effects, qualitative active and inactive classes). These efforts will be designed to complement and augment projects in NCCT (ToxCast™, ACToR, and Tox21) that are working to improve capabilities to access, mine, and integrate chemical-biological activity information from existing and new data, both within and outside EPA, in support of toxicity prediction efforts. In close coordination with ACToR, which is the primary informatics resource for ToxCast™ and Tox21 chemical and biological data, DSSTox will provide the initial chemical registration identifications, structure annotation, and quality review of ToxCast™ and ToxRef inventories, as well as the expanded Tox21 chemical testing library, helping to ensure quality and consistency of chemical information across the various NCCT programs.

3. ToxRefDB—Toxicity Reference Database
Lead/Principal Investigator: Matthew Martin

Thirty years of registration toxicity data and open literature studies have been archived as hardcopy and scanned documents by the EPA and others. A significant portion of these data now have been processed into standardized and structured toxicity data within EPA's ToxRefDB, including chronic, cancer, developmental, and reproductive studies from laboratory animals (*Martin et al., 2009a* ; *Martin et al., 2009b*; *Knudsen et al., 2009*). ToxRefDB is a collaborative project between NCCT and OPP. These data are now accessible and mineable within ToxRefDB and are serving as a primary source of validation for EPA's ToxCast™ research program in predictive toxicology. In addition to providing reference toxicity information to research efforts, ToxRefDB will be mined for information on the role and impact of previous and current study guidelines on the regulation of environmental chemicals. The initial collection

of studies in March 2006 focused primarily on the reviews of registrant-submitted toxicity studies on pesticide active ingredients. ToxRefDB design, development, and implementation were completed in mid-FY2006 with ongoing updates to the standardized vocabulary and data entry tool interface. The entry of more than 2,000 studies spanning the majority of the ToxCast™ Phase I chemical set was completed in late FY2008. The status of these initial datasets is either published, in press, or submitted and is being made publicly available through the ToxRefDB Web site. A Web-based query tool for the entire contents of ToxRefDB will become available to the public in 2009, in conjunction with a quarterly update of the EPA ACToR program. ToxRefDB will continue to enter available data from chronic, cancer, developmental, and reproductive studies with a focus on potential ToxCast™ Phase II chemicals. The availability and entry of toxicity data into ToxRefDB also will guide the selection of ToxCast™ Phase II chemicals. Over the next year, ToxRefDB also will be expanded to capture developmental neurotoxicity (DNT) study data and possibly in vivo data submitted to the EDSP. In addition, ToxRefDB is being used for retrospective analyses by various EPA, OECD, and other groups working on revisions to animal test guidelines and other projects.

4. ChemModel—Application of Molecular Modeling to Assessing Chemical Toxicity
Lead/Principal Investigator: James Rabinowitz

This project is using modern molecular modeling methods developed for the discovery of novel pharmaceutical agents to computationally predict toxicant-biomolecular target interactions. A library of computational models of relevant biomolecular targets is being developed. Molecular modeling approaches may then be used to interrogate this library for the capacity of specific environmental molecules to interact with each target. The endocrine system provides a test for the utility of this approach because many of the pathways for toxicity and the macromolecular targets in those pathways have been identified. Appropriate experimental crystal structures of many of the receptor protein targets are available to create the computational library of targets. The ultimate objective of this research is to develop a library of biomolecular targets for chemical toxicity and the methods appropriate for their application to predicting the capacity of a chemical to interact with these targets. This library of targets then may be used in conjunction with other approaches as part of a chemical prescreen.

5. ToxCast™—Screening and Prioritization of Environmental Chemicals Based on Bioactivity Profiling and Predictions of Toxicity
Lead/Principal Investigator: Keith Houck

The objective of the ToxCast™ research program is to develop a cost-effective and rapid approach for screening and prioritizing a large number of chemicals for toxicological testing. Using data from HTS bioassays developed in the drug discovery field, ToxCast™ is generating data, constructing databases, and building computational models to forecast the potential human toxicity of chemicals. HTS bioassays for ToxCast™ also are being provided by NHEERL partners. These hazard predictions should provide EPA regulatory programs, including OPP, with science-based information helpful in prioritizing chemicals for more detailed toxicological evaluations, ultimately leading to reduced animal testing. Furthermore, the toxicity pathways identified from this dataset and project will be critical to transforming the practice of risk assessment for environmental chemicals and contaminants (in collaboration with NCEA and EPA program offices). ToxCast™ is a multiyear effort that is divided into three distinct phases.

- Phase I: 300 Chemicals assayed in more than 600 different HTS bioassays, to create predictive bioactivity signatures based on the known toxicity of the chemicals
- Phase II: Focused on confirmation and expansion of ToxCast™ predictive signatures, generating HTS data on 700 additional chemicals

- Phase III: ToxCast™ expanded to thousands of environmental chemicals for which little toxicological information is available

Once ToxCast™ has gone through successful initiation of Phase III, the data and toxicity predictions will be ready for deployment throughout numerous EPA program offices. NCCT will work to link these hazard predictions with exposure predictions and create integrated database analysis tools facilitating customized chemical prioritizations appropriate to specific programs. Beyond the initial application of ToxCast™ data and tools to prioritizing chemicals for further screening, testing, and monitoring, secondary applications will include the Virtual Tissues systems modeling projects and next-generation risk assessments with NCEA, NHEERL, and EPA program offices.

6. ExpoCast™—Exposure Science for Screening, Prioritization, and Toxicity Testing

Lead/Principal Investigator: Elaine Cohen Hubal

ExpoCast™ will provide an overarching framework for the science required to characterize biologically relevant exposure as a critical part of the CTRP (*Cohen Hubal, 2009*). The ExpoCast™ program will foster novel exposure science research to (1) inform chemical prioritization, (2) understand system response to chemical perturbations and implications at the individual and population levels, and (3) link information on potential toxicity of environmental contaminants to real-world health outcomes. An important early component of ExpoCast™ will be to consider how best to consolidate and link human exposure data for chemical prioritization and toxicity testing. ExpoCast™ represents a strong collaboration between NCCT and NERL, with both parties providing leadership and critical scientific contributions toward this transformation of exposure science. Initial research will focus on identifying and evaluating novel approaches for characterizing exposure to prioritize chemicals and developing modeling approaches for considering exposure potential based on chemical properties, sources (e.g., consumer products), uses, lifecycle, and individual and population vulnerability. Later applications of ExpoCast™ data and tools will include next-generation risk assessments with NCEA, NERL, and EPA program offices.

7. v-Embryo™—Virtual Embryo

Lead/Principal Investigator: Thomas Knudsen

Motivation of the Virtual Embryo Project (v-Embyro™) is scientific needs to understand mechanisms of toxicity and predict developmental defects from complex datasets. EPA must evaluate environmental chemicals for potential effects on development. Part of this challenge is to understand mechanisms by which chemicals disrupt prenatal development. Unfortunately, the mechanisms of prenatal developmental toxicity are not understood in sufficient depth or detail for risk assessment purposes. Because embryonic tissues are regulated simultaneously by pathways that control genetic patterning, molecular clocks, morphogenetic tissue rearrangements, and cellular differentiation, there is a need for computational (in silico) models to address this complexity. EPA's v-Embyro™ will comprise a framework to merge data and knowledge about developmental processes, leading to cell-based computational models that can be used to analyze mechanisms in developmental toxicity. This is a collaborative project among NCCT, NHEERL, and NCEA. Data input is detailed knowledge of molecular embryology, high-throughput data from in vitro models, signaling pathways, and cellular phenotypes. Output models aim for the modular reconstruction of a developing embryo from cell-based models of morphogenesis and differentiation.

8. v-Liver™ —The Virtual Liver Project
Lead/Principal Investigator: Imran Shah

The Virtual Liver Project (v-Liver™) computational paradigm represents tissues as cellular systems in which discrete individual cell-level responses give rise to complex physiologic outcomes. In this model, cell-level responses are governed by a self-regulating network of normal molecular processes, and adverse histopathologic effects arise because of chronic stimulation by environmental chemicals. The v-Liver™ proof of concept is being developed by (1) focusing on environmental chemicals responsible for hepatocarcinogenesis in rodent studies, (2) organizing MOA knowledge on the relevant molecular and cellular processes perturbed by these chemicals, (3) developing a tissue simulation platform to investigate the uncertainties in MOA and neoplastic lesion formation, and (4) evaluating in vitro assays to predict lesion development across chemicals and doses. The Virtual Liver Project is collaboration between NCCT and NHEERL.

9. Uncertainty— Analysis in Toxicological Modeling
Lead/Principal Investigator: R. Woodrow Setzer

The goals of this project are to develop standardized and more efficient computational approaches for parameter estimation and model selection; standardize approaches for model evaluation for PBPK and other dynamic models; and develop methods for constructing priors (probabilistic summaries of current knowledge) for model parameters, based on existing computational methods and datasets. The initial motivation for this work was the need to standardize and make more sophisticated parameter estimation and model evaluation for PBPK models being used by OPP, and that emphasis will continue in the early phase of this project. However, all models relevant to toxicological risk assessment have similar requirements, and this project will coordinate closely with the Virtual Tissues and ToxCast™ projects. In particular, the project will collaborate with ToxCast ™ in developing approaches for quantifying uncertainty in ToxCast ™ predictions and prioritizations.

B. Intramural Projects Coordinated by NERL and NHEERL

Several key components and research projects of the CTRP are coordinated by NERL, NHEERL or both in conjunction with NCCT and other collaborators.

1. NERL

NERL is conducting human exposure research for screening, prioritization, and toxicity testing in collaboration with and complementary to the ExpoCast™ project. Exposure science is crucial for addressing many of our important and complex environmental health issues and is essential for toxicity testing to be valuable in public health protection. There is a clear need for a collaborative effort across the exposure and risk assessment community to ensure the required exposure science, data, and tools are ready to address immediate needs resulting from application of high-throughput in vitro technologies for toxicity testing. A coherent program is required to formulate significant exposure questions posed by these novel in vitro toxicity data; develop creative approaches for applying existing exposure information and tools to address these questions; and, finally, identify key exposure research needs to interpret the toxicity data for risk assessment. The authors of the National Academies report (NRC, 2007) emphasize that population-based data and human exposure information are required at each step of their vision for toxicity testing and risk assessment. The collaborating NERL and NCCT scientists have

identified and will be conducting research in the following priority exposure research areas to support chemical screening, prioritization, and toxicity testing *(Sheldon and Cohen Hubal, 2009)*: (1) accessible and linkable exposure databases, (2) exposure screening tools for accelerated chemical prioritization, (3) computational tools for dose reconstruction and source-to-outcome analyses, (4) tools for understanding the fundamental processes and factors influencing human exposures, and (5) efficient monitoring methods to measure and interpret biologically relevant exposure metrics. Susceptibility, vulnerability, and life-stage aspects are integral to each of these.

The NERL-directed aspects of the ExpoCast™ program will include research required to understand fundamental processes and factors influencing human exposures, as well as development of the tools required to facilitate efficient exposure assessment. This research will be implemented in the following broad areas.

Prioritization and screening. Two related research activities will be implemented: (1) developing high-quality, high-quantity exposure databases aligned with the NCCT databases and (2) developing and evaluating screening models for risk assessment. NERL will inventory, compile, and organize available environmental and exposure data into readily accessible databases. These databases will be organized efficiently so that they are aligned and integrated with other NCCT databases, allowing the users to link source, environmental, exposure, and effects data for chemicals and degradates/metabolites. Research will be conducted to develop the next generation of predictive environmental fate and transport, exposure, and dose-screening models that can be linked with the corresponding NCCT, NHEERL, and other Agency toxicity screening-level models. Available screening models will be inventoried and assessed. A workshop of international experts will be scheduled for evaluating the available models currently being used by various international organizations. New and refined models will be developed, based on the evaluation results, and linked with appropriate Agency toxicity models for future risk assessments.

Linked exposure-dose models. Research will be conducted to efficiently link NERL's environmental, exposure and dose models and databases for supporting program office specific exposure and risk assessments. Emphasis will be on developing tools and approaches to facilitate rapid assessment.

Biomarkers. Collaborative research with scientists from ORD, CDC, and academia will be implemented to design studies for developing and evaluating tools to interpret the results of exposure biomarker studies and link these results to indicators of first biological response. The Metabolic Simulator and Metapath research tools will be upgraded, and their utility for risk assessments will be evaluated using industry-provided data. Collaborative observational studies will be conducted with CDC, NHEERL, and others to develop and refine models for relating measured exposure biomarker results with environmental exposures (both forward and reverse dosimetry). Research will be conducted with NCCT and NHEERL to understand how "omics"-based exposure biomarker data, combined with chemical biomarker results, can be used to link exposure with indicators of effects.

Observational studies. Collaborative research with The National Children's Study and STAR awardees will be conducted to identify and characterize the key factors influencing children's exposures to pesticides and other chemicals. Research activities also will be implemented to characterize real-world exposures to multiple chemicals (mixtures) in targeted communities and vulnerable populations (including children). Tools will be developed for predicting high

exposures, understanding the factors contributing to these exposures, and providing input for the development and evaluation of risk reduction strategies.

2. NHEERL

Research within NHEERL is both parallel to and integrated with the CTRP. Although some of this research has been ongoing for a number of years, a new effort to expand this program currently is underway. The overall goal is the prediction of chemical toxicity to humans and wildlife based on understanding fundamental biology and its perturbation by toxicants. The approach is based on the elucidation of key events that link initiating events to adverse outcomes and leverages expertise in human studies, whole animal toxicology in a wide range of species, and cellular and molecular biology to identify toxicity pathways associated with adverse health and ecological outcomes. The five focus areas for NHEERL research and the integration of these efforts into the CTRP are described below.

(1) *Linkage of environmental exposure to perturbation of toxicity pathways.* For the appropriate application of high-throughput assays based on toxicity pathways to chemical screening (hazard identification) and for risk assessment, the environmental exposure levels and their relation to the exposure at the cellular level must be known. Pharmacokinetic studies and modes will be used to determine the relationship between external exposures and tissue dose in vivo, whereas cellular and molecular biology studies of in vivo effects and subsequent development of toxicity pathway assays will broaden the scope of screening assays.
(2) *Linkage of toxicity pathway perturbations to adverse outcomes.* Research here is focused on identifying toxicity pathways, key events, and MOAs as they relate to adverse outcomes and disease. *MOA* is defined as a sequence of key events and processes, beginning with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in an adverse health effect. Global biology measures ("omics") will be used to discover new toxicity pathways that then will be translated into medium-and high-throughput in vitro and non-mammalian screening assays. Results from these new technologies will be compared with predictions from in vivo experiments. In cases where molecular targets are established for chemical classes, quantitative structure-activity relationship (QSAR) models and read-across methods will be developed to predict the toxicological potential of untested chemicals. These efforts will be coordinated with activities in OPPTS, OECD, and the European Union to ensure the efforts meet Agency and international needs for regulatory purposes.
(3) *Development of toxicity pathway assays.* NHEERL is developing assays for neurodevelopmental, immunotoxicity, and cellular stress responses. As these assays are established, these will be used to expand the breadth of assays in the ToxCast testing program. To date, the ToxCast™ Phase I chemicals have been tested in several neurodevelopmental and cellular stress assays. Through participation in the Tox21 MOU, NHEERL has contributed additional toxicity pathway assays related to the assessment of stress responses in cellular systems. In addition, NHEERL will be performing secondary screening and targeted testing to explore insights and hypotheses generated from the ToxCast and Tox21 HTS efforts. These follow-up studies are designed to evaluate findings from the screening and provide quantitative in vivo relationships.
(4) *Quantitative models for risk assessment.* As quantitative relationships are established between assay conditions and environmental exposures for humans and wildlife, a transition to toxicity-pathway-based risk assessment becomes technically feasible. Data to define these quantitative relationships will be generated in collaboration with NCEA and other ORD partners to ensure the suitability for use in quantitative modeling. Research integrated with

NERL modeling efforts will make PK models compatible with exposure models. Modeling of MOA is being conducted in collaboration with NCCT through modeling of virtual tissues/systems/organisms, as well as less detailed BBDR modeling projects. Through the integrated nature of this research, it is anticipated that in vivo models will be generated for comparison with in vitro toxicity pathway screening results. In addition, models will be developed that can take in vitro results directly as input and make quantitative in vivo predictions for target organisms. The v-Liver™ and v-Embryo™ projects are two NCCT-NHEERL collaborations currently underway in this area. An additional virtual cardiopulmonary project currently is being developed based on extensive NHEERL research in this area and the CTRP "new start" project is looking at the mechanistic indicators of childhood asthma (MICA).

(5) *Special considerations for ecology research.* The actions presented thus far are applicable to both health and ecological problems. Ecology has some specific issues, however, that must be addressed in parallel with the efforts described above. First, although human health risk assessment can be conducted on the basis of a susceptible subpopulation's risk of an adverse outcome, most ecological concerns relate to the effects on population viability. There has been demonstrated success within NHEERL of linking MOA models to population models that will be extended to toxicity pathway-based models to be used for ecological risk assessment as relevant. Second, the NRC report highlights the many problems in extrapolating among species and recommends that species extrapolation be avoided by focusing on human cells for toxicity pathway assays. This is not possible for ecological risk assessment, as the number of relevant species is much too large for direct testing in each one. Therefore, the identification of appropriate sentinel species and development of toxicity pathway assays in these species will be coupled with the development of methods for species extrapolation.

C. Extramural STAR Grantee Projects

Implicit in many of the research projects contained within the CTRP, bioinformatics is one area of research that needs more focus and attention. This rapidly emerging technology is crucial to the computational toxicology program and there remains a large gap in ORD relative to the ability to analyze the high volumes of molecular data and to predict potential toxicity; MOAs; and, ultimately, risk. To help bridge this gap, NCER has supported the establishment of two STAR Environmental Bioinformatics Research Centers. The Research Center for Environmental Bioinformatics and Computational Toxicology at the University of Medicine and Dentistry of New Jersey (UMDNJ), Piscataway, NJ, and The Carolina Environmental Bioinformatics Research Center at the University of North Carolina, Chapel Hill, are operating as cooperative agreements and helping to facilitate the application of bioinformatics tools and approaches to environmental health issues supported by the CTRP.

In the next year, UMDNJ researchers will begin the design of new ebTrack interfaces to open source databases and to various "external" and center-developed modeling tools for facilitating wider deployment and applicability of the ebTrack/ArrayTrack system for integrative analyses of various types of genomic, proteomic, and metabonomic data. Additionally, plans are underway to refine the environmental bioinformatics knowledgebase (ebKB) and to make a public beta version of ebKB available; implement a modular "Virtual Liver" with alternative levels of detail in describing physical structure of the liver with respect to toxicokinetic and toxicodynamic processes with case studies focusing on environmentally relevant chemicals; and refine the framework for Dose-Response Information Analysis (DORIAN) modules representing different

scales of biological complexity, ranging from molecule-molecule interactions to biochemical networks to virtual organs and systems.

For the Carolina Bioinformatics Center, goals for the next year include (1) continuing progress in dose-response pathway modeling and analysis of ToxCast™ Phase I data; (2) continuing QSAR modeling of multiple animal toxicity end points and (3) the development of QSAR and other statistical models to use in vitro biological data to predict in vivo toxicity end points. Other plans include the development of specific data-mining algorithms for genomic databases, and extending computational work on fast approaches for genome-wide expression QTL analysis to human haplotypes.

The third STAR center is the Carolina Center for Computational Toxicology at the University of North Carolina, Chapel Hill. This center is developing fine-scale predictive simulations of the protein-protein/-chemical interactions in nuclear receptor networks, mapping chemical-perturbed networks and devising modeling tools that can predict the pathobiology of compounds based on a limited set of biological data, building tools that will enable toxicologists, to understand the role of genetic diversity between individuals in responses to toxicants; and creating unbiased discovery-driven prediction of adverse chronic in vivo outcomes based on statistical modeling of chemical structures and HTS.

Another high-priority for EPA is to understand the molecular and cellular processes that, when perturbed, result in developmental toxicity. In response to this need, NCER has funded the Texas-Indiana Virtual STAR Center; Data-Generating in vitro and in silico Models of Developmental Toxicity in Embryonic Stem Cells and Zebrafish at the University of Houston – University Park, Indiana University – Bloomington, and Texas A & M University Institute for Genomic Medicine. As chemical production increases worldwide, there is a concordant increased need for determining the hazard and risk to human health at realistic exposure levels. The main objective of the proposed multidisciplinary Texas-Indiana Virtual STAR Center; Data Generating in vitro and in silico Models of Developmental Toxicity in Embryonic Stem Cells and Zebrafish (TIVS) is to contribute to a more reliable chemical risk assessment through the development of high-throughput in vitro and in silico screening models of developmental toxicity. Specifically, the TIVS Center aims to generate in vitro models of murine embryonic stem cells and zebrafish for developmental toxicity. The data produced from these models will be f exploited urther to produce predictive in silico models for developmental toxicity on processes that are also relevant for human embryonic development.

D. Summary Integration of the CTRP Projects for FY2009 to 2012

The CTRP spans several ORD laboratories and centers, as well as the extramural STAR grants program. Collectively, these various components of the CTRP are developing new methods and tools that will enhance our ability to predict adverse effects and understand the mechanisms through which chemicals induce harm. Advances from the CTRP will give EPA the ability to screen and assess a larger number of chemicals than traditional methods enable. In addition, EPA is collaborating with other governmental and private organizations to leverage resources and access complementary expertise to accelerate progress in high-priority research areas. Throughout the various components of the CTRP, focus is maintained on addressing a number of key science questions.
What are the key linkages in the continuum between the source of a chemical in the environment and its adverse outcomes?
How can we develop predictive models for screening and testing?

How can we improve quantitative risk assessment and reduce uncertainty by using advanced computational techniques?
How can we enhance dose-response modeling, especially in low-dose ranges, to include knowledge of molecular events?

To address these questions, ORD's CTRP will continue to provide informatics, chemical prioritization, and systems modeling solutions for EPA. ACToR, DSSTox, ToxRefDB, ToxCast™, and ExpoCast™, v-Liver™, and v-Embryo™ are all excellent examples of extensive, multidisciplinary and integrated projects that bring together the talents of ORD, along with extramural scientists from EPA-funded STAR centers to provide the high-throughput decision support tools for screening and assessing chemical exposure, hazard, and risk.

IV. APPENDIXES

A. Intramural CTRP Projects

1. Project Plans

a. ACToR—Aggregated Computational Toxicology Resource

Lead/Principal Investigator: Richard Judson

Version: March 4, 2009

Research Issue/Relevance: EPA faces a significant issue in that there are many chemicals in wide-spread use, and which the Agency regulates, for which there is little or no toxicology information. The EPA ToxCast™ program is one effort that is addressing this problem by screening many of these chemicals using high-throughput techniques and helping prioritize which ones are candidates for more detailed testing. To be effective, ToxCast™ needs to know what chemicals are in need of screening and needs to know what is already known (or not) about these chemicals. ACToR is providing this information. In addition, the ToxCast™, ToxRefDB, and Tox21 projects each have need for unified, sophisticated informatics support and management of the large amount of chemical and biological information that is central to these projects. Additional capabilities tied to this informatics resource will be needed to address the data analysis and toxicity prediction challenges of the ToxCast™/Tox21 projects.

A related issue is that other EPA programs, as well as external stakeholders, need easy access to information on environmental chemicals both within and beyond the set of interest to ToxCast™. Currently, toxicity and exposure data associated with environmental chemicals does not adhere to standardized representations and are dispersed widely across many databases and Internet resources, many of which are difficult to access or search. The ACToR project is addressing this challenge by creating a central, standardized, publicly accessible chemical-informatics platform to enable searching and cross-referencing of chemical-associated toxicity information to aid prioritization and hazard identification of environmental chemicals.

Purpose/Objective/Impact: ACToR aims to provide a unified, centralized resource of data on environmental chemicals, including toxicology, in vitro assay data, and chemical structure information. By gathering information on the type and location of toxicity or exposure data associated with environmental chemicals into a single, searchable, publicly accessible Web-site, ACToR is providing the basis for chemical selection and screening within NCCT projects, such as ToxCast™ and Tox21. ACToR also is coordinating with the DSSTox project to incorporate quality chemical review and structure-annotation for the chemical datasets of highest interest to the various NCCT projects. In addition to its use in supporting various NCCT and EPA projects, ACToR is a publicly available EPA resource that enables other government agencies, industry, and academic researchers to quickly search and collate toxicity-related information on chemicals of interest. As such, it will promote and encourage other entities to adopt standards for chemical representation and broadly survey chemical information pertaining to toxicology resources on the Internet.

Synopsis: ACToR is a Web-based informatics platform, organized at the top level by chemical and chemical structure that is indexing, collecting, and organizing many types of data on environmental chemicals. Environmental chemicals are defined as those likely to be in the

environment, including all chemicals regulated or tracked by EPA, as well as related chemicals, such as pharmaceuticals that find their way into water sources. ACToR is indexing and linking to data from hundreds of sources, including the EPA, FDA, CDC, NIH, academic groups, other governmental agencies (State and national) and international organizations, such as the WHO. Information being indexed and gathered includes in vivo toxicity, in vitro bioassay data, use levels, exposure information, chemical structure, regulatory information, and other descriptive data. Planning for the project began in mid-FY2007; beta versions were available inside EPA beginning in early FY2008, and a public version became available in December 2008. ACToR consists of a back-end database and a front-end Web interface built on low-cost, publicly accessible applications and tools. Over the next 3 years, ACToR will expand to include more publicly available resources and data, including more information extracted from text reports and tabularized, and more information on chemical use and exposure. The latter effort will be coordinated with the efforts of ExpoCast™ and NERL to identify, index, and extract data from exposure-related resources of highest interest and importance to EPA programs. In planned upgrades to ACToR, the ability of users to perform flexible searches across different layers of data will be enhanced, and customized data downloads will be implemented. ACToR will serve as the primary vehicle to aggregate and publicly disseminate all published data associated with the ToxCast™, ToxRef, and Tox21 research projects. Additionally, the ToxMiner and NCCT Chemical Repository systems are being developed as part of ACToR. These are data repositories and data analysis engines for the ToxCast/Tox21 projects.

Partnerships/Collaborations (Internal & External):

(1) EPA ToxCast™—program—provide data for use in selecting chemicals and providing toxicology data for validation; provide route for publication of data
(2) Tox21 partnership—provide data for use in selecting chemicals and providing toxicology data for validation; provide route for publication of data
(3) DSSTox coordination—align methods for registering high-interest chemical inventories (ToxCast™, ToxRefDB, Tox21, DSSTox published data files), utilizing DSSTox chemical information quality review and structure-annotation within ACToR
(4) EPA centers and offices (OPPT/OPP/NCEA/OW)—provide data on chemicals of interest

Milestones/Products:

FY09

(1) Initial public deployment
(2) Significant version 2, including refined chemical structure information
(3) Develop workflow for tabularization of data buried in text reports
(4) Integrate all ToxCast™ and ToxRefDB data
(5) Quarterly releases with new data

FY10

(1) Quarterly releases with new data
(2) Implementation of a process to gather tabular data on priority chemicals from text reports
(3) Survey sources of chemical use and exposure data and import any remaining sources
(4) Develop flexible query interface and data download process
(5) Develop process to extract data from open literature

FY11

(1) Quarterly releases with new data

FY12

(1) Quarterly releases with new data

Keywords: computational toxicology, ACToR, ToxCast™, DSSTox, database

QA Project Plan: Category II. ACToR development is guided by a series of standard operating procedures. These govern all aspects of the project, including data acquisition, formatting, quality assurance, database filling and maintenance, and system administration. All quality assurance (QA) plans are archived in the EPA internal QA system.

b. DSSTox—Chemical Information Technologies in Support of Toxicology Modeling

Lead/Principal Investigator: Ann M. Richard

Research Issue/Relevance: A central regulatory mandate of EPA is to assess the potential health and environmental risks of large numbers of chemicals released into the environment, often in the absence of relevant test data. Significant advances in toxicity prediction capabilities are predicated on the ability to store, mine, and analyze information on many levels in relation to chemicals and their effects on biological systems. Standardized high-quality chemical structure annotation and searchability of toxicity-related information across the Internet and within EPA programs is a crucial requirement for creating effective data linkages and gathering relevant data. Equally important is the need to incorporate meaningful chemical structure and property representations, based on principles of organic chemistry and biologically informed measures of chemical similarity, into toxicity modeling efforts. Finally, successful modeling efforts will depend on suitable representations of biological activity, both HTS and in vivo, in relation to chemical structure. NCCT's ToxCast™ and Tox21 projects are employing HTS tests to probe biochemical target interactions, chemical pathways, and cellular responses potentially relevant to toxicity for thousands of chemicals of high potential exposure and environmental interest. The goal is to use these data, in conjunction with legacy data and chemical structure considerations, to infer meaningful patterns and to develop models to predict a range of *in vivo* bioassay responses. Biologically informed toxicity prediction models that incorporate chemical structure-activity considerations are likely to provide the best means for prioritizing large lists of chemicals for potential hazard—a pressing need for many EPA programs—and charting a path forward for a more efficient and cost-effective screening and testing paradigm.

Purpose/Objective/Impact: The current research will use the DSSTox project framework to incorporate strict quality standards for chemical information across NCCT projects (ToxCast™, ACToR, and Tox21) and expand comparability and linkages of summary toxicity data in the context of standardized cheminformatics environments. This project also will use these data foundations to promote and explore new ways to associate chemical structure with biological activity, extending traditional SAR toward new paradigms for biologically informed structure-based toxicity prediction. These efforts have the potential to impact a wide variety of EPA program offices that rely heavily on chemical information resources and have a need for structure-based data exploration, analog searching, and improved toxicity prediction models when limited test data are available. These include programs within OPPTS e.g., Green Chemistry, PreManufacture-Notification Program (PMN), OPP, HPV Testing Program, as well as EPA's IRIS Program, Office of Water, and Office of Environmental Information. New information technologies that incorporate strict quality standards and more flexible and diverse means for assessing biological and chemical similarity also will improve the identification of toxicologically relevant analogs by enhancing the ability to explore data and quantify associations across diverse chemical and biological data domains.

Synopsis: The DSSTox project has implemented high-quality data review procedures for standardized chemical structure annotation, created linkages connecting diverse toxicity resources within and outside of EPA, and published high-quality EPA chemical inventories and

toxicity data files spanning more than 10,000 substances. The broad utility of DSSTox data files for cheminformatics and modeling applications provides significant opportunities to influence the course of predictive modeling strategies and to encourage wider engagement of toxicologists in toxicity data representation. The DSSTox project will continue efforts to expand chemical data file offerings into less well-represented areas of toxicology (immunotoxicology, toxicogenomics, etc.), and provide varied representations of summary toxicity end points. In addition, this research will explore new representations of chemical structure in relation to the biology (e.g., analog measures, chemical features, chemical classes) and new representations of biological end points in relation to modeling (e.g., quantitative end points in terms of potency, summarized or grouped effects, qualitative active and inactive classes). These efforts will be designed to complement and augment projects in NCCT (ToxCast^{TM}, ACToR, and Tox21) that are working to improve capabilities to access, mine, and integrate chemical-biological activity information from existing and new data, both within and outside EPA, in support of toxicity prediction efforts. In close coordination with ACToR, which is the primary informatics resource for ToxCast^{TM} and Tox21 chemical and biological data, DSSTox will provide the initial chemical registration IDs, structure annotation, and quality review of ToxCast^{TM} and ToxRefDB inventories, as well as the expanded Tox21 chemical testing library, helping to ensure quality and consistency of chemical information across the various NCCT programs.

Partnerships/Collaborations (Internal & External): The DSSTox project is being coordinated and linked with a number of public efforts (ILSI, ToxML, LHASA UK, PubChem, ChemSpider) and government research laboratories (NIEHS, NTP, FDA) that are promoting controlled toxicity vocabularies, adopting data standards, and migrating diverse toxicity data into the public domain. DSSTox also is aligned with major NCCT projects (ToxCast^{TM}, ToxRefDB, ACToR, Tox21), providing key quality review procedures and cheminformatics support, expanding DSSTox data file publications of toxicological data in support of predictive modeling, and enhancing linkages to public resources such as PubChem, for disseminating bioassay results to the broader modeling community. In coordination with ACToR, partnerships and collaborations with scientists across EPA (NHEERL, OPPT, OPP, and NERL) are being forged to improve cheminformatic capabilities across the Agency from a unified chemical structure perspective, most recently extending into exposure data arenas (ExpoCast^{TM}). Research collaborations are ongoing with SAR modelers at UNC (A. Tropsha, H. Zhu) and, in the data mining and SAR community (C. Yang, R. Benigni, E. Benfenati), to improve methods to incorporate biological considerations into SAR models. OECD is using DSSTox as a source of high-quality, quality-controlled chemical structures and activities in their QSAR Toolbox. Finally, we are pursuing closer collaborations with toxicogenomics resources, such as the National Center for Biotechnology Information (NCBI; GEO), the European Bioinformatics Institute (EBI: ChEBI and ArrayExpress), and the NIEHS CEBS toxicogenomics resource.

Milestones/Products:

FY09

(1) Publish paper on ToxCast^{TM} 320 chemical inventory from SAR modeling perspective
(2) Publish papers and coordinate efforts with NCBI (GEO) and EBI (ArrayExpress) to structure-annotate and provide chemical linkages to microarray data for toxicogenomics
(3) Restart Chemoinformatics CoPs using EPA's Science Portal
(4) Publish DSSTox files for ToxRef and ToxCast^{TM} inventories and selected summary end points and facilitate publication and linkage to the NLM PubChem project. Compile and publish public genetic toxicity data and SAR predictions for ToxCast 320
(5) Continue expansion of the DSSTox public toxicity database inventory for use in modeling
(6) Perform primary chemical review and structure annotation of the ToxCast^{TM}/Tox21 chemical testing libraries, coordinating with ACToR and within a central chemical registry

FY10

(1) Publish DSSTox files for Tox21 inventory and selected summary end points, and facilitate publication and linkage to the NLM PubChem project
(2) Publish DSSTox files for NTP study areas (Immunotox, Genetox, etc.) to facilitate incorporation into ACToR and encourage broader SAR examination; explore new approaches to SAR modeling based on feature categories within existing DSSTox files and ToxCast™ data
(3) Explore new approaches to SAR modeling based on classifiers and feature categories
(4) Expand CEBS collaboration to incorporate DSSTox GEO and ArrayExpress files; create chemical linkage to ILSI Developmental Toxicity database and facilitate structure-searching
(5) Advise and assist efforts within ExpoCast™ to identify and chemically annotate important exposure-related public data resources

FY11

(1) In collaboration with ACToR, establish procedures and protocols for automating chemical annotation of new experimental data generated by NCCT programs (ToxCast™ and Tox21) and in collaboration with CEBS or NHEERL
(2) Document and employ PubChem analysis tools in relation to published DSSTox and ToxCast™ data inventory in PubChem
(3) Collaborate with SAR modeling efforts to predict ToxCast™ end points using in vitro data
(4) Continue expansion of DSSTox public toxicity database inventory for use in modeling with co-publication and linkage to ACToR and PubChem

FY12

(1) Redesign DSSTox Web site to provide hosting of donated chemical descriptors, properties, and predictions for high interest inventories
(2) Publish master tables of DSSTox IDs and high-quality structures to serve as public data registry for toxicology, particularly for EPA, FDA, and NTP datasets
(3) Promote use of chemical registry system from ToxCast™/Tox21, linked to DSSTox content and integrated into ACToR, more broadly within EPA
(4) Collaborate with SAR modeling efforts to expand modeling to address Tox21 chemicals and end points
(5) Continue expansion of DSSTox public toxicity database inventory into toxicity and exposure areas not effectively linked to current databases

Keywords: DSSTox, prediction, cheminformatics, structure-activity, SAR

QA Project Plan: Category II. The DSSTox project, involving the compilation, standardization, and review of chemical data largely extracted from secondary public data sources has a large intrinsic QA component. All QA plans are archived in the EPA internal QA system. Documentation and procedures contributing to overall QA objectives include

- maintenance of a DSSTox Web page describing QA procedures for obtaining and reviewing chemical information prior to inclusion in the DSSTox Master File (http://www.epa.gov/ncct/dsstox/ChemicalInfQAProcedures.html);
- file versioning, error tracking within data files, publication of log files with version history, and full documentation of published DSSTox data files;
- maintenance of the DSSTox Master File tables in ACCESS, cross referencing, and use of automated scripts to perform field content error checks for new file generation;
- coordinated publication in ACToR and PubChem allows for checks on structure consistencies;
- review of all newly published DSSTox data files by Source collaborators;

- error-reporting system associated with DSSTox published files and structure browser.

c. ToxRefDB—Toxicity Reference Database

Lead/Principal Investigator: Matthew Martin

Version: April 02, 2009

Research Issue/Relevance: As EPA moves toward a new chemical toxicity testing paradigm, the animal toxicity data the Agency has received will provide context for assessing many of the new HTS technologies for toxicity testing and screening. ToxRefDB is the relational database designed and developed to electronically capture all of the relevant information spanning 30 years of health effects data from the Agency and beyond. The EPA ToxCast™ program is one effort using high-throughput techniques to prioritize chemicals for further testing. To be effective, ToxCast™ needs reference toxicity information to provide the interpretive context for the large amounts of screening data. ToxRefDB is providing the reference in vivo toxicity data for programs such as ToxCast™ in a searchable and computable format.

Additionally, the utility of ToxRefDB is broader than use as a validation dataset for ToxCast™. Regulatory scientists have begun to assess the role and impact of previous and current guideline studies and components of those studies in the regulation and assessment of chemicals. ToxRefDB will be the primary data source for numerous retrospective analyses and may have a large impact on future revisions to existing guideline studies.

Purpose/Objective/Impact: The ToxRefDB project has provided access to a wealth of in vivo toxicity data in a structured and searchable format. These data are being released through a series of manuscripts that currently are submitted for publication or in preparation. In addition, ToxRefDB data will be publicly available through the ToxRefDB Web site. This will fill a major gap in the environmental toxicology community, as very limited resources in this area exist in the public domain. Such information should have high utility in building and interpretation of predictive toxicology models. In addition, researchers and regulatory scientists can access the toxicity information to address numerous questions specific to hazard identification and characterization of environmental chemicals, along with retrospective analyses that will direct and evaluate possible changes to toxicity studies guidelines.

Synopsis: Thirty years of registration toxicity data and open literature studies have been stored historically as hardcopy and scanned documents by EPA and others. A significant portion of these data, including chronic, cancer, developmental, and reproductive studies from laboratory animals, has now been processed into standardized and structured toxicity data within EPA's ToxRefDB. These data are now accessible and mineable within ToxRefDB and are serving as a primary source of validation for EPA's ToxCast™ research program in predictive toxicology. In addition to providing reference toxicity information to research efforts, ToxRefDB will be mined for information on the role and impact of previous and current study guidelines on the regulation of environmental chemicals. The initial collection of studies in March 2006 focused primarily on the reviews of registrant-submitted toxicity studies on pesticide active ingredients. ToxRefDB design, development, and implementation were completed in mid-FY2006, with ongoing updates to the standardized vocabulary and data entry tool interface. The entry of more than 2,000 studies spanning the majority of the ToxCast™ Phase I chemical set was completed late FY2008. The statuses of these initial datasets are either published, in press, or submitted and are being made publicly available through the ToxRefDB Web site. Over the next year, a Web-based query tool for the entire contents of ToxRefDB will become available to the public, and

this will be performed in conjunction with a quarterly update of the EPA ACToR program. ToxRefDB will continue to enter available data from chronic, cancer, developmental, and reproductive studies, with a focus on potential ToxCast™ Phase II chemicals. The availability and entry of toxicity data into ToxRefDB also will guide the selection of ToxCast™ Phase II chemicals. Over the next year, ToxRefDB also will be expanded to capture DNT study data and, possibly in vivo data submitted through the Agency as part of the EDSP. The completion of retrospective analyses on reproductive toxicity studies will be completed in FY2010, and the use of ToxRefDB for additional analyses including rat and mouse chronic/cancer studies, and rat and rabbit development study assessments will also be undertaken during the period of this implementation plan.

Partnerships/Collaborations (Internal & External):

(1) EPA ToxCast™ program—provide the reference toxicity information for interpreting the screening data with respect to animal toxicity information
(2) Tox21 partnership—provide the reference toxicity information for interpreting the screening data with respect to animal toxicity information
(3) EPA ACToR program—provide ACToR with ToxRefDB data for chemical indexing and searchability
(4) EPA centers, offices, and laboratories (OPPT/OPP/OSCP/NHEERL)—provide legacy toxicity data; searchable and computable toxicity data to offices; and entry of additional study types and from various sources
(5) OECD (including BfR, RIVM, PMRA)—evaluation of current testing guidelines and assessment of proposed new guidelines (e.g., extended one-generation reproduction toxicity study)

Milestones/Products:

FY09

(1) Publication on ToxRefDB
(2) Release of stand-alone ToxRefDB data entry tool
(3) ToxRefDB Web page online
(4) Initial public release of selected chronic/cancer end points
(5) Public release of selected reproductive toxicity end points
(6) Public release of selected developmental toxicity end points
(7) Collection of ToxCast™ Phase II chemical toxicity data
(8) Public release of ToxRefDB Web-based query tool
(9) Complete entry of targeted set of chemicals and study types for Phase II of ToxCast ™
(10) Complete reproductive toxicity study retrospective analysis

FY10

(1) Quarterly releases with new data in conjunction with ACToR
(2) Implementation of a process to gather and enter open literature studies
(3) Expansion of ToxRefDB to capture DNT studies and EDSP data
(4) Complete retrospective analyses on other major study types
(5) Release of ToxRefDB live data entry tool

FY11

Quarterly releases with new data in conjunction with ACToR

FY12

Quarterly releases with new data in conjunction with ACToR

Keywords: computational toxicology; ToxRefDB; ToxCast™; ACToR; database

QA Project Plan: Category II. ToxRefDB development is guided by a series of standard operating procedures. These govern all aspects of the project, including data acquisition, formatting, quality control and assurance, data entry and maintenance, and system administration. All QA plans are archived in the EPA internal QA system.

d. ChemModel—The Application of Molecular Modeling to Assessing Chemical Toxicity

Lead/Principal Investigator: James Rabinowitz

Research Issue/Relevance: Insufficient experimental information exists for the evaluation of the potential of a large number of environmental chemicals to cause toxicity and other environmental effects. Where data does exist, often it is not ideal for this task. The Agency often must make decisions about specific chemicals when lacking an ideal data set. Molecular modeling approaches provide an approach for estimating relevant missing information. One approach to this problem is to estimate the relevant missing information by extrapolation from existing information on the chemical of interest and other similar chemicals, making use of molecular modeling approaches. Knowledge of the mechanisms of toxicity provides a rational basis for application of these computational tools. The results of these models may be used in conjunction with experimental information to inform decisions about the relevant chemical and to prioritize the requirements for obtaining missing experimental data.

Purpose/Objective/Impact: The overall objective of this research is to develop an approach (including the necessary tools) for the application of molecular modeling methods to Agency problems, particularly problems resulting from the requirement to make preliminary decisions about chemicals in a data-poor environment. This includes the preliminary evaluation of chemical toxicity and the prioritization of chemicals testing needs. Knowledge of the potential mechanisms of toxicity provides a rational basis for extrapolation from existing information to derive information about chemicals for which little data exist. The differential step in many mechanisms of toxicity may be generalized as the interaction between a small molecule (a potential toxicant) and one or more macromolecular targets. (The small molecule may be the chemical itself or one of its descendants). Using modern molecular modeling methods developed for the discovery of novel pharmaceutical agents, it is possible to computationally predict these toxicant-biomolecular target interactions using a combination of direct computer modeling of atomic interactions between the toxicant-target pair and correction factors derived from experimentally derived interactions with similar targets. To employ this approach, a library of computational models of relevant biomolecular targets is being developed. Molecular modeling approaches then may be used to interrogate this library for the capacity of specific environmental molecules to interact with each target. These approaches were developed for the discovery of new pharmaceuticals, where the objective is to discover molecules that interact most potently with the target. However, the Agency's need is to discover whether chemicals of environmental interest interact with the target, even if their interaction is much weaker than seen with potential pharmaceutical agents. An objective of this research is to evaluate the relevant molecular modeling methods in relationship this Agency requirement.

The endocrine system provides a test for the utility of this approach because many of the pathways for toxicity and the macromolecular targets in those pathways have been identified. Appropriate experimental crystal structures of many of the receptor protein targets are available to create the computational library of targets. Additionally, experimental data of the capacity of a library of chemicals to displace the natural ligand from the rat estrogen receptor is available from a single source. Although most of the chemicals in this library have been shown not to interact with the receptor in this laboratory assay, the few that displace estrogen are orders of

magnitude less potent than the natural ligand. The data from this chemical library provides an opportunity to test the toxicant-target approach and its capacity to separate less potent chemicals from a large number of similar inactive chemicals. In addition, this exploration addresses the specific Agency need to evaluate the potential of chemicals to disrupt the endocrine system. A similar approach may be used to investigate other health effects and fate and metabolism.

The ultimate objective of this research is to develop a library of biomolecular targets for chemical toxicity and the methods appropriate for their application to predicting the capacity of a chemical to interact with these targets. This library of targets may then be used in conjunction with other approaches as part of a chemical prescreen.

Synopsis: The differential step in many mechanisms of chemical toxicity may be generalized as the interaction between a small molecule (a potential toxicant) and one or more macromolecular targets. The small molecule may be the chemical itself or one of its descendents. Describing the potential of a molecule to participate in interactions of this type is a source of insight chemical toxicity. In this project, a series of molecular models (148) for critical toxicity targets is being developed, and methods to evaluate the capacity of a small molecule to interact with these targets assessed. These methods are adopted from those used in the design of novel pharmaceutical. A study of a library of 280 environmental chemicals interacting with the estrogen receptor target is in the final stages of completion. In this library, 14 of the chemicals are weakly active (3 to 5 orders of magnitude less active than estrogen), and the others are inactive. Modeling the potential interaction of these chemicals with the rat estrogen receptor provides an ordered list of molecules. The best results are achieved using a pharmacophore filter. With that approach, all 14 active chemicals are identified in the first 22 chemicals. In addition to the importance of these results relative to potential binding to the environmentally important estrogen receptor, they indicate that this approach may be used to find chemicals that interact weakly with the target. All 150 of the targets have been interrogated with the ToxCast^{TM} chemicals. Based on the results from the estrogen receptor study, pharmacophores for as many of the targets as possible will be developed. The analysis of this data will proceed by comparison with specific ToxCast^{TM} end point data and in concert with short-term data to evaluate more complex biological end points. The logical extension is to consider the androgen receptor, where relevant data for comparison and developing a pharmacophore are available. When sufficient data on other biological macromolecules that are relevant to the Agency requirements become available, the current library of targets will be expanded. This library of targets will be used to study chemicals and families of chemicals of importance to the Agency. The toxicant-target approach described above models molecular identification processes. In collaboration with other EPA scientists, similar approaches are being applied to the steps that follow identification. A study on the differential metabolism of pyrethroids and the effect of stereo-structure on biological clearance is underway, as is a study of perfluorinated chemicals.

Partnerships/Collaborations (Internal & External): Scientists from NHEERL/RTD have provided the database of the interactions of molecules with the estrogen receptor. We continue to interact with them relative to this data and the biological details of the computational modeling effort. Scientists from NERL/HEASD are collaborators on the study of the metabolism and fate pyrethroids. Collaboration with CDC scientists relative to using the target-toxicant approach to investigate the interaction of environmental chemicals with nervous system enzymes and receptors is being developed. As described above, these studies apply methods developed for pharmaceutical discovery to model the capacity of an environmental chemical to interact with a macromolecular target.

Milestones/Products:

FY09

(1) Report on the capability of the target-toxicant paradigm to identify chemicals that bind weakly to the estrogen receptor, including a description of the method
(2) Description of the library of 148 biological macromolecule targets
(3) Report on molecular modeling studies of the potential biological effects of the perfluoro compounds

FY10

(1) Report on the metabolism of pyrethroids and the effects of three dimensional chemical structures
(2) Description of additional targets added to the target library
(3) Report on the interaction of ToxCast™ chemicals with nuclear receptor targets and the importance of pharmacophore filters

FY11

(1) Report on the integration of results from the target library and available experimental parameters
(2) Report on the comparison of results with and without pharmacophore filter for ToxCast™ chemicals

FY12

(1) Comparison of results using the target library with experimentally determined activities, particularly when observed at the molecular level
(2) Report on the potential use of molecular modeling and the target toxicant paradigm for regulatory purposes, including a discussion of the OECD principles either as they currently exist or relative to molecular modeling specific principles

Keywords: molecular modeling, protein binding, toxicity prescreening, weak interactions

QA Project Plan: Category IV. The quality objectives for molecular modeling are to achieve the best balance between reasonable computational speed and model performance. Development is guided by a series of standard operating procedures. These govern all aspects of the project, including data acquisition, formatting, quality assurance, database filling and maintenance, and system administration. All QA plans are archived in the EPA internal QA system.

e. ToxCast™— Screening and Prioritization of Environmental Chemicals Based on Bioactivity Profiling and Predictions of Toxicity

Lead/Principal Investigator: Keith Houck

Research Issue/Relevance: The objective of the ToxCast™ research program developed by NCCT of EPA's ORD is to develop cost-effective innovative approaches to efficiently screen and prioritize a large number of chemicals for toxicological testing. Using data from state-of-the-art HTS bioassays developed by the pharmaceutical industry, ToxCast™ is building computational models to predict the potential human toxicity of chemicals. These hazard predictions should provide the Agency's regulatory programs with science-based information that will be helpful in setting priorities for more targeted toxicological evaluations that will help the Agency focus on those chemicals and end points with the greatest potential for causing adverse effects in humans. The ultimate goal of ToxCast™ is to deliver an affordable, efficient, science-based system for categorizing chemicals according to their predicted toxicities.

An essential component of the ToxCast™ research program is the development of a standardized, reference database containing animal toxicity studies called ToxRefDB.

ToxRefDB is being populated with the results of guideline animal toxicity studies on pesticidal active chemicals that are submitted to the Agency by manufacturers as a requirement of licensing a pesticide product. ToxRefDB is, for the first time, providing a searchable, mineable historical database for accessing a wealth of reference in vivo study data. Most importantly, ToxRefDB will provide the essential interpretive context to anchor ToxCast™ in vitro data (i.e., HTS and genomic data) to animal toxicity endpoints with selected ToxRef *in vivo* outcomes serving as the basis for developing predictive in vitro bioactivity profiles and signatures. Equally essential to the overall success of this project will be the development of a suitable informatics and analysis infrastructure for storing, relating, and extracting patterns from all data associated with the ToxCast™ project, including chemical, HTS, and in vivo data elements.

ToxCast™ databases and predictive models for the potential toxicity of environmental chemicals will be useful to EPA program offices for chemical prioritization. For example, OPP and the OPPT anticipates taking advantage of ToxCast™ models and datasets to prioritize in vivo animal testing of products that have limited toxicity data available such as:

- antimicrobial pesticides,
- inert ingredients in pesticide products,
- manufacturing process impurities,
- metabolites and environmental degradates of concern, and
- new and existing industrial chemicals.

After internal clearance and external peer review, the information in ToxRefDB and HTS data generated on the chemicals screened in ToxCast™ will be publicly available at www.epa.gov/ncct/toxrefdb and www.epa.gov/ncct/toxcast.

Purpose/Objective/Impact: The objective of the ToxCast™ research program is to develop a cost-effective and rapid approach for screening and prioritizing a large number of chemicals for toxicological testing (Dix et al., 2007). Using data from HTS bioassays developed in the drug discovery community, ToxCast™ is generating data, constructing databases, and building computational models to forecast the potential human toxicity of chemicals. These hazard predictions should provide EPA regulatory programs, including OPP, with science-based information helpful in prioritizing chemicals for more detailed toxicological evaluations, ultimately leading to reduced animal testing. ToxCast™ is currently in the PoC phase, wherein more than 300 chemicals have been assayed in more than 600 different HTS bioassays, creating rich bioactivity profiles for these chemicals. The Phase I chemicals are primarily conventional pesticide actives that have been extensively evaluated using traditional mammalian toxicity testing and, hence, have a number of well characterized toxicity outcomes (e.g., carcinogenicity; and developmental, reproductive, and neural toxicity). These in vivo data, in turn, have been extracted from the evaluations conducted by OPP scientists and were used to construct and populate ToxRefDB. Comparable toxicity data from other toxicity sources (e.g., NTP) also are being captured in ToxRefDB. A broader and more diverse set of complementary data on thousands of chemicals is being identified and collated in EPA's ACToR. ACToR (as well as the analysis component, ToxMiner) is providing the essential informatics infrastructure for housing, integrating, and analyzing all chemical and assay data associated with the ToxCast™ project, also in the context of a much larger world of Web-accessible chemical-toxicological information. DSSTox, in turn, is providing high-quality, standardized chemical structure indexing for ACToR and ToxCast™, including other high-interest Agency chemical data inventories.

ToxRefDB is critical to developing predictive signatures, because it links ToxCast™ HTS in vitro data to in vivo toxicity end points associated with the same chemicals. The toxicity data in ToxRefDB and the HTS data generated in ToxCast™ will be made publicly available through

EPA Web sites and databases. The first manuscript on ToxRefDB recently was published (Martin et al., 2008), presenting toxicity profiles from 2-year rodent bioassays on 310 chemicals. A similar analysis is nearing completion on multigeneration reproduction and prenatal developmental test data for the ToxCast™ chemicals in ToxRefDB, profiling the toxicity potential of this chemical set across generation, life stage, and different classes of end points.

ToxCast™ is a multiyear effort that is divided into three distinct phases:
- Phase I: 300 chemicals assayed in more than 600 different HTS bioassays, to create predictive bioactivity signatures based on the known toxicity of the chemicals;
- Phase II: focused on confirmation and expansion of ToxCast™ predictive signatures, generating HTS data for at least 300 additional chemicals; and
- Phase III: ToxCast™ expanded to thousands of environmental chemicals for which little toxicological information is available.

Once ToxCast™ has gone through successful initiation of Phase III, the data and toxicity predictions will be ready for deployment throughout numerous EPA program offices. NCCT will work to link these hazard predictions with exposure predictions and create integrated database analysis tools facilitating customized chemical prioritizations appropriate to specific programs.

Synopsis: The primary goals for ToxCast™ are the completion of Phase I data collection and, concurrently, development of the informatics infrastructure to store and analyze these data; derivation of predictive signatures from Phase I data and validation of these signatures with Phase II data; and the application of these predictions to the prioritization of chemicals in various chemical and nanomaterial testing programs. Success in meeting these four principal goals will lead to secondary applications in developing human toxicity pathway analyses, and in high-throughput risk assessments.

Partnerships/Collaborations (Internal & External):
- Tox21 with NTP and NCGC
- OECD Molecular Screening Project
- OECD Enhanced One-Generation Reproductive Test Guideline
- EPA/ORD/NHEERL DNT Team
- EPA/ORD/NCEA Phthalate Team
- MOUs and MTAs with over 20 external organizations collaborating on ToxCast™ assays, chemicals, and data analysis.

Milestones/Products:
FY09
(1) Completion of ToxCast™ Phase I data collection
(2) Provide annotated Phase I datasets for public access
(3) Derivation of predictive signatures from ToxCast™ Phase I data
(4) Multiple publications on ToxCast™ datasets
(5) Publication on signature generation (SCOUT item, April 2009)
(6) Publication on analysis of NR pathways and toxicity
(7) Convene first ToxCast™ Data Summit for identifying promising prediction models from intramural and extramural sources
(8) Finalize selection of chemicals for next 3 to 5 years of ToxCast™ and Tox21 projects
(9) Prioritize and order 4000 to 6000 chemicals in collaboration with other Tox21 partners

FY10

(1) Publications describing approaches to combining exposure, PK, and in vitro assays to do risk prioritization
(2) Evaluate compatibility of nanomaterials of diverse classes with ToxCast™ assays
(3) Prioritize and select assays to be run for ToxCast™ Phase II

FY11

(1) Confirmation of ToxCast™ predictive signatures with Phase II data
(2) Publications on signature confirmations and applications
(3) Completion of ToxCast™ Phase II data collection

FY12

Application of ToxCast™ predictions to the prioritization of chemicals in various EPA chemical and nanomaterial testing programs

Keywords: ToxCast™, high-throughput screening, hazard, predictive toxicology, chemical prioritization

QA Project Plan: Category I. ToxCast™ Quality Management Plan, includes: Information Management QA Project Plan, NCGC QA Project Plan (for IAG), and nine separate contractor QA plans and records. All QA plans are archived in the EPA internal QA system.

f. ExpoCast™—Exposure Science for Screening, Prioritization, and Toxicity Testing

Lead/Principal Investigator: Elaine Cohen Hubal

Research Issue/Relevance: High-visibility efforts in toxicity testing and computational toxicology raise important research questions and opportunities for exposure scientists. There is a clear need for a collaborative effort across the exposure and risk assessment community to ensure that the required exposure science, data, and tools are ready to address immediate needs resulting from application of high-throughput in vitro technologies for toxicity testing. A coherent program is required to formulate significant exposure questions posed by these novel in vitro toxicity data; develop creative approaches for applying existing exposure information and tools to address these questions; and, finally, identify key exposure research needs to interpret the toxicity data for risk assessment. The authors of the National Academies report (NRC, 2007) emphasize that population-based data and human exposure information are required at each step of their vision for toxicity testing, and that these exposure data will continue to play a critical role in both guiding the development and use of the toxicity information. Exposure research questions posed in this report include how to (1) use information on host susceptibility and real-world exposures to interpret and extrapolate in vitro test results, (2) use human exposure data to select doses for toxicity testing so information on biological effects pertains to environmentally relevant exposures and (3) relate human exposure data from biomonitoring surveys to concentrations that perturb toxicity pathways to identify biologically relevant exposures. NCCT has identified the need to include exposure information for chemical prioritization, modeling system response to chemical exposures across multiple levels of biological organization (through to the population level), and linking information on potential toxicity of environmental contaminants to real-world health outcomes (Cohen Hubal et al., 2008). Together, scientists from NCCT and NERL's Human Exposure Research Program have identified and will be conducting research in the following priority exposure research areas to support chemical screening, prioritization, and toxicity testing (Sheldon and Cohen Hubal, 2009): (1) accessible and linkable exposure databases, (2) exposure screening tools for accelerated chemical prioritization, (3) computational tools for dose reconstruction and source-to-outcome analyses, (4) tools for understanding the fundamental processes and factors influencing human exposures, and (5) efficient monitoring methods to measure and interpret biologically relevant

exposure metrics. Susceptibility, vulnerability, and life-stage aspects are integral to each of these research areas.

Purpose/Objective/Impact: The ExpoCast™ program is being initiated in FY09 to ensure the required exposure science and computational tools are ready to address global needs for rapid characterization of exposure potential arising from the manufacture and use of tens of thousands of chemicals and to meet challenges posed by new toxicity testing approaches. The overall goal of this project is to develop novel approaches and tools for screening, evaluating, and classifying chemicals based on the potential for biologically relevant human exposure, to inform prioritization and toxicity testing. An emphasis will be placed on conducting research to mine and translate scientific advances and tools in a broad range of fields to provide information that can be used to support enhanced exposure assessments for decisionmaking and improved environmental health. Advanced exposure databases, computational tools, and analysis approaches are required to prioritize chemicals, to design effective in vitro screening protocols, and to interpret the results of these screening tests for human health risk assessment. Approaches for integrating information on genetic susceptibility and life-stage and population-level vulnerabilities with in vitro toxicity data also are required to improve public-health decisionmaking. This initiative will advance Agency tools for efficiently characterizing and classifying chemicals based on potential for biologically relevant exposures. The improved exposure science and knowledge will subsequently inform the characterization of environmentally-relevant toxicity.

Synopsis of NCCT Directed Research: ExpoCast™ will provide an overarching framework for the science required to characterize biologically relevant exposure in support of the Agency computational toxicology program. Broadly and long term, the ExpoCast™ program will foster novel exposure science research to (1) inform chemical prioritization, (2) understand the systems response to chemical perturbations resulting from environmentally relevant exposures and how these translate to relevant biological changes at the individual and population levels, and (3) link information on potential toxicity of environmental contaminants to real-world health outcomes. The NCCT-directed aspects of the ExpoCast™ program will have a strong focus on research required to interpret and translate in vitro hazard data in the context of real-world exposures for risk assessment. Research will be conducted jointly with NERL to leverage expertise and resources required to meet objectives of this multidisciplinary project.

An important early component of ExpoCast™ will be to consider how best to consolidate and link human exposure and exposure factor data for chemical prioritization and toxicity testing. Under the ExpoCast™ program, NCCT and NERL scientists will collaboratively

- evaluate and recommend approaches for improving accessibility to EPA human exposure and exposure factor data and for facilitating links between exposure and toxicity data (e.g., through DSSTox and ACToR systems),
- advocate for the creation of a consolidated EPA exposure database focused on measured and predicted concentrations in exposure and biological media, and
- propose standards for human exposure data representation.

Early research activities will focus on identifying and evaluating novel approaches for characterizing exposure to prioritize chemicals and developing modeling approaches for considering exposure potential based on chemical properties, sources (e.g., consumer products), uses, life cycle, and individual and population vulnerability. Specific tasks pertinent to these goals include:

- analysis of extant exposure data to identify the critical metrics and develop simple indices for representing biologically-relevant personal exposure over time, place, lifestage, and lifestyle

or behavior.
- development of novel approaches for characterizing biologically-relevant exposure to prioritize chemicals, some examples follow:
  - application of residential models for prioritizing SVOCs,
  - development of dermal uptake model suite,
  - application of biomonitoring equivalent (BE) approach to interpret ToxCast™ data;
- development of human exposure knowledgebase; and
- application of genomic tools and other biomarkers of exposure and susceptibility to consider population-level vulnerabilities for toxicity testing and risk assessment.

NCCT Partnerships/Collaborations (Internal & External): The ExpoCast™ project will be conducted in close collaboration with the ToxCast™ program and through extensive collaboration with NERL principal investigators in the Human Exposure Research Program. Integration of research directed by the both NCCT and NERL will be enhanced by activities such as the Computational Toxicology Rotational Fellow Program. To jump start joint research activities, a NERL investigator will join the rotational fellows program in the summer of 2009 to focus on improving access to human exposure data. Partnerships with the other laboratories and centers also will be developed as the ExpoCast™ program advances. For example, collaboration with NHEERL on the MICA study is providing the opportunity to pilot advanced computational approaches for evaluating multifactorial biomarker data (including genomic data) across the exposure-outcome continuum to investigate the interplay of environmental and genetic factors on complex diseases.

External collaborations include research with Dr. John Little of Virginia Tech, to develop improved tools for rapidly predicting exposure associated with SVOCs used or emitted in the residential environment. Dr. Sean Hays and coworkers of Summit Toxicology will be exploring application of the BE approach for interpretation of ToxCast™ data. In a collaboration established through Bio-chem Redirect Program and implemented through the International Science and Technology Center (ISTC), Dr. Petr Nikitin of the Natural Science Center (NSC) of A.M. Prokhorov General Physics Institute, Russian Academy of Sciences is leading research to develop a multichannel immunosensor for detection of pyrethroids. The primary goal of this project is development of a biochip technology that avoids sophisticated labeling steps. This is an example of the type of research required to address needs for advanced exposure monitoring tools. In collaboration with the ICCA-LRI, we are participating in planning of a workshop focused on developing innovative tools to characterize biologically relevant environmental exposures and implication of these for health risks. Finally, in collaboration with the Environmental Bioinformatics STAR center at UMDNJ, we will be presenting a symposium at the ISES 2009 annual meeting.

Input to and feedback on ExpoCast™ will be solicited through the ExpoCoP to facilitate integration and collaboration across the broader scientific community. ExpoCoP includes representatives from the ORD laboratories and centers, Agency program offices, other Federal government agencies, academia, industry, and environmental advocacy groups. International representatives also participate in the ExpoCoP.

Milestones/Products:

FY09

(1) EHP paper with NERL, “Exposure as part of a systems approach for assessing risk”
(2) Tox. Sci. Forum paper “Biologically Relevant Exposure for Toxicity Testing”
(3) ExpoCoP monthly teleconference, ESC resource, face-to-face meeting at ISES 2009
(4) ICCA-LRI workshop, “Connecting Innovations in Biological, Exposure and Risk Sciences: Better Information for Better Decisions”

(5) SVOC workshop, “Semi-Volatile Organic Compounds (SVOCs) in the Residential Environment”
(6) ISES 2009 Annual Conference, “Transforming Exposure Science for the 21st Century”
(7) Symposium at ISES 2009, “Integrative Exposure Biology and Computational Toxicology for Risk Assessment”
(8) Survey and identify high-priority exposure data resources for initial chemical indexing in collaboration with ACToR and DSSTox
(9) ExpoCast™ conceptual framework and research plan

FY10

(1) Workshop to review and evaluate current exposure prioritization tools
(2) Position paper recommending standards for exposure data representation
(3) White paper defining exposure space and plan for assessing exposure data landscape
(4) White paper exploring development and application of human exposure knowledgebase
(5) Begin implementation of standards across exposure databases of highest interest and utility for NCCT projects

FY11

(1) Manuscript describing extant data analyses to identify critical determinants for exposure classification and chemical prioritization based on potential for exposure
(2) Guide incorporation and further development of simple exposure estimation tools within the ACToR system for use in prioritization

FY12

Apply exposure index for prioritization to subset of ToxCast compounds to evaluate concept.

Keywords (three to five): exposure science, chemical prioritization and toxicity testing, vulnerable populations, susceptibility

QA Project Plan: Category III. QA of modeling projects serves at least two overlapping goals: (1) Verification - Reproducibility of results is essential for the scientific method, and (2) Continuity Proper documentation of results allows future researchers (or the same researcher after a long period of time) to return to a project without excessive amounts of time spent understanding what was done before. All QA plans are archived in the EPA internal QA system.

g. v-Embryo™—Virtual Embryo

Lead/Principal Investigator: Thomas B. Knudsen, PhD

Research Issue/Relevance: Research Issue: The Virtual Embryo project (www.epa.gov/ncct/v-Embryo/) is motivated by scientific and regulatory needs to understand mechanisms of developmental toxicity. A key research issue is to model how the embryo reacts to environmental chemicals as a “complex system.” Navigating this complexity requires detailed knowledge of molecular embryology, data on cellular systems using HTS approaches, and computational models of network dynamics and multicellular function.

Morphogenesis entails a dynamic tissue flow that is driven by conserved cell signaling pathways and cellular reaction networks that follow these pathways during stimulus, mutation, or injury [1]. Our strategy is to modularize the embryo as a collection of tractable models that represent the cell as a computational unit [2]. In this strategy, the cell is treated as an autonomous agent that processes local signals and selects from a repertoire of core behaviors that include growth, differentiation, mitosis, apoptosis, migration, adhesion, and cell-shape changes. Specific rules for signal-response are programmed for molecular pathways, cellular dynamics, and unique

biology per morphogenetic system [3]. Sophisticated imaging techniques can reveal complex dynamics of cell-cell relationships and a ‘’morphogenetic blueprint” of early development [4].

Although much is known about molecular signaling networks that drive morphogenesis, considerably less is known about the nature of “higher-order” processes that control collective cellular behavior [3]. In complex systems, molecular networks can invoke higher level processes through signal-input and response-output relationships, as determined by the timing and function of signal strength and dynamic range [5]. We take this hypothesis in the context of environmental stressors to embryonic development. Understanding network-state relationships will be required to predict nonlinear dose-response relationships and when a breakdown of higher order control systems may occur [6]. Cell-based computational models have been used to predict emergent properties that arise from cooperative transactions of cell groups behaving as a self-regulating system [7]. Homeostasis, adaptation, and repair are a few examples of emergence in a perturbed system [8].

Relevance: A new strategy for developmental toxicity testing involves screening multiple chemicals through cell-based in vitro assays [1]. The goal is to build robust signatures of toxicity that translate into in vivo predictions [9]. Because tissues are more than a cumulative sum of individual cell behaviors, computational models can accelerate this effort [7]. A “virtual tissue” (VT) representation reconstructs a broad range of biological responses following cell-based rules and systems-level controls. VT models rapidly can sweep parameter space following chemical or genetic perturbation to predict aggregate cellular behaviors and higher order responses [10].

Purpose/Objective/Impact: Purpose: EPA’s Virtual Embryo (v-Embryo™) will comprise a knowledgebase (VT-KB) of relevant information and simulation engine (VT-SE) on the front end of the modeling software. Proof-of-principle is underway to explore the range of potential applications where comprehensive simulation is reliable and to find the limit in scale or stages where studying the real embryo is not cost effective. Computational models have been built by others for in silico reconstruction of chondrogenesis [11], gastrulation [12], angiogenesis [13], and somitogenesis [14]. These models were implemented as hybrid cellular automata using CC3D open-source tissue-simulation environment [www.CompuCell3D.org], which is being evaluated for the Virtual Embryo. Initial models will focus on specific morphogenetic systems that replay important concepts in experimental embryology and that are targets in developmental toxicity. The end goal is a library of computer-driven simulations that can be manipulated in silico and correlated with in vitro responses or in vivo phenotypes to predict developmental toxicity. Applications for developmental toxicity align with EPA’s strategy on the future of toxicity testing [15] and can leverage unique pathway-based data for numerous chemicals tested in mouse embryonic stem cells (mESC), free-living zebrafish (ZF) embryos, and ToxCast™ assays [16] to

simulate key signaling pathways, interlocking genetic networks and cellular dynamics in developing tissues;
model how embryonic cells react as agents to chemical exposure individually and collectively as a complex system;
analyze emergent behaviors and canalizing influences following stimulus, injury, and perturbation; and
understand how this complexity contributes to the differential susceptibility of embryonic tissues across chemical, dose, stage, genetic makeup, and time.

Objective: The main objective is to build data-rich models that can be used to analyze causal relationships during environmental and genetic perturbation. One initial prototype will focus on

the powerful sine oculis network that controls early eye development - conserved morphogenetic pathways [17], patterns of malformation and sensitivity to chemical perturbation [18]. There are many advantages of focusing on eye malformations and, specifically, the molecular events related to Pax6 leading to this end point: strong knowledgebase, relevant human phenotypes, and underlying genetic susceptibility and environmental sensitivity. The underlying molecular pathways and signaling networks scale to tissue-level developmental effects in many different systems. A second prototype will focus on the patterning systems controlling early limb-bud development. The specific objectives are as follows.

1. Build knowledgebase (VT-KB) and front-end simulation engine (VT-SE) for developmental processes and toxicities.

   Rationale: VT-KB is required to initially store gene-gene and gene-phenotype associations that will be used to provide the rules for cell-based modeling in the VT-SE. The framework will rely on information from the literature, data from national repositories (GO, EMAGE, GXD, MPO, ZFIN, and OMIM) and pathway analysis software. VT-KB design, development, and implementation are supported through ITS-ESE contract no. 68-W-04-005, task order no. 058: Technical Support for Development of Developmental Systems Toxicity Network (DevToxNet). Perl-scripts written for information extraction and assisted curation of relevant facts from scientific literature returned an output matrix loaded to MySQL. Initial application will build gene-gene and gene-phenotype associations during eye and limb development for rat, mouse, zebrafish, or human species. VT-SE comprises a front end to the modeling software (DDLab, CC3D, C++, Python, Blender3D, and GanttPV). An interactive tool is being developed with support from the Environmental Modeling and Visualization Laboratory under ITS-ESE contract no. 68-W-04-005, task order no. 02: Virtual Embryo: Simulation and Visualization Project Management Plan. Initial application of VT-SE is to construct a cell-based, network-driven model for lens-retina induction that reconstructs the cellular dynamics and morphogenetic blueprint of ocular dysmorphogenesis. This model can be quantitative in terms of the degree of severity of chemical-induced defects (graded dose response) and relative risk for incidence of responding embryos (quantal dose response).

2. Construct cell-based computational models for prototype morphogenetic processes and embryonic modules.

   Rationale: The second specific aim uses the VT-SE to model modular embryonic systems and specific morphogenetic events and their perturbation. The strategy will apply agent-based models (ABMs). Initial prototypes (optic cup and limb bud) have well-characterized signaling networks and differential susceptibility to chemical disruption; other systems will be added over time. Both small prototypes are organized by complex self-regulating networks of signal molecules commuted from cell-signaling centers and described mathematically as Turing gradients. Prevailing models entail reciprocal induction in which heterotypic interactions between presumptive lens epithelium and prospective neural retina lead to formation of the optic cup, and interactions between apical ectodermal ridge and underlying mesenchyme drive polarized outgrowth of the paddle-shaped limb bud. Both processes are organized by a self-regulating network of genes and signaling gradients (FGFs, BMPs, SHHs). Although the dual-reciprocating models set the stage for emergence of the optical neuraxis and appendicular skeleton, respectively, they do not explain higher order processes that control geometry and size of these rudiments, nor do they account for differential susceptibility to teratogens [18]. For this purpose, we propose the extended cellular large-Q Pott's model (CPM) implemented in CC3D [19] and managed with Python software as part of the VT-SE.

3. Specify rules for component interactions of developmental pathways at the cellar and molecular scales.

   Rationale: Network structures for regulatory pathways and cellular systems will be portrayed using a Boolean (on-off) formalism. A Boolean Network (BN) qualitatively captures system behavior probabilistically (PBN) or deterministically (DBN); the former is more biologically plausible, whereas the latter is a modeling tool of the whole process, which enables us to simulate, analyze, and manipulate different parts of the system. Both models incorporate rule-based dependencies for gene-gene and cell-cell interactions that can be built with information from the VT-KB. States of the network, as determined by wiring and rules, will be characterized using DDLab software, which identifies stable attractors in complex systems [20]. The attractor concept implies that a finite number of stable cell states exist in a complex system as pathways of differentiation or canalization. Order and timing have great importance for the predictive modeling of genetic errors or cellular disruptions in the embryo caused by phase-specific chemical effects. As such, the ability to introduce predefined or stochastic lesions will enhance the functionality of VT-SE. Virtual Embryo is building a prototype interface that manages the order and timing of gene expression and signaling events for Python-based components in the VT-SE. This tool is being developed under ITS-ESE contract no. 68-W-04-005, task order no. 02: Virtual Embryo—Simulation and Visualization.

4. Analyze abnormal developmental trajectories predicted from cellular MOAs that follow chemical perturbations.

   Rationale: A high-fidelity computer program that links cellular processes with network-level function can be evaluated for its capacity to evolve features not explicitly coded in the cell-based model. As noted above, this emergence is important for manifesting the response to genetic errors and cellular disruptions that are introduced to the model as targets of developmental toxicity, based on simulated and experimental data. Such a computational model can reveal an interaction of mechanisms at the cellular and molecular scales to produce emergent phenomena that manifest as abnormal developmental phenotypes [10]. Rules will derive from simulated data and semiarbitrary parameters in ocular or related systems because it will take a major experimental effort to model parameters kinetically for all relevant pathways, reactants, and interactions. Eventually, such information would be helpful to build a quantitative model. CC3D software advancements will be needed to implement molecular motors for core cell behaviors and to parallelize this implementation.

Impact: This research aims to improve mechanistic understanding and predictive modeling of developmental toxicity. Biological models that are simple enough so as to be computationally feasible (tractable) and yet complex enough to compute integrated cellular behaviors (rational) can reveal key events in multicellular organization, classify abnormal developmental trajectories from genetic network inference, and predict chemical dysmorphogenesis from pathway-level data. The initial focus on existing data, with use of the modeling effort to identify data gaps and to help guide the design of experiments for generating additional data as needed, can produce results that provide significant new information on likely dose-response and time-course behaviors of developmental toxicants. Most developmental modeling has been qualitative, showing the link between fundamental processes and morphogenesis. Virtual Embryo is moving to a different, quantitative level through knowledge of molecular embryology and pathway-level data from HTS efforts. That resource can have an impact on HTP hypothesis testing (parameter sweeps) to inform experimental design or to dry-run intractable experiments complicated by

time, scale, and cost (monetary and animal). Over the short term, we anticipate the work will draw greater attention to integrative thinking, the application of computational models to understand mechanisms, and approaches for uncertainty analysis and understanding how large uncertainties about parameter values will affect quantitative prediction. Expanding the prototype models to broader representation of stages, tissues and species will be an intermediate step toward the more visionary reconstruction of a “Virtual Embryo.”

Synopsis: Motivation of the Virtual Embryo is scientific needs to understand mechanisms of toxicity and predict developmental defects from complex datasets. The research goal is to simulate embryonic tissues reacting to perturbation across chemical class, system, stage, genetic makeup, dose, and time. Data input is detailed knowledge of molecular embryology, high-throughput data from in vitro models, signaling pathways, and cellular phenotypes. Output models aim for the modular reconstruction of a developing embryo from cell-based models of morphogenesis and differentiation.

Milestones/Products:
FY09-10
(1) Project Plan: Category III QAPP
(2) Recruit: postdoctoral fellow
(3) Manuscript: application of VT-KB to analyze ToxRefDB developmental toxicity studies
(4) Model: VT-KB based qualitative (structural) model of self-regulating ocular gene network
(5) Model: VT-SE based cell-based computational model of lens-retina induction
(6) Manuscript: ocular morphogenesis, gene network inference, analysis, and modeling
FY10-11
(1) Project plan: extend lens-retina model to other stages and species
(2) Model: incorporate pathway data from ToxCast™, mESC, and ZF embryos
(3) Manuscript: sensitivity analysis for developmental trajectories and phenotypes
(4) Project plan: integrate with other morphogenetic models (ES cells, Zfish)
FY11-12
(1) Manuscript: test model against predictions for pathway-based dose-response relationship
(2) Manuscript: uncertainty analysis of models for complex systems
(3) Model: computer program of early eye development, using rules-based architecture, cell-based simulators, and systems-wiring diagrams

Keywords: embryo development, systems biology, computational modeling

QA Project Plan: Category III. The proposed designation for Virtual Embryo is Quality Assurance Category III. This designation recognizes its origin as a basic research project (Category IV) that is moving into proof of concept phase (Category III). A Virtual Embryo Quality Management Plan (QMP) will be constructed as the project moves into the proof of concept phase.

Phase-I (development): first-generation ABMs based on the small prototype systems of lens induction and polarized limb outgrowth (2009-10).

Phase-II (evaluation): sensitivity analysis using data for ToxCast™ chemicals that disrupt eye and/or limb development or Tox21 assays of relevant signaling pathways (2010-11).

Phase-III (expansion): uncertainty analysis of quantitative models that simulate reaction to perturbation across chemical, system, stage, genetic makeup, dose, and time (2011-12).

The anticipated Date of Elevation to Category-II is 2012. This is based on the premise that research enabled by these models will reduce uncertainty in risk assessment for prenatal developmental toxicity through understanding of complex mechanisms of environmental chemicals and their impact on complex developing systems. We also anticipate a successful Virtual Embryo in the long-term can reduce the reliance on animal testing for prenatal developmental toxicity. Many of the 10,000 chemicals with which EPA is concerned do not have such information available.

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h. v-Liver™ —The Virtual Liver Project

Lead/Principal Investigator: Imran Shah

Research Issue: The Virtual Liver project (http://www.epa.gov/ncct/virtual_liver) is aimed at providing decision support tools for evaluating chemical-induced adverse liver outcomes across chemicals, doses, and species using in vitro data. Considering nuclear-receptor (NR)-mediated liver cancer as an archetypal chronic adverse outcome, we focus on the research issues: Which molecular circuits and cellular states altered by chemicals lead to cell damage, death, and proliferation? How are these cellular perturbations propagated across tissues as lesions? How can we organize this complexity computationally to develop Virtual Tissues?

Two important perturbed cellular phenotypes, or states, in carcinogensis are (1) initiation, in which chemical mutagens cause DNA damage rendering a cell resistant to apoptosis, inhibition of cell proliferation; and (2) promotion, in which mitogenic signals persistently stimulate the initiated cell creating focal proliferation. Increasing evidence suggests that the NR superfamily mediates rodent hepatocarcinogenesis for a number of environmental chemicals (Butler, 1996). For example, di(2-ethylhexyl)-phthalate (DEHP) and perfluorooctanoic acid (PFOA) are PPAR-α activators (Maloney and Waxman, 1999); whereas pesticides like conazoles and pyrethroidsactivate are either PXR or both PXR and CAR (Kretschmer and Baldwin, 2005). The role of NR-mediated activity in molecular circuits is being explored actively through genomic profiling, and the dose-dependence of specific molecular switches is being assayed across hundreds of environmental chemicals in ToxCast™.

Propagating cellular alterations spatially requires information flow between cells, which normally occurs on a backdrop of microanatomic spatial zones with heterogenous levels of nutrients and distinct spatial distribution of intracellular states (Pette and Wimmer 1979; Oinonen et al. 1998). The microanatomic distribution of xenobiotics causes zonal alterations in cell states (Kato et al., 2001) that can progress to cell injury and even death. Hepatocyte (HC) death stimulates neighbouring cells to replicate (regenerative proliferation). Necrotic death also can lead to Kupffer cell (KC) activation, migration, and release of inflammatory cytokines, which locally can accelerate cell injury. There is evidence for such HC-KC interactions in PPAR-α mediated hepatotoxicity and cancer (Rusyn, 1998; Roberts, 2007). Mitogens also have been shown to disrupt gap junction communication between cells (Krutovskikh et al., 1995), which can reduce their homeostatic capacity. Advanced imaging, histomorphometry (HMP), and molecular assays are making it feasible to extract local information on cells in a microanatomic context.

To computationally model this level of biological complexity requires some simplifying assumptions about the modular organization of physiologic events across scales. We

hypothesize a cell-oriented abstraction for developing “Virtual Tissues” with the following assumptions: (a) tissues can be represented as a complex cellular system, (b) cells are the unit of function and can be modeled as autonomous agents that use molecular circuits to make decisions, and (c) injury is a collective response of the multiagent system to persistent stress.

Relevance: Current approaches for assessing the risk of adverse effects in humans are based on animal testing, which is time consuming, resource intensive, and fraught with uncertainty. Novel strategies are necessary to efficiently and effectively evaluate the risk of thousands of environmental chemicals. Integrative computational systems, in vitro models, and assays offer an avenue for more cost-effective and humane alternatives for the future of toxicity testing. Liver toxicity is a frequent outcome in rodent testing, and it is difficult to evaluate its relevance in humans.

Purpose: The v-Liver™ will provide in silico decision support tools to analyze the MOA in light of available data and prior knowledge, and quantitatively simulate an MOA at environmentally relevant tissue doses. This will inform/evolve biologically based dose-response models to include more relevant physiologic details necessary for predicting the human risk of injury at low doses.

Objective: The primary objective is to develop an integrated in silico/in vitro framework that aids intelligent hypothesis generation about the plausible sequence of molecular, cellular, and tissue events perturbed by a test chemical, and quantitatively simulates the risk of these events in humans at environmentally relevant tissue doses using in vitro data. The v-Liver™ PoC will focus on a subset of 20 environmental chemicals with known rodent toxicity in ToxRefDB and in vitro data in ToxCast ™. There are two specific goals of the PoC:, which are described below.

I. The v-Liver™ Knowledgebase

Knowledgebased, or semantic, approaches (Karp, 2001) are important for computationally modeling incomplete and evolving insight on complex processes. They enable integration of disparate biological information from literature, omic data, or pathway databases at different scales into coherent computable representation that is flexible, extensible, and transparent. A more important advantage of semantic approaches is their support for automated reasoning, which is important for inferring plausible sequences of events perturbed by new chemicals. Large-scale knowledgebased approaches have become feasible because of semantic Web technology and available ontologies for different levels of biological organization. The v-Liver™ Knowledgebase (v-Liver-KB) will represent normal hepatic functions and their perturbation by chemical stressors into pathophysiologic states using description logic expressed in OWL and stored in the Sesame semantic repository. To facilitate the construction of the v-Liver-KB, a Cytoscape plug in is being developed to synthesize information from different biological databases into OWL using a custom ontology. This system also supports SPARQL-based queries, interactive visualization, and information export in RDF. Information about the 20 PoC chemicals will be represented in the KB at multiple biological levels describing events and causal relationships between these events based on evidence from experiments or the literature. The main outputs of the KB will be

(1) a computable logical description of the molecular circuits and cellular states involved in normal hepotocyte and Kupffer cell function based on literature;
(2) a computable logical description of perturbations in molecular circuits and cell states due to by test chemicals;
(3) interactive web-based tools to browse and interactively query/explore the v-Liver-KB to analyze alternative MOA in light of HTS, *omic* or other cell based assays; and

(4) intelligent inference tools to explore alternative pathways perturbed by chemicals based on existing information on partial orders in the KB.

II. The v-Liver™ Simulator (Sim)

The spatial model of the hepatic lobule will be developed using a multi-agent system (MAS) (Axelrod, 1997; Epstein and Axtell, 1996; Athale et al., 2005) in which hepatocytes and Kupffer cells will be modeled as autonomous agents. Information on molecular circuits in the KB will be used to describe chemical-induced molecular perturbations of NRs namely, CAR, PXR, and PPAR-α. We are developing a variation of Probabilistic Boolean Networks (Kauffman, 1993; Shmulevich et al. 2002) to describe the dynamics of individual agent decisions regarding state of stress, injury, cell cycle progression, apoptosis, necrosis, or migration (KC). These will be augmented and calibrated using available literature and/or ToxCast™ data on the PoC chemicals. We believe this work will advance the two-stage clonal growth models of cancer (Conolly, and Andersen, 1997) by including relevant information on molecular pathways and cell communication. The agent population initially will be situated in a 2-dimensional regular spatial grid to model in vitro conditions and a simplified cross-section through the hepatic lobule. Portal-to-centrilobular blood flow initially will be represented as a gradient of nutrients and xenobiotics (estimated from organ dose), which can be extended to model more complex flows if necessary. Simulating the MAS will generate a spatial distribution of cellular alterations that can be interpreted as tissue lesions. Hence, the v-Liver™ Simulator (v-Liver-Sim) will dynamically simulate the key molecular and cellular perturbations leading to adverse effects in hepatic tissues. The predictions will be evaluated for the PoC chemicals using ToxCast™ data. The main outputs of the v-Liver™ Simulator are

- a large-scale tissue simulation engine to enable quantitative exploration of alternative physiologic processes and their histopathologic outcomes.
- a computational interface to integrate the tissue simulator with a PBPK modeling system to investigate individual exposure / population variability.
- interactive tools to communicate with the simulation engine and to visualize results of simulations across chemicals, MOAs, doses and species.

Impact: The v-Liver™ will impact the future of toxicity testing by providing computational tools to explore the MOA for new environmental chemicals using background knowledge, chemical structure, and/or in vitro assays and to provide an initial assessment of hepatic lesion formation. Focusing on 20 NR activating environmental chemicals and their hepatic lesions through a subset of molecular pathways will demonstrate the v-Liver ™ PoC. The project also is expected to contribute to ongoing assessments of pesticides and persistent toxics by providing useful information about the human relevance of any liver effects and their putative dose-response. If successful, the Virtual Tissues will be able to leverage available screening data from ToxCast™; fill any data gaps with targeted studies; and reduce the time, the cost, and the requirement for as many animal studies.

Synopsis: The v-Liver™ computational paradigm represents tissues as cellular systems in which discrete individual cell-level responses give rise to complex physiologic outcomes. In this model, cell-level responses are governed by a self-regulating network of normal molecular processes, and adverse histopathologic effects arise because of chronic stimulation by environmental chemicals. The v-Liver™ PoC is being developed by focusing on environmental chemicals responsible for hepatocarcinogenesis in rodent studies, organizing MOA knowledge on the relevant molecular and cellular processes perturbed by these chemicals, developing a tissue simulation platform to investigate the uncertainties in MOA and neoplastic lesion

formation, and evaluating in vitro assays to predict lesion development across chemicals and doses.

Partnerships/Collaborations (Internal & External): EPA Collaborations: NCCT ToxCast™ in vitro assays and their linkage with physiologic outcomes, NCCT v-Embryo™ and cell-level modeling of tissue responses, NCCT/NHEERL/PBPK modeling to infer internal dose, NHEERL Genomics Core on MOA for PPAR-α activators, and NHEERL PK Branch on hepatic xenobiotic and T3/T4 metabolism. External Collaborators: UNC Carolina Center for Computational Toxicology; UMDNJ PBPK Modeling.

Milestones/Products:

FY09

(1) Prioritize PoC environmental chemicals with clients
(2) KB: Information about PoC chemicals using ToxCast assays
(3) KB: Cytoscape KB visualization and analysis tool
(4) Cell Response: Initial molecular circuit describing hepatic cell functions
(5) Tissue Simulator: Develop and use MAS framework

FY10

(1) Tissue Simulator: Test liver lesions formation
(2) Integrate molecular circuits for MOA chemicals in tissues
(3) Evaluate simulator using PoC chemicals and ToxCast™ data to predict outcomes

FY11

(1) KB inference tool for analyzing MOA for new chemicals and mixtures
(2) Extend lobule simulator to liver and integrate with PBPK model

FY12

(1) Evaluate impact of genomic variation on cellular responses and lesion formation
(2) Evaluate v-Liver™ for simulating human pathology outcomes using clinical data

Keywords: virtual tissues, knowledgebases, MOA, dose-response modeling, nuclear receptors mediated hepatocarcinogenesis

QA Project Plan: Category III. QA of modeling projects serves at least two overlapping goals: (1) Verification reproducibility of results is essential for the scientific method, and (2) continuity **-** proper documentation of results allows future researchers (or the same researcher after a long period of time) to return to a project without excessive amounts of time spent understanding what was done before. Because the v-Liver™ project requires multiple researchers working over several years, ensuring both continuity of modeling efforts and reproducibility of modeling results is vital. All QA plans are archived in the EPA internal QA system.

i. Uncertainty —Analysis in Toxicological Modeling

Lead/Principal Investigator: R. Woodrow Setzer

Research Issue/Relevance: The analysis of uncertainty in toxicological modeling is critical to EPA because the Agency is increasing its use of toxicological models in regulatory decisions, and any use of model predictions in a rational decision process must consider the uncertainty of those predictions. The recent National Academy report on risk assessment activities in EPA emphasizes the importance of incorporating a quantification of uncertainty in risk assessments.

In the context of models, the easiest form of uncertainty to address is that about parameter values, assuming we have the correct model. This is the sort of uncertainty that statistical

methodologies were designed to estimate and is typically quantified through confidence intervals or probability distributions. However, we are rarely completely confident about the models we use, and often, the uncertainty about the underlying processes taking place or the best way to characterize those processes in a model can be quite substantial. This form of uncertainty is prevalent throughout dose-response analysis, from simple empirical modeling used in benchmark dose analysis, to pathway modeling used in virtual tissues, and is called model uncertainty.

Both forms of uncertainty are quantified through comparisons of models with data, by quantifying the degree to which different parameter values for a given model, and the best-fitting parameter values among different models, yield model predictions that are consistent with the data. “Consistency” is quantified through the mediation of an additional statistical model that relates the biological model to the data by describing the variability in the data as it is affected by the biological model.

In principle, this process is straightforward, and there are standard statistical procedures for carrying out the process. However, toxicological models comprise a wide array of modeling techniques, for example, simple algebraic expressions and systems of ordinary or partial differential or difference equations, and may involve agent-based or stochastic process models. Such models can be quite complex, with many parameters whose values are known with varying degrees of uncertainty. Statistical models usually need to accommodate multiple hierarchical levels of variability: variation among studies and among individuals, in addition to the usual measurement error, which should be allowed to differ among studies and end points. Information for estimating parameters and evaluating models themselves may come from well-characterized experimental data, as well as from tabulated (for example, physiological parameters like organ weights) or computed (for example, computed partition coefficients for a PBPK model).

Thus, despite the existence of sound statistical theory, the application of good statistical practice for these models can be difficult, requiring thoughtful application of both statistical and computational expertise and quite a bit of “art” to get good results. The key challenges in uncertainty analysis for toxicological models in risk assessment are to develop computationally efficient, transparent, and statistically valid approaches that may be implemented without the development of extensive case-specific programming.

Purpose/Objective/Impact: The objective of this project is to develop tools and best practices to facilitate the quantification of uncertainty in toxicological models. Early efforts will focus on PBPK models, specifically models being developed as part of a joint project between the Agency’s ORD and OPP to develop a cumulative risk assessment for the pyrethroid pesticides. However, similar methods are applicable to other forms of toxicological models, and examples will be pursued in the virtual tissue projects in NCCT and in evaluating uncertainty in the ToxCast™ predictors.

In recent years, it has become clear that Bayesian statistical methods are ideal for estimating parameters for PBPK models. For three reasons: (1) the ease with which partial information about parameters is included, through informative priors; (2) the ease with which hierarchical models are constructed; and (3) the fact that, as long as informative priors are used, lack of identifiability of model parameters, which is not generally possible to diagnose a priori, is not an impediment to completing a valid analysis (failure of identifiability of parameters may be diagnosed from analyses of the posterior parameter distribution).

The same logic should apply to other toxicological models. Thus, this project will center on tools to apply Bayesian methods to such models.

More specific objectives of this project are those that follow.

- Develop the computational tools to carry out Bayesian analyses of large dynamic models as efficiently as possible. The standard approach to evaluating the posterior in a Bayesian analysis of complex models is to generate random samples from the distribution by Markov Chain Monte Carlo (MCMC) methods. For models that are expensive to compute, such as those (e.g., PBPK models) expressed as solutions to systems of differential equations, an implementation that takes advantage of cluster computing may take advantage of some parallel structures in the problem. This objective will develop a standard computational approach to parallelizing such problems and will explore alternative approaches to implementing MCMC methods, with an eye towards computational efficiency. This objective also includes the development of modeling tools to facilitate dynamic models in the statistical language R.
- Describe a language specifically for expressing PBPK models. The language should be extensible, be capable of incorporating systems models expressed in SBML, and should use semantics specific to PBPK models to facilitate model checking. The language would be ideal as a way to archive PBPK models and as the definitive way to communicate PBPK models in the literature.
- Adapt statistical model evaluation approaches to complex toxicological models, and develop examples to demonstrate the behavior of different model evaluation methodologies in the face of various model failures.
- Develop a general approach to developing priors for chemical-specific PBPK model parameters. There are already methods for computing chemical-specific parameters either from physical chemical properties or in vitro assays (depending on the parameter). For this objective, datasets of measured chemical-specific parameters will be compared to values predicted from computational or in vitro methods. Statistical methods, such as regression will be used to adjust the predictions and the variance about the resulting regression, lines used to characterize the prior uncertainty about such predictions.
- Develop approaches for quantifying uncertainty of ToxCast™ like predictors involving HTS data as inputs, explicitly evaluating the importance of variability and design of HTS assays on prediction and prioritization uncertainty.
- Identify examples for parameter estimation, model evaluation, and overall uncertainty analysis drawn from PBPK models for pyrethroid pesticides and the encompassing cumulative risk analysis being conducted in collaboration with NHEERL, NERL, and OPP; selecting among molecular and cellular models used in developing a virtual liver model, and others developed in collaboration with the virtual tissues projects in NCCT.

Synopsis: This plan will target three areas: (1) standardizing and making more efficient computational approaches for parameter estimation and model selection; (2) standardizing approaches for model evaluation for PBPK and other dynamic models; and (3) development of methods for constructing priors (probabilistic summaries of current knowledge) for model parameters, based on existing computational methods and datasets. The initial motivation for this work was the need to standardize and make more sophisticated parameter estimation and model evaluation for PBPK models, and that emphasis will continue in the early phase of this project. However, all models relevant to toxicological risk assessment have similar requirements, and this project will coordinate closely with the virtual tissue and dose-response projects.

Partnerships/Collaborations (Internal & External): Internal: Jimena Davis (postdoctoral Fellow); Richard Judson, John Wambaugh, Imran Shah, and Thomas Knudsen.
External: ORD/NHEERL: Mike Hughes, Kevin Crofton, Tim Shafer, Ginger Moser, Rory Conolly; ORD/NERL: Rogelio Tornero, Valerie Zartarian, Xianping Xue; OPPTS/OPP: Anna Lowit, David Miller, Ed Scollon; and NIEHS/NTP: Mike DeVito.

Milestones/Products:

FY09

(1) Contribution to 2009 SOT CED course, "Uncertainty and Variability in PBPK Models"
(2) Submission of manuscript(s) on improvement of computational efficiency in Bayesian analyses of PBPK models using MCMC, and assessment of convergence (Wambaugh, Davis, Garcia, and Setzer)
(3) Submission of manuscript(s) on assessing fit of PBPK models (and, through example, other complex mechanistic models) to data, whether the models are "fit" to the data using Bayesian or other methods are parameterized a priori from in vitro data (Wambaugh, Davis, Garcia, and Setzer)
(4) Submission of manuscript(s) reviewing approaches for constructing priors for PBPK model parameters (i.e., establishing a priori estimates for model parameters in advance of using in vivo PK data, with a characterization of uncertainty) (Davis, Setzer, Tornero, and DeVito)
(5) Draft manuscript(s) on the estimation of model parameters and comparison of alternative model forms for a PBPK model for permethrin (in preparation of SAP review in FY10) (Davis, Setzer, Tornero, and DeVito)
(6) Draft manuscript(s) on global sensitivity analysis for exposure-dose model for permethrin, in preparation for SAP review in FY10. (Davis, Setzer, Tornero, Zartarian, and Chiu)
(7) Estimation of PBPK parameters for deltamethrin and two other pyrethroids complete Davis, Setzer, and Tornero)

FY10

(1) Manuscript on permethrin PBPK parameter estimation and model comparison submitted
(2) Manuscript on global sensitivity analysis for permethrin exposure-dose model submitted
(3) SAP review of permethrin PBPK exposure-dose model
(4) Submission of manuscript on parameter estimation for deltamethrin and two other pyrethroids
(5) Completion of exposure-dose-effect model for "mini-cumulative" risk assessment and draft document for SAP review in early FY11, including global "exposure to effect" sensitivity analysis (Davis, Setzer, Tornero, DeVito, Crofton, Shafer, Lowit, Scollon, Miller, etc.)
(6) Substantial completion of modeling and uncertainty analysis for v-Liver™ (Setzer, Shah, and Wambaugh)
(7) R package "RDynamic", for simplifying dynamic modeling in the statistical language R, submitted to CRAN
(8) Description of ontologically aware PBPK language working name "SemanticPK" drafted, and translator to R completed (using code developed for RDynamic)
(9) Discussion of uncertainty about ToxCast™ phase I predictions at 2010 SOT.
(10) Problem identification and early stages of evaluating alternative pathway formulations in v-Liver™ model (Setzer, Shah, and Wambaugh).

FY11

(1) Problem identification, timelines, and early stages of evaluating alternative model formulation for BBDR and v-embryo™ models completed (Setzer, Conolly, and Knudsen
(2) SAP on "mini-cumulative" risk assessment for pyrethroids
(3) Preparation for SAP for cumulative risk assessment for pyrethroids
(4) Publication of virtual tissue and BBDR modeling results
(5) Initial steps in constructing test datasets for analysis "competition" recommended by UVPKM

(6) Submission of manuscript on “RDynamic” to the *Journal of Statistical Software*

FY12

(1) Submission of manuscript on “SemanticPK”
(2) R package “RDynamic”, for simplifying dynamic modeling in the statistical language R, submitted to CRAN

Keywords: uncertainty analysis, physiologically based pharmacokinetic models, statistics, Bayes methods, priori, Markov Chain Monte Carlo

QA Project Plan: Category III. This project is a Category III QA category because of the significance of the pyrethroid cumulative risk assessment to which this plan contributes. Several quality objectives apply to this project (1) Computer code developed for the project must faithfully execute the intent of the code, whether that intent is described mathematically or in terms of other software (for example, implementations of the same model in different programming languages must give identical results for identical inputs); (2) distribution versions of software packages must be installable and useable by a reasonably sophisticated person; (3) analyses must be transparent: It must be possible to replicate all analyses from the archived files and information; and (4) data sources must be transparent; in particular, data from the literature must be annotated to be adequate for purpose, and extent of literature searches must be documented. All QA plans are archived in the EPA internal QA system.

IV. APPENDICES (cont'd.) - 2. Project Outcomes

2. Project Outcomes - Providing High-Throughput Computational Tools for the Identification of Chemical Exposure, Hazard, and Risk

Project Title	Outcomes FY09	Outcomes FY10	Outcomes FY11	Outcomes FY12	Expected Impacts
ACToR – Aggregated Computational Toxicology Resource	• Initial public deployment. • Significant version 2, including refined chemical structure information • Develop workflow for tabularization of data buried in text reports • Integrate all ToxCast and ToxRefDB data • Quarterly releases with new data	• Quarterly releases with new data • Implementation of a process to gather tabular data on priority chemicals from text reports • Survey sources of chemical use and exposure data and import any remaining sources • Develop flexible query interface and data download process • Develop process to extract data from open literature	• Quarterly releases with new data	• Quarterly releases with new data	Enables access to cross-chemical data by EPA program offices, NCCT, and other ORD organizations and external stakeholders. Improves EPA data transparency.
DSSTox- Chemical Information Technologies in Support of Toxicology Modeling	• MS: SAR Perspective of ToxCast 320 chemical inventory • MS(s): NCBI (GEO) and EBI (ArrayExpress) structure annotations and linkages to microarray data • Restart Chemoinformatics CoP • Publish files for ToxReDB and ToxCast inventories and selected summary end points, and facilitate publication and linkage to PubChem • Publish public genetic toxicity data and SAR predictions for ToxCast 320 • Continue expansion of DSSTox public toxicity database inventory. • Primary chemical review and structure annotation of ToxCast/Tox21 libraries within a central registry	• Publish files for Tox21 inventory and selected summary end points and facilitate linkage to PubChem • Publish files for NTP study areas • Explore new approaches to SAR based on feature categories • Expand CEBS collaboration to incorporate DSSTox GEO and ArrayExpress files and create chemical linkage to ILSI Developmental Toxicity database • Assist efforts within ExpoCast regarding chemically annotation	• Establish procedures and protocols for automating chemical annotation of new experimental data generated by NCCT and in collaboration with CEBS or NHEERL • Document and employ PubChem analysis tools in relation to published DSSTox and ToxCast data • Collaborate with SAR modeling efforts to predict ToxCast end points • Continue expansion of DSSTox public toxicity database inventory for use in modeling with co-publication and linkage to ACToR and PubChem	• Redesign DSSTox Website to provide hosting of donated chemical descriptors, properties, and predictions • Publish master tables of DSSTox IDs and high quality structures • Promote use of chemical registry system from ToxCast/Tox21 more broadly within EPA • Collaborate with SAR modeling efforts to expand modeling to address Tox21 chemicals and endpoints • Continue expansion of DSSTox public toxicity database inventory into new toxicity and exposure areas	Adoption of DSSTox chemical standards more broadly within EPA and across other government Agencies to improve quality and read-across capabilities. Promote public data dissemination and encourage greater public participation of industry and commercial sources in public toxicity database and modeling efforts. Facilitate improved toxicity prediction models and data mining capabilities across wider span of end points and chemicals impacting hazard ID and risk assessment.

IV. APPENDICES (cont'd.) - 2. Project Outcomes

2. Project Outcomes - Providing High-Throughput Computational Tools for the Identification of Chemical Exposure, Hazard, and Risk

Project Title	Outcomes FY09	Outcomes FY10	Outcomes FY11	Outcomes FY12	Expected Impacts
ToxRefDB-Toxicity Reference Database	• MS(s): Chronic/cancer, multigenerational and developmental modules • Release of stand-alone data entry tool • ToxRefDB Webpage online. • Collection of ToxCast Phase II chemical toxicity data • Public release of ToxRefDB Web-based query tool • Complete entry of targeted set of chemicals and study types for Phase II of ToxCast • Complete reproductive toxicity study retrospective analysis	• Quarterly releases with new data in conjunction with ACToR (Q1,Q2,Q3,Q4) • Implementation of a process to gather and enter open literature studies (Q1) • Expansion of ToxRefDB to capture DNT studies and EDSP data (Q1) • Complete retrospective analyses on other major study types (Q2) • Release of ToxRefDB live data entry tool	• Quarterly releases with new data in conjunction with ACToR	• Quarterly releases with new data in conjunction with ACToR	Enables access of traditional toxicological data in a structured and computable format extending the utility of the data beyond chemical risk assessment and into broad research applications, including guiding novel toxicity characterization methods and transparent data-driven retrospective analyses leading to refined animal use, a more predictive toxicology paradigm, and more efficient chemical safety assessments.
ChemModel -The Application of Molecular Modeling to Assessing Chemical Toxicity	• MS: Capability of the target-toxicant paradigm to identify chemicals that binds weakly to the estrogen receptor, including a description of the method • Description of the library of 148 biological macromolecule targets • MS: Molecular modeling studies of the potential biological effects of the perfluoro compounds	• MS: The metabolism of pyrethroids and the effects of three-dimensional chemical structure • Description of additional targets added to the target library • MS: The interaction of ToxCast chemicals with nuclear receptor targets and the importance of pharmacophore filters	• MS: The integration of results from the target library and available experimental parameters. • MS: The comparison of results with and without pharmacophore filters for ToxCast chemicals	• Comparison of results using the target library with experimental determined activities • MS: The use of molecular modeling and the target toxicant paradigm for regulatory purposes, including a discussion of the OECD principles relative to molecular modeling specific principles	An approach will be provided to prioritize chemicals for their ability to influence the endocrine system by competing with natural ligands for the binding sites of receptors. The application of a method used to find strong-acting drug-like chemicals will be evaluated for its capability of discovering weakly active chemicals. The capability of these methods will be used for finding weakly active chemicals. Parameters based on the capacity of a chemical to interact with macromolecular targets for toxicity will be available for applications of computational methods to screen for or, eventually, predict chemical toxicity.

IV. APPENDICES (cont'd.) - 2. Project Outcomes

2. Project Outcomes - Providing High-Throughput Computational Tools for the Identification of Chemical Exposure, Hazard, and Risk

Project Title	Outcomes FY09	Outcomes FY10	Outcomes FY11	Outcomes FY12	Expected Impacts
ToxCast™-Screening and Prioritization of Environmental Chemicals Based on Bioactivity Profiling and Predictions of Toxicity	• Completion of ToxCast Phase I • Provide Phase I data sets to public • Derivation of predictive signatures from ToxCast Phase I data • MS(s): ToxCast Phase I data sets • MS: Signature generation • MS: NR pathways and toxicity • Convene first ToxCast Data Summit for identifying prediction models • Finalize selection of chemicals for Phase II of ToxCast and Tox21	• MS(s): Describing approaches to combining exposure, PK, and in vitro assays to do risk prioritization • Evaluate compatibility of nanomaterials of diverse classes with ToxCast assays • Prioritize and select assays to be run for ToxCast Phase II	• Completion of ToxCast Phase II data collection • Confirmation of ToxCast predictive signatures with Phase II data • MS(s): Signature confirmations and applications	• MS(s): Profiling of large chemical sets for potential for hazard	Program offices will have tools to prioritize chemicals for targeted toxicity testing and insight on potential mechanisms of toxicity.
ExpoCast™: Exposure Science for Screening, Prioritization, and Toxicity Testing	• ExpoCoP monthly teleconference, ESC resource, face-to-face meeting at ISES 2009 • MS(s): EHP and ToxSci on role of exposure in the transforming toxicology • SVOC workshop, "Semi-Volatile Organic Compounds (SVOCs) in the Residential Environment" • Survey and identify high priority exposure data resources for initial chemical indexing in collaboration with ACToR and DSSTox • ExpoCast conceptual framework and research plan	• Position paper recommending standards for exposure data representation • White paper defining exposure space and plan for assessing exposure data landscape • White paper exploring development and application of human exposure knowledge base • Begin implementation of standards across exposure databases of highest interest and utility for NCCT projects • Award contracts	• MS: Describing extant data analyses to identify critical determinants for exposure classification and chemical prioritization based on potential for exposure • Guide incorporation and further development of simple exposure estimation tools within the ACToR system for use in prioritization	• Apply exposure index for prioritization to subset of ToxCast compounds to evaluate concept	Advance Agency tools for efficiently characterizing and classifying chemicals based on potential for biologically relevant exposure and inform characterization of environmentally relevant toxicity.

IV. APPENDICES (cont'd.) - 2. Project Outcomes

2. Project Outcomes - Providing High-Throughput Computational Tools for the Identification of Chemical Exposure, Hazard, and Risk

Project Title	Outcomes FY09	Outcomes FY10	Outcomes FY11	Outcomes FY12	Expected Impacts
v-Embryo™-Virtual Embryo	• MS: Application of VT-KB to analyze ToxRefDB developmental toxicity studies • VT-KB based qualitative (structural) model of self-regulating ocular gene network • VT-SE based cell-based computational model of lens-retina induction • MS: Ocular morphogenesis, gene network inference, analysis and modeling	• Extend lens-retina model to other stages and species • Incorporate pathway data from ToxCast, mESC and ZF embryos • MS: Sensitivity analysis for key biological pathways • MS: Developmental trajectories and phenotypes in computational models • Integrate with other morphogenetic models (ES cells, Zfish)	• MS: Test of model against predictions for pathway-based dose-response relationship • MS: Uncertainty analysis of models for complex systems model • Model: Computer program of early eye development using rules-based architecture, cell-based simulators, and systems-wiring diagrams	• MS: Integrated eye morphogenesis • Integration of computational models of different systems • Evaluation of integrative model with data from ES cells, Zfish	Framework for in silico reconstruction of the embryo to facilitate navigation of complex relationships and predict systems-level behavior (outcome) from data on biochemical, molecular, and cellular changes.
v-Liver™ – The Virtual Liver Project	• Prioritize proof of concept (PoC) environmental chemicals with clients. • Knowledge Base (KB): Information about PoC chemicals using ToxCast assays • KB: Cytoscape KB visualization and analysis tool • Cell response: Initial molecular circuit describing hepatic cell functions • Tissue Simulator: Develop / use MAS framework	• Tissue Simulator: Test liver lesions formation • Integrate molecular circuits for MOA chemicals in Tissues • Evaluate simulator using PoC chemicals and ToxCast data to predict outcomes	• KB inference tool for analyzing MOA for new chemicals/mixtures • Extend lobule simulator to liver and integrate with PBPK model	• Evaluate impact of genomic variation on cellular responses and lesion formation • Evaluate v-Liver for simulating human pathology outcomes using clinical data	Proof-of-concept decision support tools to enable tiered-testing:- (1) reduce uncertainty in evaluating the effect of chemicals on normal hepatic pathways and (2) estimate dose-dependent adverse hepatic effects in individuals and variability in populations.
Uncertainty - Analysis in Toxicological Modeling	• MS: Improvement of computational efficiency in Bayesian analyses of PBPK models using MCMC • MS: Assessing fit of PBPK models to data using Bayesian or other methods • MS: Reviewing approaches for constructing priors for PBPK model parameters • MS: Estimation of model parameters and comparison of alternative model forms for permethin PBPK model • MS: Global sensitivity analysis for exposure-dose model for permethrin • Estimation of PBPK parameters for deltamethrin and two other pyrethroids complete	• MS:Permethrin PBPK • MS on global sensitivity analysis for permethrin exposure-dose model submitted • MS: Parameter estimation for deltamethrin and two other pyrethroids • Completion of exposure-dose-effect model for "mini-cumulative" risk assessment • Substantial completion of uncertainty analysis for v-Liver • Description of ontologically aware PBPK language and translation to R • Problem identification for evaluating alternative pathway formulations in v-Liver model	• Problem identification evaluating alternative model formulation for BBDR and virtual embryo models • SAP on "mini-cumulative" risk assessment for pyrethroids • Preparation for SAP for cumulative risk assessment for pyrethroids • Publication of virtual tissue and BBDR modeling results • Initial steps in constructing test datasets for analysis "competition" recommended by UVPKM • Submission of ms on "RDynamic" to the Journal of Statistical Software	• Submission of MS on "Semantic-PK" • R package "RDynamic", for simplifying dynamic modeling in the statistical language R, submitted to CRAN	Improved cumulative risk assessment for pyrethroid pesticides, based on realistic quantitative assessment of uncertainties.

IV. APPENDICES (cont'd.)

B. Extramural STAR Centers Projects

1. The Research Center for Environmental Bioinformatics and Computational Toxicology at the University of Medicine and Dentistry of New Jersey (UMDNJ), Piscataway, brings together a team of computational scientists with diverse backgrounds in bioinformatics, chemistry, and environmental science, from UMDNJ, Rutgers, and Princeton Universities, and the U.S. Food and Drug Administration's Center for Toxicoinformatics. The team is addressing multiple elements of the source-to-outcome sequence for toxic pollutants, as well as developing tools for toxicant characterization. The computational tools developed through this effort will be evaluated extensively and refined through collaboration between STAR Center scientists and with colleagues from the three universities and EPA. Particular emphasis is on methods that enhance current risk assessment practices and reduce uncertainties. Researchers also are developing the Web-accessible Environmental Bioinformatics Knowledge Base that will provide a user-oriented interface to an extensive set of information and modeling resources.

2. The Carolina Environmental Bioinformatics Research Center at the University of North Carolina, Chapel Hill, is developing new analytic and computational methods, creating efficient user-friendly tools to disseminate the methods to the wider community, and applying the computational methods to molecular toxicology and other studies. The center brings together multiple investigators and disciplines, combining expertise in biostatistics, computational biology, chemistry, and computer science to advance the field of computational toxicology. Researchers focus on providing biostatistician support to the center by performing analyses and developing new methods in computational biology. The center is also creating a framework for merging data from various technologies in a systems-biology approach.

3. The Carolina Center for Computational Toxicology at the University of North Carolina, Chapel Hill, will advance the field of computational toxicology through the development of new methods and tools, as well as through collaborative efforts. The center is utilizing a bottom-up approach to predictive computational modeling of adverse effects of toxic agents. The emphasis spans from the fine-scale predictive simulations of the protein-protein/-chemical interactions in nuclear receptor networks, to mapping chemical-perturbed networks and devising modeling tools that can predict the pathobiology of the test compounds based on a limited set of biological data, to building tools that will enable toxicologists to understand the role of genetic diversity between individuals in responses to toxicants, to unbiased discovery-driven prediction of adverse chronic in vivo outcomes based on statistical modeling of chemical structures, high-throughput screening and the genetic makeup of the organism. In each project, new computer-based models will be developed and published that represent the state of the art. The tools produced within each project will be disseminated widely, and the emphasis will be placed on their usability by the risk assessment community and investigative toxicologists alike. The synthesis of data from a variety of sources will move the field of computational toxicology from a hypothesis-driven science toward a predictive science.

4. Texas-Indiana Virtual STAR Center; Data-Generating *in vitro* and *in silico* Models of Developmental Toxicity in Embryonic Stem Cells and Zebrafish at The University of Houston, Texas A&M Institute for Genomic Medicine, and Indiana University. The project period is from November 1, 2009 to October 31, 2012.

Objectives/Hypothesis:

As chemical production increases worldwide, there is increasing evidence as to their hazardous effects on human health at today's exposure levels, which further implies that current chemical regulation is insufficient. Thus, a restructuring of the risk assessment procedure will be required to protect future generations. Given the very large number of man-made chemicals and the likely complexity of their various and synergistic modes of action, emerging technologies will be required for the restructuring. The main objective of the proposed multidisciplinary Texas-Indiana Virtual STAR (TIVS) Center is to contribute to a more reliable chemical risk assessment through the development of high-throughput in vitro and in silico screening models of developmental toxicity. Specifically, the TIVS center aims to generate in vitro models of murine embryonic stem cells and zebrafish for developmental toxicity. The data produced from these models will be exploited further to produce predictive in silico models for developmental toxicity on processes that are relevant also for human embryonic development.

Approach:
The project is divided into three investigational areas; zebrafish models, murine embryonic stem cells models, and in silico simulations. The approaches are as follows.

(1) Generate developmental models suitable for high-throughput screening. Zebrafish developmental models (transgenic GFP/EGFP/RFP models of crucial steps in development) and embryonic stem cell (ESC) differentiation models (transgenic beta-geo models of crucial steps in differentiation) will be generated. Important morphology features and signaling pathways during development will be documented. The impact of environmental pollutants on development and differentiation will be assessed in the models. Finally, the models will be refined for high-throughput screening and automation.
(2) Generate a computational model that faithfully recreates the major morphological features of normal wild-type zebrafish development (i.e., segmentation into somites, proper patterning of vascular and neural systems) and the differentiation to three primitive layers (endoderm, mesoderm, and ectoderm) in mouse embryonic stem cells. The data for simulations are produced from developed high-information-content zebrafish and ESC models. Once a working model of normal development has been generated, we will carry out a directed series of parameter sweeps to try to create developmental defects in silico. We will compare the results of computationally created defects with experimentally generated defects in zebrafish and embryonic stem cells. Best matches between the two datasets will suggest hypotheses about possible mechanisms by which defects occur.
(3) Perform proof-of-concept experiments of the in vitro and in silico test platforms with a blind test of chemicals.

Techniques will be molecular biology techniques on zebrafish and ESC models, such as cloning, imaging, in vitro differentiation and in vitro exposure studies, and in silico mathematical simulations.

Expected Results (Outputs/Outcomes):
In collaboration with other initiatives taken in the field of chemical safety, our generated results and models will contribute to large screening effort to prioritize chemicals for further risk assessment. We will contribute specifically with

- 9 transgenic fish lines validated for toxicity screening;
- 16 embryonic stem cell models validated for toxicity screening;
- High-information-content models on development and differentiation to produce data for in silico simulations, within the project and elsewhere;
- computational models for developmental toxicology of normal development and of mechanisms by which chemical perturbations cause experimentally observed developmental defects; and
- Information on developmental toxicity on 39 compounds.

C. FY2004 "New Start" Award Bibliography

Project Title: Linkage of Exposure and Effects Using Genomics, Proteomics, and Metabolomics in Small Fish Models

Peer-Reviewed Publications

Ankley, G.T., K.M. Jensen, E.J. Durhan, E.A. Makynen, B.C. Butterworth, M.D. Kahl, D.L. Villeneuve, A. Linnum, L.E. Gray, M. Cardon and V.S. Wilson. 2005. Effects of two fungicides with multiple modes of action on reproductive endocrine function in the fathead minnow (Pimephales promelas). Toxicol. Sci. 86, 300-308.

Ankley, G.T., K.M. Jensen, M.D. Kahl, E.A. Makynen, L.S. Blake, K.J. Greene, R.D. Johnson and D.L. Villeneuve. 2007. Ketoconazole in the fathead minnow (Pimephlaes promelas): reproductive toxicity and biological compensation. Environ. Toxicol. Chem. 26, 1214-1223.

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