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BOARD OF SCIENTIFIC COUNSELORS

Computational Toxicology Subcommittee Meeting Summary
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Welcome and Introductions

Dr. George P. Daston, Miami Valley Laboratories, The Proctor & Gamble Company

The meeting was called to order at 10:05 a.m. by Dr. George Daston, Chair of the Computational Toxicology Subcommittee. He introduced himself, welcomed participants to the meeting, and asked the other subcommittee members and the Designated Federal Officer (DFO) to introduce themselves:

- Dr. James R. Clark, Exxon Mobil Research and Engineering Company
- Dr. Richard T. Di Giulio, Duke University
- Ms. Lorelei Kowalski, DFO, BOSC Executive Committee

Dr. Daston informed the participants that this meeting of the U.S. Environmental Protection Agency (EPA) Board of Scientific Counselors (BOSC) Computational Toxicology Subcommittee would be different from those of other subcommittees because it offered the subcommittee and the public an opportunity to examine a new program, see how it fits into other EPA programs, and comment on the program. The computational toxicology program is a unique concept because computational toxicology represents the merging of many sciences and scientific processes, interacts with all of them, and offers a fresh way to manage large data sets.

DFO Welcome and Remarks

Ms. Lorelei Kowalski, Designated Federal Officer for the BOSC Executive Committee, Office of Research and Development (ORD), EPA

Ms. Lorelei Kowalski, DFO for the BOSC Executive Committee, thanked the chair, the subcommittee members, and the public for their attendance at the meeting. She mentioned that two subcommittee members, Dr. Michael Clegg and Dr. Ken Ramos, were not present. Ms. Kowalski noted for the record that Dr. Ramos had recused himself due to a potential conflict of interest, and Dr. Clegg had a scheduling conflict. She also thanked the EPA staff for developing the materials for the meeting. Ms. Kowalski then reviewed the administrative procedures and Federal Advisory Committee Act (FACA) rules and described the objectives of the subcommittee and its charge.

The DFO works with EPA officials to ensure that all appropriate ethics regulations are satisfied. Each subcommittee member has filed a confidential disclosure form. These reports are reviewed by the Deputy Ethics Officer of ORD's Office of Science Policy (OSP) and the DFO, in consultation with the Office of General Counsel, to ensure that all ethics requirements are met. In addition, the subcommittee members have completed their ethics training. The subcommittee members must inform the DFO of any potential conflicts of interest in any of the topics discussed at this meeting.

Ms. Kowalski described the process for agenda development and public comment. She stated that the meeting was being recorded and a summary of it would be posted on the BOSC Web Site (http://www.epa.gov/osp/bosc/subcomm-ctox.htm). Because it was a public meeting, she asked all persons speaking to identify themselves for the record. She said that background materials were provided to the subcommittee members and that anyone who would like copies of that material should contact her. She noted that there would be time during breaks and lunch to view the posters on display in the Atrium. As indicated in the agenda, time was set aside for public comment, however, no one had contacted Ms. Kowalski to request time to speak during that period. If anyone present wishes to do so, they should contact her immediately. Ms. Kowalski concluded her presentation by informing participants that any questions for or about the subcommittee or the contractor should be directed to her. She also reminded everyone to register by signing in so the record of attendance would be accurate.

Acting Deputy Assistant Administrator for Management, ORD, EPA Remarks
Mr. Lek Kadeli, Acting Deputy Assistant Administrator for Management ORD, EPA

Mr. Lek Kadeli presented a basic overview of the NCCT in relation to the other EPA centers. He stated that, initially, ORD recognized the need to develop computational and molecular approaches to environmental issues, which led to the development of the computational toxicology program, and shortly thereafter, the NCCT. As the newest of the EPA centers, the NCCT is well positioned to work with other ORD components in fulfilling the Agency's mission. Because the NCCT (also referred to as the Center) is still developing, it views peer review by BOSC as a process from which helpful advice and input can be gleaned, so the subcommittee's work will assist the Center, especially in the area of program evaluation.

Mr. Kadeli defined ORD's two major research components as problem-driven research and core research. Problem-driven research identifies existing and emerging issues, but uses risk assessment to prioritize those issues, and narrows the focus of the issues based on the Agency's mission. Research efforts then focus on the most salient issue. Core research has more broad applicability. It looks beyond the present based on relevance to EPA and scientific merit.

The external review of ORD research programs was spawned by recommendations from the National Academy of Sciences (NAS) for independent expert review for evaluating federal research programs and the Office of Management and Budget (OMB) recommendations. ORD is strongly committed to independent and objective evaluation of research at the program level and asked the BOSC to participate in the review of scientific programs. Recommendations from the BOSC review will strengthen accountability and provide guidance to ORD to help: (1) implement and strengthen the research program; (2) verify that clients have applied research

to strengthen environmental decisions; (3) make decisions about research investments/ disinvestments over the next 5 years; and (4) prepare EPA's performance and accountability reports to Congress as required by the Government Performance and Results Act (GPRA).

Evaluation criteria for ORD research programs include relevance to national priorities, Agency missions, customer needs, quality maximization, and demonstrated performance that encourages research managers to characterize scientific leadership. Because the Center has been in existence for just over 1 year, the BOSC program review charge is for prospective analysis, rather than the customary retrospective analysis. The evaluation will examine whether the Center is establishing effective collaborations, staffing as needs and direction indicate, and staying current with technology. In evaluating themes, the review will: (1) look for clear research rationale and direction, (2) consider whether NCCT research programs are collaborating effectively and taking advantage of potential partnerships, and (3) determine whether sufficient depth of effort is being expended.

Mr. Kadeli discussed the themes for each day of the meeting and closed by saying that the BOSC is not the only entity evaluating the NCCT's programs. The Center would welcome any advice from other EPA centers or staff to ensure that its programs are of the highest quality and relevant to the Agency's mission.

Questions

Dr. Clark asked whether NCCT has the full support of Agency management, including that of the EPA Administrator. Mr. Kadeli replied that, although computational toxicology is relatively new, NCCT is an equal partner within ORD.

Dr. Daston commented that it would be interesting to see how the subcommittee approaches the evaluation charge questions considering that the review is prospective rather than retrospective. Although the subcommittee's ability to predict is limited, the only way to prepare for the future is to identify and develop core competencies, which are the keys to building any new program. The subcommittee has the opportunity to assist NCCT staff in establishing the NCCT's core competencies. Mr. Kadeli responded that the breadth and depth of expertise that would comprise the Center's core competencies are issues being addressed in the meeting.

Background and Direction of ORD's National Center for Computational Toxicology
Dr. Robert Kavlock, Director, National Center for Computational Toxicology, EPA
Dr. Jerry Blancato, Deputy Director, National Center for Computational Toxicology, EPA

Dr. Kavlock thanked the participants for attending the meeting and taking an interest in the newest EPA center. He outlined the developmental history of the Computational Toxicology Program and the NCCT by defining computational toxicology as integrating modern computing and information technology with molecular biology to improve Agency prioritization of data requirements and risk assessment of chemicals. Computational toxicology uses novel technologies derived from computational chemistry, molecular biology, and systems biology in conducting toxicological risk assessment.

Dr. Kavlock said that, from a regulatory perspective, EPA's need to conduct quantitative risk assessment and establish methods of risk management for priority pollutants had led to the development of methods to detect and characterize those pollutants, and then evaluate them, one chemical at a time. Newer mandates have created the need to make this process more efficient and effective, as increasing numbers of chemicals need to be assessed for hazard and risk. The challenges of conducting such research include the following: (1) many priority pollutant lists existed across the environmental field, but no standard criteria for testing them existed; (2) different authorities had different testing requirements; and (3) the field lacked the data to reduce uncertainties quantitatively. As an example, Dr. Kavlock described problems experienced with respect to pesticidal inerts. These are additives to registered chemical formulas, and there are legislative mandates to reissue chemical registrations. However, although there are no formal data requirements, there is an associated legal burden-of-proof of "reasonable certainty of no harm" that complicates the process of conducting risk assessments for pesticidal inerts. In addition, an August 2006 deadline was established for completion of testing on nearly 1,000 chemicals. The computational toxicology program emerged to address the needs such as those presented by this situation.

Three phases of development have brought the Computational Toxicology Program from idea to implementation. Dr. Kavlock described each phase.

Phase I began in Fiscal Year (FY) 2002, when Congress directed the EPA to provide funds for the research and development of alternatives to traditional toxicological testing procedures. The first research projects were devoted to impaired reproduction and development and consisted of five types of initial proof-of-concept (PoC) studies related to endocrine disrupting chemicals. These projects were chosen because their known mode of action made such research a target with reasonable certainty of success. The five types of PoC studies were: (1) estrogen receptor (ER) binding data refinement, (2) ER quantitative structure activity relationships (QSAR) enhancement, (3) steroid docking models, (4) H295R assay evaluation, and (5) hypothalamic-pituitary-gonadal axis systems models. The PoC studies were expanded later in the fiscal year to include ER and androgen receptor (AR) transcription assay scale-up, predictive toxicogenomics evaluation, and long-term research on higher throughput screening and systems biology initiated under the Science to Achieve Results (STAR) program.

Phase II was initiated in FY 2003. The foundation of the Computational Toxicology Program was laid with the establishment of a design team that developed a framework document intended to identify the research needs and unique capabilities of ORD laboratories. The framework provided the basis for a more focused and integrated research program in the future. The framework document was brought to the EPA Science Advisory Board (SAB) for a consultation, and it was endorsed enthusiastically. It also was presented to the Board of Scientific Councilors, where it received similar support. The design team conducted a workshop to introduce the framework to the entire Agency, developed a bibliographic inventory of publications and a Web site, and released a Request for Applications (RFA) to conduct additional research projects under the STAR program.

In FY 2004, Phase III moved the program from planning to implementation. The design team was superceded by the Computational Toxicology Implementation and Steering Committee

(CTISC) in January 2004. The CTISC was charged with implementing the broader computational toxicology program while continuing to work on existing PoC activities related to endocrine disrupting chemicals. In February 2004, the CTISC initiated an internal competition by issuing two RFAs, one for augmentation awards to existing projects, and the other for new projects. Augmentation awards allow current EPA projects to increase the application of computational toxicology tools and techniques and showcase examples of how the Computational Toxicology Research Program is addressing its objectives. The areas covered include genomics, proteomics, metabonomics, and database development. The CTISC also funded seven New Start projects, which collectively showed broad, multidisciplinary efforts supporting the objectives of the framework. The CTISC also provided support for several workshops that helped to increase awareness and understanding of the Computational Toxicology Research Program. Partnerships were (or are being forged) with the U.S. Department of Energy, U.S. Department of Defense, National Institute of Environmental Health Sciences (NIEHS), National Center for Toxicological Research (NCTR), IBM, Affymetrix, Chemical Industry Institute of Toxicology Centers for Health Research, and a former Soviet Union scientist redirection program.

In Phase IV of program development and institutionalization, the establishment of the National Center for Computational Toxicology was announced in October 2004. Through a series of meetings and "inventory" sessions, the Center's work and staff roles have emerged. The Center was formally launched on February 20, 2005. Its mission is to:

- Provide scientific expertise and leadership related to the application of mathematical and computational tools and models.

- Improve the predictive capabilities of the methods, models, and measurements that form the input materials to the computational models.

- Conduct and/or sponsor research to provide models for fate and transport of chemicals, environmental exposures to humans and wildlife, delivery of the chemical to the target site of toxicity, molecular and cellular pathways of toxicity, and ultimately systems level understanding of biological processes and their perturbation.

- Maintain a strong emphasis on the development of partnerships with other government and private organizations.

Recently completed work on building the foundation of the NCCT includes: (1) development of a Memorandum of Agreement with the National Exposure Research Laboratory (NERL) and the National Health and Environmental Effects Research Laboratory (NHEERL) to provide administrative support functions; (2) outreach to the National Toxicology Program (NTP) in the National Institute of Environmental Health Sciences (NIEHS) for collaborations in systems biology research in the chemical screening tools; (3) engaging the assistance of the National Computer Center and its Environmental Modeling and Visualization Center to assist the core projects being identified within the Center; (4) staff recruitment; and (5) development of an RFA for the STAR Center for Environmental Bioinformatics. That RFA, which closed on February 24, 2005, will support an institution at $1M per year for 5 years through a cooperative agreement

to help develop ORD's bioinformatic capabilities. Application review is planned for June or July 2005. Dr. Kavlock also mentioned that additional research was solicited in March 2005 through a Small Business Innovation Research (SBIR) program solicitation addressing exposure diagnostics, biotransformations, docking models, QSAR databases and models, molecular signatures, and "omic" integration. This solicitation closes on May 25, 2005.

Concerning staffing, Dr. Kavlock outlined the NCCT staff of 19 full-time equivalents (FTEs). Current positions include: four administrative staff, four systems modelers, and five computational chemists. A senior systems biologist will join the staff in May 2005, and the NCCT is recruiting two bioinformatics experts, an expert in high throughput screening tools, two additional systems biology modelers, and an ecological modeler (tentative). In addition, postdoctoral fellows and staff detailed from other areas or agencies are being recruited or identified. The emerging focal areas for the NCCT staff are information technology, prioritization and screening, biological models, and cumulative risk assessment.

The NCCT was launched as a full-fledged Center for a variety of reasons. Most important, ORD saw the need to commit to what computational toxicology could do to advance EPA's overall abilities. In addition, regulatory needs and their associated support required Agency expansion into areas of crosscutting expertise. The development of a center gave visibility and stature to the Computational Toxicology Research Program, allowed dedicated resources to be allocated in a protected budget, allowed the staff to focus on themes, and emphasized the urgency of the work. Equally important was the fact that development of a center brought a freshness to the work by allowing staff to branch out into new areas, and to do so in a very collaborative and interdisciplinary fashion.

In summary, Dr. Kavlock stated that the Computational Toxicology Program had a solid start, has been responsive to input from other areas within and outside the EPA, clearly addresses human and ecological health, and is enhanced by the establishment of the NCCT. The NCCT is staffed by talented, motivated, and enthusiastic people with a solid understanding of critical issues in computational toxicology. Currently, the Center and its staff are establishing focal areas, developing working relationships, delivering interim products, and building confidence in research-based predictions and extrapolations. All of this is adding value to ORD's efforts.

Questions

Dr. Daston commented that the NCCT staff plan showed the need for expertise in modeling, informatics, and chemistry, and asked what fraction of the expertise needed resides inside the Center and outside of it. Dr. Kavlock replied that it would be a hard number to estimate, but perhaps approximately 30 percent of the expertise existed between Center staff and the rest elsewhere in ORD.

Dr. Daston asked how the Center staff plans to interact with other laboratories and centers at EPA. Dr. Kavlock responded that sections of upcoming presentations speak to that issue directly.

Dr. Daston acknowledged the individual program milestones discussed and commented that there was a need for Center-wide milestones. Dr. Kavlock replied he will be addressing the issue of a system of accountability and creation of milestones for the Center very soon.

Dr. Daston said that the NCCT plans to leverage significant expertise and resources from other areas within EPA and asked if there was any plan to track that. Dr. Kavlock replied that there is no tracking plan in place.

Direction

Dr. Jerry Blancato thanked the subcommittee members and other participants for coming to the meeting and exchanging ideas with the NCCT staff. To begin his presentation, he said that the NCCT's direction was based on its interactions with other ORD laboratories and centers and other entities in the scientific community. At this early stage of development, such interactions are extremely important. To his knowledge, the Center is the first to have interaction and collaboration as part of its mandated tasks. Much of the NCCT's work is in the same topical areas or overlaps with work being done by the National Risk Management Research Laboratory (NRMRL), NERL, and NHEERL. Each does a significant amount of work in measurement and modeling, PoC, and exposure, so nearly everything done at the NCCT impacts other Centers and is connective as to overlap, with considerable collaboration taking place at all levels.

The NCCT focuses on four major topical areas: (1) informatics, (2) prioritization, (3) systems modeling, and (4) cumulative risk assessment. Dr. Blancato explained that informatics covers several areas in the source-to-outcome continuum, such as structural activity relationship (SAR) structured activity with toxic endpoints, and computational chemistry, to help provide *in-silico*-derived parameters to exposure, biologically based dose response, and systems models. Interactions outside and within different scientific components of programs include databases to associate structure with endpoints and databases to organize, characterize, and analyze the "omics" information, which is a large part of informatics. The NCCT staff is beginning to look at information from databases derived from unstructured data. The other ORD laboratories and the Center have or will have informatics experts to handle "omics" information. Within ORD, a working group is being formed to address informatics. NCCT's senior informatics expert will interact with the STAR informatics grantee under a cooperative agreement. Computational chemistry scientists work with informatics experts to connect structure with toxic endpoints, "omics" information, and physical properties. Both NHEERL and NERL will be impacted. The NCCT exposure, dose response, and systems modelers will work with informatics to develop key governing parameters for models, working with modelers in the National Center for Environmental Assessment (NCEA) and elsewhere.

Prioritization encompasses predictive models and methods for screening and testing. NCCT is working on this and plans to hire new staff with expertise in this area during 2005. Several ongoing projects in NHEERL are directed toward prioritization, and NCCT will work to coordinate with them. Although they exist in other areas of ORD, all of the New Starts projects addressing prioritization were funded by the NCCT, and NCCT staff will cooperate with outside groups in several areas of computational toxicology, including prioritization. ORD tracks prioritization outputs, and several workshops in this area are planned.

Dr. Blancato said that systems biology helped to make the linkages in the source-to-dose-to-outcome continuum by harnessing the power of mathematics, engineering, and computer science to analyze and integrate data on understanding normal physiology. This elucidates the mechanisms of the "abnormal." Systems biology is a new way to work with biology. Among the systems to be targeted are: key molecular pathways of functioning cells, interaction of cells of a tissue, organ systems, morphogenesis, whole organisms, ecosystems, and the exposure-dose response continuum. There are expert systems modelers in all ORD laboratories and centers. There also are several projects in other laboratories and centers to which the NCCT can contribute by developing more systems biology approaches and bringing together exposure models, dose models, and biologically based dose response models to do predictive work. NHEERL, NERL, NCEA, and the Office of Pesticide Programs (OPP) are involved in these studies. Because several members of the NCCT staff transferred from NERL and NHEERL, there are natural working connections that will assist collaborations.

As to specific mechanisms for collaboration, Dr. Blancato stated that an expert systems modeler will help design the research program in modeling and coordinate with other laboratories and centers. The NCCT proposes a cross-ORD modeling workgroup that has regular communication and sharing of ideas and work loads. A cross-ORD working group of informatics specialists will create synergy and avoid "reinventing the wheel." Periodic scientist-to-scientist meetings with ORD laboratories and centers and NIEHS will identify projects of interest and implement them jointly to strengthen the connection with the STAR program. Adjunct staff from other EPA laboratories, including some assigned to the NCCT on detail, will work on specific projects. Associate staff from other laboratories who work on projects related to computational toxicology or who have a direct interest in it, currently work with the NCCT staff informally. An Agency Risk Assessment Forum looks at crosscutting issues, and two NCCT staff are members of the Forum. Other staff serve as members of other forums and workgroups.

Questions

Dr. Clark commented that he did not see any collaborative links to the offices and laboratories handling waste and asked if they had been included. Dr. Blancato said that the NCCT had not yet had a chance to talk to those offices and acknowledged the need for additional outreach. He also mentioned that as other offices hear about the computational toxicology program and the new Center, some have contacted members of the NCCT staff to express their interest. For example, he was recently contacted by the Office of Water.

Summary of FY04 ORD Computational Toxicology Activities
Dr. Robert Kavlock, Director, National Center for Computational Toxicology, EPA

Dr. Kavlock gave brief descriptions of the research being done by 19 PoC, Augmentation, and New Start research studies funded by the Computational Toxicology Program or the NCCT within the past 3 years. The projects are classified under three objectives of the Computational Toxicology Framework: Linkages, Prioritization and Screening, and Quantitative Risk Assessment.

The following are Linkages projects.

- *A Systems Approach to Characterizing and Predicting Thyroid Toxicity Using an Amphibian Model*. This is a New Start project that focuses on building linkages between early molecular events associated with exposure and organism-level effects mediated via alterations in the action of thyroid hormones. Understanding these linkages and their relative importance will be assessed through development of a hypothalamic-pituitary-thyroid (HPT) systems model in the developing amphibian. The ultimate goal is to use the systems model for relating predicted activity (via QSAR, based on chemical-biological target interaction) to whole organism outcomes.

- *Linkage of Exposure and Effects Using Genomics, Proteomics, and Metabonomics in Small Fish Models*. This project's focus is identifying new molecular biomarkers of exposure to endocrine disrupting compounds (EDCs) representing several modes/mechanisms of action (estrogens and anti-androgens). The goal is to link those biomarkers to effects that are relevant for both diagnostic and predictive risk assessments using small fish models. This is a New Start project.

- *Metabonomic Studies of the Effects of Bioaccumulated Conazoles on Endogenous Metabolites in Rainbow Trout Using NMR*. This Augmentation research study is measuring the bioaccumulation of nine conazoles (enantiomeric forms) by rainbow trout and assessing endogenous metabolite profiles using high resolution nuclear magnetic resonance (NMR).

- *Chemical Screening and Prioritization–Protein Expression Profiling Using a Small Fish Model.* This research provides *in vitro* and short-term *in vivo* assays needed to support hypothesis-based risk assessment and enable EPA to incorporate a proteomics-based approach into chemical screening and assessment programs. This is an Augmentation project.

- *Discovering the Mode(s) of Action of Conazole Toxicity Using the Tools of Toxicogenomics and Toxicology for Harmonization, Interspecies Extrapolation, and Computational Toxicology*. This Augmentation study is attempting to answer the following four questions for a class of pesticides that exhibit characteristic, but not identical, manifestations of toxicology that vary from one chemical to another:
    - Is there a common mode of action for the observed toxicities?
    - Is P450/XME modulation a common critical event?
    - Can the information be used to reduce uncertainties in interspecies extrapolation?
    - Can we then predict toxicities of new conazoles?

- *Gene Expression Profiling to Assist in the Development of In Vivo and In Vitro Toxicity Tests.* The issues addressed by this Augmentation project are rodent to human extrapolation, mechanisms of toxicity, screening, and prioritization. It compares the ability of toxicogenomic analysis of rodent and human cells *in vitro* to predict responses of the whole organism using a parallelogram approach.

- *A Systems Biology Approach to Improve the Predictive Value of Biomarkers for Assessing Exposure, Effects, and Susceptibility in the Detroit Children's Health Study—Mechanistic Indicators of Childhood Asthma (MICA).* This New Start project is exploring the use of genomics in conjunction with a variety of biomonitoring tools to address the following major research questions in a population of third and fourth grade children in Detroit:
    - What role do neighborhood differences in urban air pollutants play in the development of allergies and asthma?
    - Are ambient pollutant exposures reflected in clinical/biological markers of exposure?
    - Are exposure biomarkers predictive of differences in biomarkers of early effect?

- *Global Analyses of Proteins and Lipids from Diesel Exhaust Exposed Human Subjects*. The study increases the components (typically protein expression and gene activation) used in assessing the biology of human lung fluids in controlled exposure to air pollutants by adding the dimension of analysis of the lipid component of the fluid. It will determine whether "lipidomics" is useful in assessing the toxicity of diesel exhaust, and whether it provides a rationale for future examination of this portion of the metabolome. This is an Augmentation project.

- *Identification of Fecal Anaerobic Bacterial Markers for Microbial Source Tracking.* The research will continue to identify the origin of fecal pollution impacting watersheds and evaluate the use of 16S rDNA sequences of fecal anaerobic bacteria as potential indicators of pollution from specific animal hosts. This project received Augmentation and New Start funding.

- *Endocrine Disruptor Elicited Gene Expression Network Elucidation in the Rat Uterus*. In this STAR grant project, the objective is to use a systems biology approach to integrate, computationally, complementary gene expression and histopathology data to develop a model that can predict the uterotropic effects of environmental estrogens.

- *Systems Biology Modeling of Fathead Minnow Response to Endocrine Disruptors.* This STAR grant's hypothesis is that one can determine specific gene expression patterns for typical steroid hormones in exposed fish, and that these patterns will predict gene expression and protein synthesis patterns, and physiological outcomes for specific classes of environmental EDCs. The model compounds will have unique gene expression patterns that can be used to "train" a mathematically derived algorithm to predict exposure outcomes of other environmentally relevant compounds.

- *Chemical Induced Changes in Gene Expression Patterns Along the HPG Axis at Different Organizational Levels Using a Small Animal Model (Japanese Medaka).* A systems approach is being used in this STAR grant study to identify effects of endocrine modulating compounds (EMC) on the HPG axis in a small fish model. *In situ* hybridization and *in situ* RT-PCR immuno-histochemical staining (IHCS) will be used to obtain results and provide increased information on the anatomical distribution of the organism's responses to exposure to endocrine disrupting compounds.

Projects in the Prioritization and Screening category are described below.

- *Simulating Metabolism of Xenobiotics as a Predictor of Toxicity.* At issue in this project are methods and tools needed to prioritize chemicals for toxicity testing and hazard assessment. The particular approach involves the use of computational advances to develop a simulator of metabolism for identification of chemical metabolites and to link output to a toxic effects model to elucidate metabolites of greater toxicity than the parent chemical (in this case, binding to the estrogen receptor). This is a New Start project.

- *ASTER (Assessment Tools for Evaluation of Risk).* ASTER is an integration of the aquatic component of the ECOTOX database and a QSAR-based expert system. When empirical data are not available, mechanistically based predictive models are used to estimate ecotoxicology endpoints. The system includes a database and models to estimate chemical properties, biodegradation, and environmental partitioning. ASTER is designed to provide high quality data for discrete chemicals, when available in the associated databases, and QSAR-based estimates when data are lacking.

- *A Bioluminescent Yeast Reporter System for Screening Chemicals for Estrogenic Effects.* The objective of this research is to develop, validate, and automate yeast-based bioluminescent bioreporters for the rapid detection of estrogenic and androgenic compounds. This is a STAR grant project.

- *A High Throughput Zebrafish Embryo Gene Expression System for Screening Endocrine Disrupting Chemicals.* The goals of this STAR grant study are to:
    - Predict more accurately which chemicals in the environment have the potential to disrupt hormone-dependent processes of physiology, reproduction, and development.
    - Provide biologically relevant criteria for prioritizing chemicals for further testing.
    - Help interpret reports of reproductive and developmental abnormalities in wildlife and humans by developing an assay based on altered gene expression in living zebrafish embryos as a whole animal, *in vitro* screening system for simultaneous detection of multiple subsets of EDCs.

- *Using a Sensitive Japanese Medaka (Oryzias latipes) Fish Model for Endocrine Disruptors Screening.* The overall goal of this project is to develop and validate a high-throughput EDC screening assay using a microarray gene chip applied to a small fish model.

- *Mechanistic Approach to Screening Chemicals and Mixtures for Endocrine Activity Using an Invertebrate Model.* For this research project, staff are developing a mechanism-based high-throughput screening approach for evaluating endocrine activities of chemicals using an invertebrate species (Daphnia) and adapting the approach for use in evaluating interactive effects of endocrine-active chemicals.

Quantitative Risk Assessment projects are described below.

- *Risk Assessment of the Inflammogenic and Mutagenic Effects of Diesel Exhaust Particulates: A Systems Biology Approach*. This New Start project is building a cross-species computational model describing the relationship between the physicochemical composition of diesel exhaust particles (DEP) and their mutagenic and inflammogenic health effects.

In closing, Dr. Kavlock said that collectively, these projects presented a diverse portfolio of research endeavors covering both human and ecological health issues. A strong underlying theme is to increase the predictive value of biological indicators and to help position them in context of the overall assessment of adversity and risk.

Questions

Dr. Clark asked whether funding was received for all approved programs or whether choices about funding had to be made program by program. Dr. Kavlock replied that all projects that passed the scientific and relevancy reviews could not be funded, and that some choices had to be made in light of available resources.

Dr. Daston commented that the NCCT was doing much work on endocrine disruption and asked if this would continue to be a focal area of study. Dr. Kavlock replied that it would be to the extent that findings from current studies indicated, and added that some significant work on other signaling pathways also is anticipated.

Dr. Di Giulio asked if the Center were involved in the research projects beyond providing the funding. Dr. Kavlock said that a Center scientist is a member of the research collaboration team for many of the projects (those underlined in the figure listing the projects arrayed around the triangle), although a number of them do no have such a level of interaction.

Dr. Daston commented that NCCT personnel were represented on the staffs of several research projects. Dr. Kavlock agreed and identified the Center personnel working with each project.

Dr. Daston asked if the audience had any questions. No questions were posed from the audience.

Research Theme I: Information Technology
Dr. Ann Richard, National Center for Computational Toxicology, EPA

Dr. Richard cited a QSAR meeting held in Bulgaria as the starting point for the computational toxicology information technology project, which began approximately 4 years ago. The problem being addressed by this theme is the lack of information on the toxic effects of specific chemicals. Currently, the Agency has mandates to evaluate multiple lists of chemicals and many toxicity endpoints to assess, but lacks sufficient and relevant data with which to conduct these assessments. This situation creates the need to prioritize assessment efforts and focus limited resources on the chemicals and problem areas with the potential to make the greatest impact on

health and the environment. To assess various chemicals, one first searches for chemical-specific data, such as Chemical Abstract Services (CAS) registry numbers. Because data on new chemicals undergoing screening often are not available, most of the data applied to screening assessments is inferred from data on analogs of chemicals.

The concept of "data mining" involves use of analog approaches to place chemicals for which data are unavailable in a broader context (i.e., involving the collection of closely related and similar data from multiple sources). Past and current work in the pharmaceutical industry that focuses on drug development and toxicity assessment has been a driving force for development of computational methodologies for screening the toxicity of environmental chemicals.

Dr. Richard informed participants that "chemoinformatics" is a generic term that encompasses the design, creation, organization, management, retrieval, analysis, dissemination, visualization, and use of chemical information. In practice, chemoinformatics can be defined further as the use of information technology in chemistry-based data mining and exploration. To some extent, chemoinformatics is being used in:

- Pharmaceutical Sciences
- Drug Discovery
- Chemical Design
- Materials Science
- Green Chemistry
- Agriculture
- Pesticides
- Food Science
- Polymers
- Atmospheric Chemistry
- Environmental Studies
- Green Chemistry
- Predictive Toxicology

Chemoinformatics will be expanded even further by the Computational Toxicology Program.

The toxicity prediction problem deals with extrapolations across levels of increasing chemical and biological complexity (from chemical structure, to biochemical interactions, to cell-based *in vitro* responses, to whole animal *in vivo* studies, to human health effects). With increasing levels of complexity comes increasing degrees of uncertainty, accompanied by increasing relevance to risk assessment at each level. To explain this progression in detail, Dr. Richard showed a series of slides depicting relational graphics, formulas, and scientific procedures, and discussed their specific relationships to and effects on each other. She concluded Part I of her presentation by citing the limitations of public toxicity data for use in SAR: sources are scattered, formats are nonstandard, information content is diverse, and there is a lack of chemical structure annotation. Suitable databases are unavailable for many types of toxicity endpoints because often, the toxicology domain experts do not understand the needs of computational toxicology models. Overall, sufficient data are lacking and available data are difficult to find and use for improving predictive toxicology models. In addition, the existing data are not being efficiently utilized due to problems with standardization and availability. Large, public databases containing chemical information, private databases compiled by pharmaceutical and chemical industry corporations, and scientific databases compiled by universities or science-based associations offer the greatest potential for contributing chemical information to larger public resources capabilities. Hope exists in standardizing such databases, publishing new files, and providing new ways to link and access relevant information.

In Part II of her presentation, Dr. Richard discussed the Distributed Structure-Searchable Toxicity (DSSTox) public database network project, providing details on file structure, record, content, and coordination with other databases, as well as the steps involved in working with several specific databases and efforts to standardize toxicity databases across domains of toxicology. Dr. Richard closed this part of her presentation by stating that the information technology component of the NCCT is headed toward cultivation of expanded data offerings; automation, particularly of the DSSTox master list; integration with other public databases; coordination with public data standards; creation of an EPA-wide structure browser; and development of linkages to several toxicogenomics projects and databases.

Part III of Dr. Richard's presentation discussed the meeting and merging of bioinformatics with chemoinformatics to form a new field of study that she titled "Toxico-chemoinformatics." Toxico-chemoinformatics is concerned with data standardization, integration, and exploration. In practice, it would make better use of all available data, overcome data limitations by exploring diverse domains of data from multiple perspectives, develop expanded definitions of chemical analogs, and employ both biological and chemical information to develop predictive toxicity signatures. Dr. Richard concluded her presentation by stating that the field of chemoinformatics needs to address each of the aforementioned areas.

Questions

Dr. Daston commented that although chemical information is getting better, not much of it is in the realm of toxicology. Dr. Richard agreed and said she had not devised an approach to this issue other than acknowledgment of what has been done and publishing databases with caveats.

Dr. Daston noted that the presentation concentrated on obtaining data from well known, high quality databases and asked why small databases from industry or universities were not included. Dr. Richard responded that the data being sought at this time is confined to information that will prove most useful to the public.

Regarding the UniLever's Skin Sensitization Database, Dr. Daston asked if there was a way to encourage other information sources to add data to that system. Dr. Richard replied that she and others are trying to find a way to encourage industry to do so.

Dr. Clark asked about the possibility of coordination with various private and government databases from other countries. Dr. Richard said that there were some linkages. European countries are doing some work in this area. It is up to entities like EPA and the Food and Drug Administration (FDA) to improve or increase their marketing efforts to get other countries interested in sharing data.

Dr. Daston asked whether the Computational Toxicology Program offers any information on the value of structured data. Dr. Richard responded that part of her job is to educate staff and other scientists on this subject and give examples to other scientists that will stimulate their thinking. The NCCT Web Site and Dr. Richard's publications are part of this educational effort.

Dr. Daston asked whether there is a means of expanding EPA's Integrated Risk Information

System (IRIS) Structure Index (known as the IRISSI project). Dr. Richard said she had attended some meetings to help people understand the uses of the index, but others would have to become more involved before expansion could be considered.

Dr. Daston asked whether there are public databases that could accept information for prospective publishers and authors who want to submit their papers and whether such interest should be encouraged by the Computational Toxicology Program. Dr. Richard stated that it would require a commitment to maintain such a public database; however, some small academic sites and chemical-based publications may have enough interest to move it forward.

Research Theme II: Prioritization

Dr. James Rabinowitz, National Center for Computational Toxicology, EPA
Dr. Robert Kavlock, Director, National Center for Computational Toxicology, EPA

Dr. Rabinowitz began with a discussion of molecular modeling's application to computational toxicology. Modeling the interaction between environmental chemicals and target macromolecules is an important computational approach to understanding key steps in the mechanics of toxicity. Modeling is a tool for the prioritization of bioassaying requirements. The conundrum is that the application of modern experimental techniques to the study of chemical toxicity has led to an explosion of data that are relevant to the risk assessment process. Often, those data are not sufficient for evaluating risk. To use the existing data to obtain the information needed or to identify key missing information, extrapolations for evaluating the risk posed by chemicals should be done. Such extrapolations could include:

- High Dose to Low Dose
- Route of Exposure to Route of Exposure
- Chemical to Chemical
- Species to Species
- Population Characteristics
- Sensitive Subpopulations
- Life Stages
- Complex Exposure
- Dose Regime
- Mixtures of Chemicals

Dr. Rabinowitz explained that knowledge of the mechanism of toxicity often provides a rational basis for extrapolation. The challenges for computational toxicology are to: (1) determine appropriate levels of generalization for prediction (e.g., in definition of chemical classes); (2) capture relevant structural determinants of activity that provide a causal basis for the activity; (3) provide rationalization and/or a basis for the model prediction, (e.g., by communicating to the user rules or model descriptors used in prediction, chemical analogues identified by the model, and statistical measures of model robustness or appropriate application); and (4) to recognize the limits of available knowledge and when these limits are sufficient to preclude a prediction, for example, the macromolecular target potential toxicant paradigm is similar to a paradigm used by drug companies to identify potential new pharmaceuticals.

Computational toxicology has existed for more than 50 years, so one might ask why molecular modeling is being raised as an application now. Besides the idea that concepts are cyclical, there has been an increase in the technology and tools available. The knowledge base has increased to make the approach more fruitful and the computational hardware and software needed for the problems has been or is being developed.

Advances in biological knowledge are a contributory factor. The methods for molecular modeling in biological systems are rapidly improving. The engine for this improvement is the pharmaceutical industry and the commercial need to develop new drugs. The NCCT can take advantage of these improvements. Assessing toxicity is similar to finding new drugs, but, in important ways, it is different. To be a viable drug, a molecule must be a strong actor while environmental agents are often weak actors. If a drug company finds one or more prospective agents, the discovery is considered a success, computational toxicology needs to find all, or almost all of a potential agent. The Center's goal is to prioritize testing.

Dr. Rabinowitz mentioned that docking is the best fit between an unknown potential ligand and the molecular target. It is obtained by using classical methods. In this manner, a large library of potential ligands could be screened. Models that include more of the underlying physics of the interaction can be used. One of the NCCT's initial studies is of polycyclic aromatic hydrocarbon (PAH) metabolites binding to the estrogen receptor. From an initial study, it was observed that many crystal structures of the estrogen receptor bound with different ligands. Targets created from different crystal structures yielded different results, showing protein flexibility. This led to the conclusion that both protein and ligand flexibility are important to docking. In traditional docking studies, there is one protein target and the comparison of an array of potential ligands is made to identify the best potential ligands for that target. The new approach to docking examines a series of related protein targets; the chemicals to be screened are docked into each target, and the most likely target for each ligand is identified. The objective is always to find the best interaction partner for each chemical.

Current conclusions are that the easily available methods for docking show promise. Most of these methods do not allow the receptor to be flexible during docking and this artificially limits the subset of chemicals that bind to the receptor. When a series of potential macromolecular targets are considered simultaneously, the results are enhanced. Including an indiscriminate receptor for comparison purposes aids in classifying chemicals relative to steroid hormone receptor binding. Well-constructed datasets obtained from a consistent source using the same protocol will help the process of developing methods for screening.

In the future, Dr. Rabinowitz stated, the NCCT proposes to develop a series of macromolecular targets against which environmental molecules can be tested. The choice of targets would result from mechanistic understanding and provide insight into the mechanisms for toxicity. The appropriate level of interaction between the target and the potential toxicant would be dictated by the mechanism. The kind of knowledge that could be provided by this approach includes: feasibility of putative mechanisms of action on the molecular level, incorporation of structural information in understanding chemical toxicity, screening of chemicals for their capacity to partake in specific mechanisms, predictions on specific chemicals, and prioritization of chemicals for testing.

Turning to the topic of collaborations, Dr. Rabinowitz said that, in the area of prioritization, NCCT collaborations exist with the Reproductive Toxicology Division (RTD) of NHEERL for advice and data; Duke University for advanced computational chemistry; and EPA's Office of Environmental Information for computing and visualization. Potentially, those applying for the SBIR projects in software development will be collaborators as well.

Questions

Dr. Clark asked whether one needed to have a series of receptors that represent the work to screen for other elements. Dr. Rabinowitz replied that without a large library of targets with which to work, a series of receptors is not needed.

Dr. Daston asked how one creates a usable prioritization scheme. Dr. Rabinowitz stated that some postdoctoral staff have come up with preliminary estimates similar to those produced by the chemical industry, but much more testing is needed. Additional work must be done in this area. Drs. Richard and Kavlock concurred.

Research Theme II: Prioritization

Dr. Robert Kavlock. Director, National Center for Computational Toxicology, EPA

Dr. Kavlock reiterated that Dr. Richard had discussed legacy data and existing databases and Dr. Rabinowitz discussed the future use of *in silico* approaches to begin to prioritize chemicals. This presentation discusses methods of obtaining data if the model is not ready or the data do not yet exist. The ToxCast concept is a forecasting procedure and screening process based on the assumptions that: (1) prioritization/categorization is needed, (2) prioritization is not equivalent to screening, (3) global coverage of potential outcomes is necessary, and (4) these outcomes are mediated by chemical-biological interactions that can be used as a prioritization tool. There is no current model for prioritization, but technological advances can be employed to work toward developing a model. Cost is the chief factor in acceptance. The pharmaceutical industry has experience in this area, but it has focused on specific drug target developments and a few off-targets. This industry also accepts a high false negative rate. It is possible to build on pharmaceutical examples where mode/mechanism of action has been, or is being, employed in hazard or risk assessment. The endocrine area has shown promise in this regard.

ToxCast consists of a number of information domains: physical and chemical properties, biocomputational properties, biochemical properties, cell-based properties, *in vitro* omics indicators, and *in vivo* omics indicators. As one crosses the information domains, one finds more biological relevance at each level, starting with physical-chemical properties. The issue of cost controls how far one progresses across domains, each of which refines knowledge. The proposal is to conduct a proof-of-concept study using a series of reference chemicals of known toxicological phenotypes, acquire information for various information domains, and use clustering techniques to seek out patterns that would hopefully reflect the groupings based on the traditional toxicological analysis. Based on a study published by Pfizer scientists using the CEREP database, there is evidence that this approach is viable.

Benefits to the field include the ability to categorize or prioritize chemicals and the creation of a tool box of indicators that could be used across information domains. Additional benefits include the potential for targeting elements and outcomes of concern to the field. The approach is flexible in its adaptability to technological advances and refinement of key indicators with experience. It lends itself to the development of predictive models as the database enlarges. The Agency is concerned with green chemistry, and involving green chemistry experts would address one information domain explicitly. It also is important to note that the work can lead to more

effective and efficient use of animals in screening and testing.

Data from the OPP indicate that it costs approximately $20 M to acquire the data necessary to apply for registration of a pesticide, and the legislative mandates, costs and sheer use of large numbers of animals prevents this approach from being utilized for other chemicals of concern. Therefore, it would be beneficial to develop a cost-effective approach for assessing any chemical's potential to be a biologically active agent. Other limitations and issues concern the chemical and assay selection processes, such as determining which chemicals would be the best to test, how several chemicals would be managed simultaneously, signal to noise, and the inclusion of metabolism studies in domains where they are lacking. The issues of where to start and where to focus are important. Some would advocate starting with a global approach; others would focus on a few specific toxicities. Another issue is how to cover developmental susceptibility.

Many discussions are in process or planned for the future. To develop a consensus on this issue, the NCCT has approached several program offices, the BOSC, and external stakeholders to help arrange a stakeholder's meeting in May 2005.

The development of partnerships around ToxCast continues. NTP and a few in the pharmaceutical industry have expressed interest in working with NCCT and sharing what they have learned. Although there is no firm commitment, NCCT anticipates interest from the chemical industry because ToxCast could offer it a fairly economical way to identify important data. Once partners are identified, NCCT would conduct a "Deep Dive," which involves setting up the problem, bringing the right experts to one place, and giving them 1 week to brainstorm and form workgroups to develop specific details.

Questions

Dr. Clark requested clarification on what the ToxCast process is trying to accomplish, considering the fact that base-level chemicals (such as Malathion) have been studied extensively, and asked whether work would start at that level. Dr. Kavlock responded that work would start at the basic level, with chemicals such as Malathion that have a rich experience.

Dr. Daston commented that a ToxCast study might require a consortium of sponsors to absorb the potential costs involved. Eventually, someone would need to decide which analyses would be done, how much data will be enough, and the minimum number of elements required to insure statistical equity. Dr. Kavlock replied that NCCT would assign two people to determine how to interpret the data from ToxCast.

Review of Day 1 Activities

Dr. George P. Daston, Miami Valley Laboratories, The Proctor & Gamble Company and Subcommittee Chair

Dr. Daston commented that the level of information provided by the day's introductory presentations had been stimulating. He said that everything was proceeding on schedule and reminded participants that the meeting would resume at 8:30 a.m. the following morning.

Finally, he reminded participants that there still was time to sign up to speak during the time allotted for public comment. The meeting recessed for the day at 5:35 p.m.

TUESDAY, APRIL 26, 2005

Research Theme III: Biological Models

Dr. Woodrow Setzer, Statistician, National Center for Computational Toxicology, EPA
Dr. Hugh Barton, Toxicologist, National Center for Computational Toxicology, EPA
Dr. Michael Zager, Postdoctoral Trainee, University of North Carolina (UNC)

Dr. Michael Zager opened the meeting by explaining that the presentation would take place in three stages: first, he would discuss the NCCT's vision of biological modeling leading to integrated quantitative systems biology; second, Dr. Setzer would discuss computational systems biology and current and future research plans; and finally, Dr. Barton would elaborate on additional research plans.

The NCCT Vision of Biological Modeling

Dr. Michael Zager, Postdoctoral Trainee, University of North Carolina (UNC)

Dr. Zager informed participants that in the biological modeling vision, NCCT takes the approach of integrating different types of toxicological data (i.e., pharmacokinetic, pharmacodynamic, "omic", etc.) and accompanying mathematical/statistical models on a systems level. Such integration will yield a better understanding of the mechanisms and modes of action in dose-response relationships. To explain the vision further, Dr. Zager discussed an example in biological modeling that addressed antiandrogens and prostate dose-response. To summarize the points of the example, he said that mathematical/statistical dose-response relationships can be explained by the underlying biology and that toxicity results from excesses or deficiencies that perturb biological pathways at critical times and can lead to a range of dose-response behaviors.

Computational Systems Biology and Research Plans

Dr. Woodrow Setzer, Statistician, National Center for Computational Toxicology, EPA

Dr. Setzer first presented information on biologically based dose-response modeling and several related issues. One issue in risk assessment is how to go beyond biologically based dose-response models and incorporate more mechanistic methods into risk assessment as the knowledge of basic biology increases. Computational systems biology modeling helps to organize and integrate data from disparate sources to improve hypothesis testing and generation. This can answer questions around consistency with existing data and what might happen under certain conditions. It also is useful for prediction and extrapolation qualitatively, related to dose-response shape, and quantitatively across species and across routes of exposure. Modeling is the basis of future of risk assessment and a conduit whereby more mechanistic research influences modern risk assessments.

Research plans were Dr. Setzer's second topic. He informed participants that he would discuss two research topics: statistical analysis of biological systems and developing new methods and technologies for modeling. In the area of statistical analyses of biologically based models, one

thing that needs to be done is to quantify uncertainty about model parameters, model predictions, and the procedures used to do extrapolations. The field needs to think of models as formal hypotheses and use statistical methods to test those hypotheses rigorously and compare alternative hypotheses-driven data. The use of models in designing experiments rather than the opposite, is important. Statistical methods must be designed to help in developing models.

Dr. Setzer also discussed several issues and problems affecting specific research plans. In the area of modeling methods and technology development, he cited model portability, linking, and archiving as key issues. Currently, a "Tower of Babel" problem exists (i.e., everyone uses their favorite software packages and languages to catalog data). Although models are seen as a means to integrate disparate data, integrating data from heterogeneous types of experiments done at different times in different places is a type of meta analysis. A major problem for biological models is the fact that they must fit into specific parameters and some parameters are not identifiable. Another problem lies in scientists' attempts to include all parameters in a physiologically based, pharmacokinetic (PBPK) model. Given the data typically available, it is not possible to include all parameters and attempting to do so can lead to highly singular data. Because models typically cover a wide dynamic range of response, model misspecification is likely. In mixed effects models, there is some variance in parameters that can cause problems with estimation. In addition, numerical solutions can cause problems.

Long-term research goals are to identify and address unsolved statistical methodological problems, work with NCEA to develop a systematic framework or handbook for statistical analysis, develop freely distributable software tools, and develop further collaborative efforts.

Questions

Dr. Clark asked whether the collaborations Dr. Setzer mentioned are formal written commitments. Dr. Setzer replied that most of the collaborations were based on handshakes, not formal documents.

Dr. Clark commented that it would best serve the interests of the Center to formalize as many collaborations as possible. Dr. Daston suggested that it would be useful to include the issue of formal collaborative relationships in the Center's long-term goals. He added that staff should give careful thought to the collaborations that are most sensible, based on what the Center is trying to achieve.

Additional Research Plans

Dr. Hugh Barton, Toxicologist, National Center for Computational Toxicology, EPA

Dr. Barton continued the discussion of research plans. He began by describing the Center's current effort in modeling technology development. NCCT is concerned with the long-standing problem of developing a means to create portability in biological models across various platforms and software packages. As mentioned previously, numerous software packages exist in the PBPK model community and are highly incompatible. A possible solution to this problem is the development and use of an extension of the Systems Biology Markup Language (SBML), a standardized XML-based markup language enabling portability of biological pathway models. NCCT is exploring whether an extension of SBML would accommodate PBPK model use.

NCCT staff have begun to collaborate with Lockheed Martin Corporation, through the EPA Office of Environmental Information, to identify limitations of the current SBML version for use in PBPK modeling. There also has been initial contact with several authors of SBML from the California Institute of Technology (Caltech).

Research plans for PBPK Modeling Across Lifestages includes improving dose-response analysis for first and second generation studies involving *in utero*, lactational, and early post-weaning exposures. The current default analyses use exposure dose/concentration to the mother. Extrapolation of PK across life stages requires awareness of the developmental windows involved. One aspect of this effort is directed toward developing a database of physiological parameters for developing rats, mice, and humans, through collaborations with NCEA and International Life Sciences Institute (ILSI). A postdoctoral student is working on experimental PK and modeling for conazoles in collaboration with human health research mode-of-action developmental studies. This effort will continue. Studies of perfluorinated compounds will continue as well. In systems modeling, two research studies are prominent: one on DEP and another on pyrethroid neurotoxicity.

In summary, Dr. Barton noted that NCCT is conducting and will continue to conduct several studies related to improving biologically based modeling methods and technologies and developing novel applications linking PK and pharmacodynamics (PD).

Questions

Dr. Di Giulio asked how the new Center has affected modeling overall. Dr. Barton replied that there has been PK modeling at NERL, NHEERL, and more recently, NCEA. PK modeling is an interface point, so it is necessary for expertise to exist in all areas of ORD. As a group, the NCCT staff needs to go beyond PK only and focus on the issues of linkage with PD, and development of biology-based dose response models. Knowing all of the pieces does not give one knowledge of systems' behavior; it is understanding how the components work together to create systemic behavior that is important. The systems biology studies used in medicine and in pharmaceutics are largely still qualitative. The quantitative parts are needed for risk assessment.

Research Theme IV: Cumulative Risk

Dr. Elaine Cohen-Hubal, National Center for Computational Toxicology, EPA
Dr. Woodrow Setzer, Statistician, National Center for Computational Toxicology, EPA
Dr. Michael Tornero, National Center for Computational Toxicology, EPA

Background

Dr. Elaine Cohen-Hubal, National Center for Computational Toxicology, EPA

Dr. Cohen-Hubal introduced the cumulative risk theme by providing background on its importance to EPA as a whole. She stated that EPA is being called on to: (1) assess cumulative risk resulting from exposures to complex mixtures, (2) identify vulnerable populations, (3) characterize life-stage risks, and (4) evaluate gene-environment interactions. To meet these increasingly complex needs for cumulative risk assessment, the Agency requires sound scientific understanding of the systems being assessed and appropriate tools and approaches for characterizing these systems. The mandate to address cumulative risk goes back to the Food

Quality Protection Act (1996), which mandates consideration of "cumulative effects" from aggregate exposures to different pesticides with the same mode of action. In 2000, EPA published its *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures*, which describes procedures for chemical mixture assessment using different levels of data. Most recently, in 2003, EPA published *A Framework for Cumulative Risk Assessment*, which defines cumulative risk assessment as the "analysis, characterization, and possible quantification of the combined risks to health or the environment from multiple agents or stressors." This broad view expands on cumulative risk assessment to include consideration of nonchemical stressors and increases emphasis on identifying and characterizing risks to vulnerable human and ecological receptors. This shifts emphasis away from traditional environmental risk assessment to characterize environmentally-related risks in the context of the larger public health paradigm. Among EPA's overall research needs are:

- Characterizing cumulative effects from exposures to complex mixtures;
- Using biomonitoring data to assess cumulative risks;
- Understanding the influence of prior exposures to one or more environmental contaminants on risks from subsequent exposures to additional stressors; and
- Understanding how to address nonchemical stressors in cumulative risk assessments.

Within NCCT, several activities are being conducted or planned to address Agency needs for characterizing cumulative risk. One group of projects focuses on characterizing effects from exposures to multiple chemicals. This research will improve quantitative risk assessment. Another area of research focuses on characterizing exposures for cumulative risk assessment. It will improve quantitative risk assessment as well as our understanding of source-to-outcome relationships. The exposure research also is very focused on source-to-outcome links.

Cumulative Risk Assessment Research Projects

Dr. Woodrow Setzer, Statistician, National Center for Computational Toxicology, EPA

Dr. Setzer discussed his work on a project entitled "When Is Dose-Additivity a Reasonable Assumption?" He stated that his work began with the assumption that if compounds act through a common mode of action, they should be expected to act in a dose-additive manner. This left open the need to prove the value of quantitative models for developing and testing generalizations about toxicological outcomes. Borrowing from PBPK/PD models, two exposure scenarios were considered, and interactions were characterized in terms of the behavior of isoboles or loci of points in "dose space" that have the same response in multichemical exposures. Non-interaction coincides with linear isoboles. Using a "Toy" OP Model, Dr. Setzer conducted experiments that resulted in curved isoboles, signifying interaction.

A second study, entitled "Cumulative Risk Assessment for N-Methyl Carbamate," is being conducted in collaboration with OPP. For this study, a probabilistic risk assessment, based on food, drinking water, and residential exposures, is being performed to determine health effects.

The study strategy requires development of a single model that describes acetylcholinesterase (AChE) activity as a function of dose and time post dosing. The model was adjusted to all relevant datasets, treating some of the variation among datasets as random (e.g., log [benchmark dose]), and others as fixed effects with specific values for each dataset and sex (e.g., background levels). The result yields a nonlinear, mixed-effect, animal dose-time response model that can be extrapolated to humans by scaling parameters. The risk assessment approach for this result uses approximate models of relationship between dose, time, and AChE inhibition and simulations of dietary exposure combined with the extrapolation model to produce estimates of human AChE inhibition time-courses. The final risk assessment is due in 2006.

Questions

Dr. Clark asked how NCCT plans to capture and disseminate the lessons learned about risk assessment from the current research project after the Center reaches the level of a standardized computational resource. Dr. Setzer responded that the Center is working on many approaches to this issue, but does not have a complete solution at present. Some patterns should emerge after the staff has completed more studies.

Dr. Clark posited that the issue for the Center is that the staff who are good problem solvers will be challenged to come up with generic practices for solving broad-based problems instead of delving into a single problem. It is difficult to visualize going from individual study results to broad concept application. Dr. Setzer agreed and added that the challenge is knowing how to give generic guidance. Until one has worked through the specifics, it is difficult to generalize.

Dr. Daston injected that he sees the problem differently and relates it to a previous discussion about developing the Center's core competencies. Serving as a resource provides a method for staff with topical expertise to link to the people who can do high-powered computational work.

Cumulative Risk Assessment Research Projects

Dr. Michael Tornero, National Center for Computational Toxicology, EPA

Dr. Tornero discussed a project entitled "Computational Solutions in the Cumulative Assessment of Pyrethroid Pesticides." He said the cumulative risk assessment of pyrethroids follows the narrow scope of the Food Quality Protection Act (FQPA), where there is a presumed common mechanism of action. One tool used to test that is dose activity. Pyrethroids are thought to act by a common mechanism. They interact with sodium channels, causing nerve firing, which manifests in tremors and other behavioral effects. The Neurotoxicology Division (NTD) is conducting an assay of behavioral effects and motor activity in a combination dose of pyrethroids administered to: (1) assess whether the chemicals act by common mechanism, and (2) if so, perform a cumulative assessment. The study also may look at the target tissue, which is closer to where the mechanism takes place. In considering the PK factors, the goal is to look at human *in vivo* target tissue data. Because it is not always available, however, the rodent data are used to inform a model composed of biological, physiochemical, and biochemical data that help devise the human model. In addition, the study is looking at computational techniques to assist in constructing models.

Cumulative Risk Assessment Research Projects
Dr. Woodrow Setzer, Statistician, National Center for Computational Toxicology, EPA

Dr. Setzer presented information on the research project entitled "Mixtures of Molecules Active Through Binding to Acetylcholine Esterase (AChE): Understanding Cumulative Effects Through Modeling Key Steps in the Mechanism of Action." The research problem is that toxic exposures are often to mixtures of chemicals that are AChE inhibitors; however, most data address single chemicals in this class. For single chemicals, the difference in the effect caused by the binding is different from that for chemical mixtures, and that difference is critical.

Exposure Research
Dr. Elaine Cohen-Hubal, National Center for Computational Toxicology, EPA

Dr. Cohen-Hubal presented information on the Agency's approach to exposure research and outlined some current activities in that area. She told participants that characterizing cumulative risk and understanding exposure-outcome relationships require the collection and analysis of a wide range of chemical, physical, biological, and psychosocial data at multiple levels of organization. These types of analyses are needed to: (1) conduct national-scale, regulatory-based risk assessments; (2) conduct community-based risk screening and remediation; (3) support epidemiology studies investigating gene-environment interactions; (4) characterize exposure and risk for public health tracking; and (5) design and test interventions. Increasingly, the Agency is being held accountable for tracking and evaluating interventions.

Dr. Cohen-Hubal has conducted some preliminary (conceptual) research on assessing children's exposures, the use of biomonitoring data to assess cumulative risk, and incorporation of psychosocial factors into cumulative risk assessments. NCCT plans to address the significant challenges associated with characterizing cumulative risks by applying a systems approach to a human-receptor-based framework and using visual analytic tools. The conceptual modeling framework that the Center proposes is advantageous in that it sets out clear assumptions, communicates assumptions, and organizes and carries out analyses and risk assessments.

A source-to-outcome approach can be quite valuable, but is difficult to implement in assessment of multiple exposures. The NCEA draft document, "A Framework for Assessing Health Risks Resulting From Exposures to Children," provides an overarching framework for a more thorough assessment of health risks from exposures to children that examines the impact of potential exposures during all stages of development. The Center contributed to the NCEA Framework by introducing use of the person/population oriented conceptual model for assessing risks resulting from exposures to children. Center contributions to the NCEA framework also included development of the exposure assessment approach that includes two-tiers and requires probabilistic methods when multiple factors are evaluated. Other current Center activities address development of a biomonitoring framework and a framework for incorporating psychosocial factors into cumulative risk assessments. Additional challenges of integrating human health and ecological risk assessment are being considered in this framework.

The Computational Toxicology Program is proposing to apply these previous activities to develop tools applicable to cohort studies of children's environmental health. Several significant

cohort studies are currently being designed and/or conducted, including the Detroit Children's Study, the North Carolina Cohort Study, and the National Children's Study. The science question for each of these studies is: "Given multiple exposures and multiple outcomes, as children grow and develop across time, how do we understand the relationships in this multidimensional space?" Center researchers propose to apply a human-receptor-based framework, a systems approach, and visual analytic tools to address the challenges associated with using results of these studies to assess cumulative risks. Visual Analytics (VA) is a new concept that merges scientific and information visualization to represent complex multidimensional data. It includes technologies from fields of information extraction, knowledge management, and statistical analysis. NCCT will take advantage of VA capabilities that are being developed in the Scientific Visualization Center at the EPA National Environmental Scientific Computing Center. As a first step, NCCT proposes to develop collaborations with the STAR Children's Center grantees. Data collected by these centers, which can be made available with appropriate human subjects clearance, will be combined with additional publicly available data and used to explore the potential of visual analytics to facilitate evaluation of the effects of environmental exposures on child health and development. Results will be used to develop concepts and tools for application to the Detroit Children's Study, the North Carolina Cohort, and the National Children's Study.

Questions and Comments

Dr. Daston commented that, for quite a while, it has been a theme of the BOSC to include social scientists in conducting risk assessments and complimented NCCT for taking the initiative to involve social science in their studies of children.

Public Comment

Ms. Kowalski indicated that no one had requested time to speak during this period reserved for public comment.

Discussion

Computational Toxicology Subcommittee

Dr. Daston stated that the remainder of the day would include developing a schedule for producing a letter report addressing the subcommittee's charge questions. He proposed a followup conference call with the subcommittee members, which was tentatively scheduled for May 20, 2005.

Dr. Daston mentioned that the present subcommittee was rather small and asked for opinions on what additional expertise might be needed. He requested suggestions of specific individuals whenever possible, citing that it will be challenging to find candidates without conflicts of interest, due to the large number of collaborations with the NCCT. He also mentioned that, given the necessity of collaboration at all levels of research, potential contribution to collaborative efforts was more important than the ease of identifying member candidates.

- Dr. Di Giulio suggested community issues and bioinformatics as two areas of expertise

that should be added. He recommended John Quakenbush as someone whose expertise could benefit the subcommittee.

- Dr. Daston raised the possibility of ecological modeling as a needed area of expertise. Dr. Clark agreed and said he did not know anyone to recommend.

- Dr. Di Giulio agreed to try to locate someone with PBPK experience.

- Dr. Kavlock suggested that the issue of cumulative risk warranted the inclusion of a computational chemist.

- Dr. Blancato mentioned that the subcommittee might want to consider a person with expertise in data use and assessment and, possibly, someone with knowledge of exposure issues.

Dr. Daston thanked the members for their suggestions. He agreed to coordinate suggestions of potential members who were external to EPA and Ms. Kowalski and Dr. Kavlock agreed to coordinate suggestions from EPA employees.

Changing the subject to preliminary development of the subcommittee's letter report, Dr. Daston requested comments on each of the charge questions from Drs. Clark and De Giulio. The subcommittee members used approximately 20 minutes to review and respond to the charge questions, ask questions of the NCCT staff, comment on the material presented during the meeting, and exchange information to assist overall preparation of the subcommittee's evaluation report. Following their discussion, Dr. Daston asked that all written comments from other subcommittee members be forwarded to him by May 15, 2005. He also said that a draft of the letter report would be circulated to all involved before the conference call on May 20.

In concluding the meeting, Dr. Daston noted that beyond the charge questions, the subcommittee was impressed by the efforts undertaken by the Center to date. Ms. Kowalski reminded the participants that the proceedings of the meeting would be posted on the BOSC Web Site (http://www.epa.gov/osp/bosc/subcomm-ctox.htm) when they became available. The next step involved subcommittee members collaborating on the report via a public conference call. She will forward the logistical information on that call to the BOSC subcommittee members and EPA staff. The meeting was adjourned at 12:08 p.m.

Action Items

- Dr. Di Giulio will obtain information on John Quakenbush, whom he recommends as a potential subcommittee member, identify someone with PBPK experience, and forward the information on both individuals to Dr. Daston.
- Dr. Daston will coordinate receipt of suggestions pertaining to potential subcommittee members from outside of EPA.
- Ms. Kowalski will coordinate with Dr. Kavlock regarding receipt of suggestions pertaining to potential subcommittee members from EPA.
- Subcommittee members will send their written comments on the evaluation charge questions to Dr. Daston by May 15, 2005.
- Dr. Daston will compose the first draft of the subcommittee's letter report based on his notes and comments from the other subcommittee members. He will forward the draft letter report to the DFO and subcommittee members before the May 20 conference call.

APPENDIX A:

Agenda
List of Subcommittee Members and Meeting Participants

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U.S. EPA BOARD OF SCIENTIFIC COUNSELORS
Computational Toxicology Subcommittee

DRAFT AGENDA
April 25-26, 2005

Environmental Protection Agency
Room C-111A
109 T.W. Alexander Drive
Research Triangle Park, NC 27711

Monday, April 25, 2005

10:00 - 10:30 a.m.	Registration	
10:30 - 10:45 a.m.	Welcome and Introductions DFO Remarks	Dr. George Daston, Subcommittee Chair Ms. Lori Kowalski, ORD
10:45 - 11:00 a.m.	Acting Deputy AA/ORD Remarks	Mr. Lek Kadeli
11:00 - 12:15 p.m.	Background and Direction of ORD's National Center for Computational Toxicology (NCCT)	Dr. Robert Kavlock, Director, NCCT Dr. Jerry Blancato, Deputy Director, NCCT
12:15 - 1:15 p.m.	Lunch*	
1:15 - 2:15 p.m.	Summary of FY04 ORD Computational Toxicology Activities	Dr. Robert Kavlock, Director, NCCT
2:15 - 2:45 p.m.	Discussion	Computational Toxicology Subcommittee
2:45 - 3:45 p.m.	Research Theme I: Information Technology	Dr. Ann Richard, NCCT
3:45 - 4:15 p.m.	Break*	
4:15 - 5:15 p.m.	Research Theme II: Prioritization	Dr. Robert Kavlock, Director, NCCT Dr. James Rabinowitz, NCCT
5:15 - 5:30 p.m.	Review of Day 1 Activities	Dr. George Daston, Subcommittee Chair
5:30 p.m.	Adjourn	

Tuesday, April 26, 2005

8:30 - 9:30 a.m.	Research Theme III: Biological Models	Dr. Hugh Barton, NCCT Dr. Woodrow Setzer, NCCT Dr. Michael Zager, NCCT/UNC Dr. Jerry Blancato, Deputy Director, NCCT
9:30 - 10:30 a.m.	Research Theme IV: Cumulative Risk	Dr. Elaine Cohen-Hubal Dr. Woodrow Setzer, NCCT
10:30 - 10:45 a.m.	Public Comment	
10:45 - 11:00 a.m.	Break*	
11:00 - 12:00 noon	Discussion - Highlights from posters - General	Computational Toxicology Subcommittee
12:00 noon - 12:30 p.m.	Future Business - Action Items - Letter Report - Next Meeting	Dr. George Daston, Subcommittee Chair
12:30 p.m.	Adjourn	

*Note that posters covering ORD Computational Toxicology Center research will be available in the meeting room and/or adjacent space and can be viewed during meeting breaks.

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Computational Toxicology Subcommittee Site Visit

Participants List
April 25-26, 2005

Environmental Protection Agency
Room C-111A
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