



PROCEEDINGS:

REGION/ORD WORKSHOP ON ENDOCRINE DISRUPTORS

May 1 - May 3, 2001
Atlanta, GA



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FOREWORD

The *Region/ORD Endocrine Disruptors Workshop* was the sixth in a series of Regional Science Topic Workshops sponsored by the Office of Science Policy (OSP) in the Office of Research and Development (ORD) at the U.S. Environmental Protection Agency (EPA). Other workshops in this series included:

- *Asthma: The Regional Science Issues*
- *Communicating Science: Waves of the Future Info Fair*
- *FIELDS*
- *Nonindigenous Species*
- *Pesticides*

The objectives of the Regional Science Topic Workshops are to: 1) establish a better cross-Agency understanding of the science applicable to specific Region-selected human health and/or ecological topics, and 2) develop a network of EPA scientists who will continue to exchange information on these science topics as the Agency moves forward in planning education, research, and risk management programs.

Each year, EPA Regions identify priority science topics on which to conduct workshops. The workshops address the science issues of greatest interest to the Regions on the selected topic area. Each workshop is planned and conducted by a team of Regional, ORD, and interested Program Office scientists, is led by a Regional chairperson and facilitated by one or more Regional Science Liaisons to ORD. Participants maintain the cross-Agency science networks they establish at the workshops through planned post-workshop projects and activities, such as the identification of collaborative research opportunities, creation of information sharing mechanisms (e.g., interactive web sites), and development of science fact sheets for Regional use.

For additional information on a specific workshop or on the Regional Science Topic Workshop series in general, contact David Klauder in ORD's Office of Science Policy (202-564-6496).

EXECUTIVE SUMMARY

The ORD/Region Workshop on Endocrine Disruptors was held on May 1 - May 3, 2001, at the EPA Region 4 Offices in Atlanta, Georgia. The workshop was chaired by Bobbye (Barbara M.) Smith (Region 9 Regional Science Liaison to ORD [RSL]), and David Klauder, Regional Team Leader in the OSP/ORD, with assistance from Thomas L Baugh (Region 4 RSL) and Patti Lynne Tyler (Region 8 RSL). The workshop was organized into seven sessions:

- I. State of the Science: General
- II. Bioassays: Development, Applications, and Use of Results
- III. State and Application of Toxicologic Data
- IV. Communicating the Science to EPA Managers and the Science Community
- V. Communicating the Science to the Public
- VI. Sources of Exposure and their Management
- VII. Next Steps

Regional and Office of Research and Development (ORD) staff presented case studies and research protocols as a way of illustrating the major science issues underlying the detection, evaluation, and regulation of endocrine disrupting chemicals (EDCs). Subsequent discussions revolved around how the information could be used in support of the emerging EDC issues. The discussions also highlighted how additional field data and other Regional information could augment the development and validation of applicable ORD and OPPT (Office of Pollution Prevention and Toxics) bioassays, models and databases.

Break-out sessions identified: 1) how the Regions could use the science presented; 2) what scientific uncertainties limit EPA's ability to conduct assessments of the endocrine effects of chemicals; and 3) what products or tools would help fill the gaps in science information. A mock public meeting was held to assist scientists in understanding the difficulty in clearly communicating the state of the science with respect to endocrine disruptors to interested and affected stakeholders and community members. The resulting discussion identified potential approaches to improved communication including Workshop products to assist Regional staff, and the need to develop lines of communication among ORD, Regional staff, and local health care professionals.

Participants expressed appreciation for the opportunity to view their own work in the context of other related activities across the Agency and for the opportunity to network with those doing supporting research. The workshop format was thought to be an excellent opportunity to identify how available science could support Regional activities and identify areas where further research is needed.

Call to Order: Bobbye (Barbara M.) Smith, (Region 9)

Welcome: Bobbye Smith, (Region 9)

Introduction: Stanley Meiburg, (Region 4)

SESSION I: STATE OF THE SCIENCE - GENERAL

Elaine Francis (ORD/NCER), Co-Chair

Cornell Rosiu (Region 1), Co-Chair

Marian Olsen (Region 2), Facilitator

Overview: What is Known and What are the Scientific Uncertainties - Bob Kavlock (ORD/NHEERL)

Endocrine disrupting chemicals (EDCs) are exogenous substances that alter the function(s) of the endocrine system and consequently cause adverse health effects in an intact organism, its progeny, or (sub)populations. EDCs are of global concern and have been the subject of more than 30 major international meetings in the last six years, in which the EPA played a key role.

There is a growing body of evidence demonstrating the deleterious effects that EDCs have on human health and the environment. MEDLINE citations, alone, have increased nearly three-fold over the last decade. Reported human health effects include increases in breast cancer, shortened lactation time in nursing women, reduction in semen quality, and developmental learning disabilities in children exposed to EDCs *in utero*. There is a larger body of evidence concerning EDCs' effects on wildlife. Eggshell thinning in raptors, limb deformities in amphibians, altered breeding behaviors in gulls, reduced reproductive success in fish, and malformations in reptiles and birds are some of the reported impacts on wildlife.

Many different classes of chemicals exhibit endocrine disrupting effects. Such chemicals occur as ingredients in flame retardants, pesticides, pharmaceuticals, plasticizers, and surfactants. There are also naturally occurring, found in such products as soy.

Conclusions and Next Steps

Major uncertainties concerning the effects of EDCs remain. More data are needed on exposure-outcome linkages and dose/response relationships. Some EDCs appear to have more than one mode of action. For example, some chemicals seem to act and interact differently in different tissues.

Questions and Responses

Question: Is there direct evidence linking endometriosis to EDC exposure?

Response: No, there is little evidence showing a relationship between EDC exposure and endometriosis. There are some data demonstrating a connection between dioxin exposure and endometriosis; however, there is not enough research at the present time to make a determination [of direct cause and effect].

Science Issues and Communication at Superfund Sites with Suspected Endocrine Disrupting Chemicals - Marian Olsen (Region 2)

The presentation focused on the issues associated with evaluating human health risks at Superfund Sites. In assessing risks associated with Superfund Sites, human health risk assessors evaluate cancer risks and non-cancer health hazards to the reasonably maximumly exposed (RME) individual, as well as to the Central Tendency (CT) or average individual. The risk assessment is performed under baseline conditions i.e., in the absence of cleanup and/or institutional controls, such as fish consumption advisories, as part of the Remedial Investigation of the Site. The examples presented were primarily of river systems – sediment sites contaminated with bioaccumulative chemicals including PCBs, a suspected endocrine disrupting chemical.

In the face of the current scientific knowledge about endocrine disrupting chemicals, the concerns raised by the community and the responsible parties often differ. Communities raise concerns about whether the chemical have a potential for endocrine disrupting effects and their potential impacts on human or environmental health. At the same time, responsible parties have identified uncertainties regarding the scientific information on endocrine effects at environmental concentrations.

Human health risk assessors, in evaluating exposure to chemicals from Superfund Sites, consider multiple exposure routes (i.e., ingestion, inhalation and dermal contact). Humans may be exposed to Site contamination through ingestion of fish and drinking water from a river that serves as a drinking water source or while swimming; inhalation of volatilized chemicals during recreation; and dermal contact (e.g., wading, boating, swimming) with water or sediment,. The determination of fish ingestion rates, swimming frequency, frequency of exposure, and duration of exposure relies on a number of different sources of information. For example, to estimate fish consumption rates, sources of information may range from Site-specific creel surveys, state-wide creel surveys, the Superfund's default exposure assumptions and information presented in EPA's Exposure Factors Handbook. The risk assessor uses all of these resources to choose the most representative value for each of the exposure parameters.

Depending on the extent of site contamination, the size of a Superfund Site may range from a small geographic area to hundreds of square miles. For large Sites, consideration should be given to individuals living in the communities surrounding the Site, since they may continue to fish within the contaminated area of the Site. Census data may be one source of information for the human health risk assessment (demographics, including how long individuals reside in the area of the Site, and income level, can inform ingestion pathway assumptions).

Cancer and non-cancer toxicity factors were discussed and data presented that emphasized the importance of including consideration of children's health in Superfund risk assessments.

Probabilistic risk assessment was described as a valuable tool. This technique can be used to evaluate variable exposure assumptions and a range of possible effects, as well as the uncertainties associated with each, based on available Site data.

Conclusions and Next Steps

The human health risk assessment provides a method for evaluating the risk associated with chemicals from a contaminated Site, based on various routes of exposure. At many contaminated sediment sites, fish ingestion has been determined to be the primary exposure pathway of concern. This conclusion is based on comparing the cancer and non-cancer health risks from ingestion of fish, with risks associated with other routes of exposure, e.g., dermal and inhalation pathways. The results of the baseline risk assessment are then compared to regulatory levels as a way to evaluate whether Remedial Action may be necessary at the Site. Using the results of the risk assessment, the next steps in the Superfund process include performing a Feasibility Study that includes development of remediation goals and Remedial Action alternatives.

At Superfund sites, public meetings are conducted throughout the Remedial Investigation/ Feasibility Study process to address the concerns of the community. After consideration of public comment, a Draft Proposed Plan is released that summarizes the cancer risks and non-cancer health hazards and the proposed Remedial Action. At public meetings, stakeholders have commented on many issues regarding endocrine effects including: concern over the known health effects of EDCs; concern over the unknown effects of EDCs; concern over the differences in scientific opinion concerning the importance of EDCs with respect to human and environmental health; and concern over the adequacy of proposed remediation goals. A comment period is available during which the stakeholders may submit comments on the Proposed Plan. EPA then develops the Responsiveness Summary that addresses these comments and this is published as part of the Record of Decision (ROD) for the Site.

Questions and Responses

Question: With all of the uncertainty concerning EDCs, do you have to respond in writing to comments regarding your decisions that address the adequacy of the protective EDC endpoints?

Response: At [Superfund] Sites, a Responsiveness Summary is developed to address comments that are submitted by the stakeholders during the comment period for the Proposed Plan.

Comment: Concerning the Responsible Party's comment that there is no credible evidence on the effects of EDCs, they must be speaking about there being no credible evidence on the effects of background levels because many studies demonstrate the impact of these chemicals on animals.

Response: In general, the comments address the uncertainties associated with both human epidemiological studies and animal studies.

Question: How do you handle, in public meetings, the question of alternative medical etiologies for, say for example, some of the thyroid conditions?

Response: Usually these issues would be discussed privately, based on concerns about personal confidentiality issues. We work with the individual and the state health department, as appropriate, to address the individual's concerns.

Question: On the issue of non Site-related exposures and cumulative effects, how does that enter into your risk assessment?

Response: Under the Superfund law, we are responsible for evaluating only Site-related exposures for multiple chemicals and multiple pathways of exposure. We do not consider exposures from other regulated sources since they are addressed by other EPA programs i.e., RCRA, CAA, CWA, etc.

Ecological Assessments of Endocrine Disrupting Chemicals - Cornell Rosiu (Region 1)

Ecological risk assessors' definitions of endocrine disrupting chemicals (EDCs) are quite diverse. The definition used for this presentation was taken from ORD's 1998 research plan on endocrine disruption:

Endocrine disrupting chemicals affect normal hormone production, release, transport, metabolism, binding, action, or elimination which maintain homeostasis and regulate development.

Ecological risk assessment examines the biological organization of communities. It involves a continuum of responses moving from those of lower ecological relevance (e.g., change in response in a single biochemical system) to those of higher ecological relevance (e.g., change in population size). Risk assessors are challenged in their attempts to determine the significance of an exposure to a chemical on populations or communities.

The presenter stressed that ecological risk assessments (ERAs) are place-based. ERAs address a specific defined habitat and contaminants. One aspect of the ERA addresses population dynamics and emigration/immigration patterns and attempts to determine what impact the Site has on populations of organisms within the defined geographic area of the Site.

Much research is currently being conducted on fish species, especially in the Great Lakes region. Much is already known about exposure to EDCs and the effects on development of age classes of identified species but the relationship to body burden is less clear. The issue of bioaccumulation in fish is a complex problem because of the need to consider various metabolic pathways including: uptake of contaminants through ingestion and absorption of contaminants through the gills; with concurrent loss of contaminants through metabolism, egestion, excretion, and dilution of tissue concentrations due to growth.

The presenter discussed the results of a baseline ecological risk assessment conducted at the Centredale Manor Superfund site on the Woonasquatucket River in Rhode Island.

Conclusions and Next Steps

The activities conducted thus far served as a pilot study. Currently, work includes preparations for a more detailed field study and laboratory research on channel catfish because bullhead catfish and carp are no longer present at this Superfund site due to their susceptibility to TCDD.

Questions and Responses

Question: You discussed analysis for TCDD. Did your analysis identify other potential contaminants that might account for the effects you are seeing?

Response: Yes, we conducted a screening level risk assessment and we weren't able to eliminate many, if any, chemicals so we've taken into our ecological risk assessment the whole range of organics and inorganics.

Validation Status of EPA's Endocrine Disruptor Screening Program - Tony Maciorowski (OPPTS/OSCP)

Tony Maciorowski described the efforts conducted to validate EDC testing protocols in support of EPA's screening and testing program, which came about as a result of the Food Quality Protection Act (FQPA) of 1996. The FQPA was the first new testing authority that the Agency had received in a number of years. The act amends the Federal Food Drug and Cosmetics Act and the Federal Insecticide Fungicide and Rodenticide Act (FIFRA). Under the FQPA, EPA has authority to require screening for any pesticide and for any chemical that is persistent or bioaccumulative. The presenter described the Endocrine Disruptors Screening Program (EDSP).

EPA was mandated to establish and implement a screening program within a three-year time frame. The main aspects of the EDSP are: development of tools to sort and prioritize chemicals; screening chemicals for endocrine activity and further testing when results are positive (Tier 1 screening); identify adverse effects and dose/response relationships for hazard assessments (Tier 2 testing).

Sorting and priority setting will be assisted by an Endocrine Disruptor Priority Setting Database (EDPSD). The purpose of the EDPSD is to rank chemicals for screening. The EDPSD will focus largely on commodity chemicals and pesticide active ingredients. Priorities for pesticide actives will be based on criteria developed from a pilot program specific to pesticides. Details regarding the EDPSD can be found at:

<http://www.epa.gov/opptintr/chemrtk/volchall.htm>

Quantitative Structure Activity (QSAR) models are also being developed for incorporation into the EDPSD. EPA has entered into partnerships with the Food and Drug Administration's National Center for Toxicological Research, and the University of Bourgas to develop and validate the QSAR models. Current efforts are focused on an estrogen receptor model (rather than the androgen receptor model) since it is more substantially developed.

In its attempts to develop the EDSP, EPA is applying a set of principles established by the Interagency Coordinating Committee for the Validation of Alternative Methods. This validation approach utilizes a defined scientific process, a member agency administrative process, and a domestic and international harmonization process.

Both Tier 1 and Tier 2 tests are currently undergoing validation by ORD. The Tier 1 screening battery currently includes seven assays: receptor binding assays, uterotrophic, Hershberger, female pubertal, steroidogenesis, frog metamorphosis, and fish reproductive screen. Alternative Tier 1 assays may include: male pubertal, placental aromatase, and *in utero* through lactation assays. Five Tier 2 or testing assays are being validated: mammalian development and reproduction; avian development and reproduction; mysid shrimp life cycle, fish reproduction and development; and amphibian development and reproduction.

Conclusions and Next Steps

EPA is bound by a settlement agreement to complete its work according to the following schedule:

- Priority Setting: complete by the end of FY2001;
- Tier 1 Validation conducted through mid-2003;
- Tier 2 Validation complete mid-2005; and
- Phase 1 implementation complete by the end of FY2005.

EPA faces the challenge of integrating the science and policy at the same time as complying with the time schedule for the settlement agreement.

Questions and Responses

Question: What is *in utero* through lactation?

Response: It's where, in mammalian reproduction, exposure occurs [to the animal] from *in utero* through the end of lactation.

Question: On the initial [*in vitro*] assays, have you already contracted out validation for those or are they already finished?

Response: There are several things ongoing. Four background review documents are being developed looking at specific methods in the literature to determine if enough data exist to validate the methods, and we have contracted with several laboratories to do some validation on those methods.

Comment: Some people have the impression that the Tier 1 Screening data will replace the testing data, and that is not correct. The Tier 1 studies are not intended for use in risk assessment.

Overview: ORD's Activities to Address Uncertainties and Regulatory Mandates for Endocrine Disrupting Chemicals - Elaine Francis (ORD/NCER)

EPA is studying endocrine disrupting chemicals (EDCs) for several reasons. Evidence indicates that EDCs may cause adverse health effects in humans and wildlife populations. The chemicals of concern are within EPA's regulatory authority. Additionally, many uncertainties exist in our knowledge of endocrine disruptors – the nature of their effects, the extent of the problem, and their dose/response relationships.

ORD has played a major leadership role in addressing EDCs. At the Agency level, ORD identified EDCs as an emerging public health and environmental issue in 1994. The Office of Science Policy's (Office) EDCs program grew out of ORD's efforts to integrate, into a single program, the research being conducted across ORD's laboratories. To accomplish this goal, the Office organized and hosted two international research needs workshops in 1995. The Office published an interim science assessment in 1997, a peer-reviewed research plan in 1998, and a draft multi-year plan in 2000.

At the federal level, ORD chairs the Committee on Environment and Natural Resources (CENR) Endocrine Disruptors Working Group which has representation from 14 federal agencies. On a national and international scale, ORD has co-sponsored a series of workshops on screening methods in collaboration with industry and the World Wildlife Fund. ORD also participates in joint collaborations with the European Union and Japan.

In 1996, ORD identified EDCs as one of its risk-based high priority areas in its Strategic Plan. ORD's Research Plan for Endocrine Disruptors incorporates the science needs for a screening and testing program and identifies a series of key research questions that the program will address. The Research Plan is divided according to the risk assessment paradigm with sections on biological effects studies, exposure studies, and linkage studies.

Intramural and extramural research programs were presented. The intramural research program is concentrating its efforts on four areas: 1. effects research which includes developing new or improved protocols for the EDC screening and testing program; 2. exposure research, including the development of predictive models to estimate the extent and magnitude of exposure; 3. risk assessment research, including developing a case study for methods on integrating human health and ecological EDC data into risk management; and 4. risk management research, including identifying major sources of EDCs entering the environment and developing tools for management of unreasonable risks. The extramural program is executed through the STAR (Science To Achieve Results) program and currently includes 32 grants and one fellowship (see www.epa.gov/ncerqa). Grantees are researching various endpoints in a wide variety of species. A total of 83 chemicals are under study.

Finally, ORD is supporting EPA's screening and testing program by conducting research, developing protocols, and serving as a consultant to OPPTS in the standardization/validation process.

Conclusions and Next Steps

Future research outcomes include:

- Developing new assays for the Agency's screening and testing program;
- Determining the magnitude of the adverse impacts of EDCs on human health;
- Estimating their impacts at the population level in wildlife species;
- Developing approaches for reducing exposures to EDCs from contaminated sediment, wastewater treatment outfalls, confined animal feeding operations, and combustion sources;
- Developing improved methods and models for exposure assessments; and
- Developing improved approaches for integrated risk assessments.

Questions and Responses

None

Roundtable Discussion - Session Presenters/Audience

Question: From a risk assessment standpoint, since endocrine disruption is a mode of action rather than an endpoint, does that imply that if a chemical is a developmental toxicant and we have traditionally looked at a chemical by the methods we used to evaluate that endpoint [developmental toxicant], does the fact that the chemical is an endocrine disruptor change our view about what the data showed us about the traditional developmental test or has it incorporated endocrine disruptor outcomes or modes of action? In other words, if we have a chemical in our system someplace that has had no substantial endocrine or developmental processes tests done on it, do we have any confidence at all that is still a good basis for making decisions concerning endocrine effects?

Response: You are asking, “have we done any standard hazard associations, and are we satisfied with the belief that we have conducted sufficient testing to justify the endocrine effects reported?” In this case, because we happen to know the mode of action, that gives us more confidence in the extrapolation that the effects are going to appear across multiple species. I am not sure we have to be more conservative because of that. I think that the traditional endpoints (if the studies are well conducted, if they have the right endpoints, if they are doing the right kind of studies, if they have done the correct assessments, the standard way we have done our calculations), are good for looking at EDCs and basically that is what peer review last fall [concluded], although they focused on making sure you had good robust hazard identification studies done on these chemicals.

Those are questions that we hope that the Endocrine Screening Program pilot study will address. The pilot study will look at 25 to 50 chemicals. We plan to evaluate the adequacy of the existing database for those chemicals. It is too early to determine how we should look at the endpoint. At this point in the process, we don't want to say that endocrine disruption is something special, as opposed to neurotoxicity or reproductive toxicity. It is too early, at least from the regulatory standpoint, to address those issues.

From an ecological standpoint, a lot of the information that has been presented is biased toward the human health side. There are a number of people that are interested in the ecological impacts and expanding the number of species that should be looked at. On the ecological side the pilot project is looking at a group of chemicals with a wide range of data in terms of quantity and quality. We will be looking in more detail at each chemical regarding the actual studies we have in our files, as well as in the literature, and will try to determine what additional data will be required to help us in assessing EDC impacts. The primary goal is to look at a sample and be able to have some type of priority setting and set some kind of criteria.

Comment: The ecological tests were designed pretty much 20 years ago, so there is nowhere near the amount of pathological examination. Thus, there is a large dichotomy in the ability to look at mechanisms. If there is an existing two-generation study and

it does not look at endocrine endpoints, what do you do? One of the things that the *in utero* lactation test or screen may do is fill some of the end point gaps without having to completely redo a two-generation study.

Comment: If you have developmental toxicology data and you are looking at an EDC, is the old database inadequate? If you only have developmental toxicity/teratology test data, then I think the data are inadequate [for EDC evaluation] because of the limitations of the older data, in that the exposure period does not include the reproductive development, and there is no assessment of the fetal animal unless you have multigenerational studies.

Question: A committee of a national research council (committee on risk management of PCBs and PCB-contaminated sediments) issued a report. They seem to be very enamored with using tree swallows as monitors over time. The report even suggests that the correlations between concentrations in tissue and sediments are such that this could put together a very effective monitoring program. What is your impression of using tree swallows as a monitoring scheme in contaminated areas or delimitating between contaminated areas?

Response: The utility of using tree swallows in doing ecological risk assessments is the fact that once they do set up in a reproductive mode they forage in a tight radius of on half kilometer. This allows you to very easily recognize differences in sites when comparing data-even from sites that are comparatively close. They are useful for that reason. The only reason I would question their use is that if you are simply looking at tree swallows and not aquatic organisms, you are not looking at this other direct contact exposure that can occur with the sediment. You would not want to rely solely on the tree swallow.

Question: On the ecological side, for some of these EDC pesticides, concerns have been expressed about the residue levels on certain crops. What sort of action is taken to confirm whether there are effects or is it just an observation? At what point does the risk become significant enough to trigger research to see if there is an EDC effect?

Response: To make any determination, you need to know how well the study was designed, what tools were used, which tools were least vulnerable to background contamination, were the selected tools the most sensitive available. We must remember that the ability to call in data is great, but the ability to regulate is not so great. A while ago, a chronic [adverse] effect on a bird species was not going to prevent registration. Today, we are more concerned with chronic effects and some regulatory decisions are being made based on those effects. In 1992, there was a policy shift away from field studies for pesticide registration. On a registration action, you are likely to get better data, more data, and more innovative data, because the registrant wants to register the compound. On a re-registration, it is very difficult to get new data.

SESSION II: BIOASSAYS - DEVELOPMENT, APPLICATION, USE OF RESULTS

Tala Henry (ORD/NHEERL), Co-Chair
Sharon Thoms (Region 4), Co-Chair
Patti Lynne Tyler (Region 8), Facilitator

Region 4 Case Study: Lake Apopka and Tower Chemical Ecological Risk Assessment – Focus on Reptiles - Sharon Thoms and Galo Jackson (Region 4)

Since the early 1980s, Florida has conducted research on alligator populations. Data show that clutch viability and juvenile recruitment in Lake Apopka have remained low compared to other Florida lakes. Field studies have shown that the Lake Apopka alligators have experienced unique changes in reproductive parameters and endocrine systems.

A 1980 storm caused a pesticide manufacturer's wastewater percolation pond to breach. The resulting spill released wastewater originally thought to contain a mixture of dicofol, dichlorobenzil, and DDT and its metabolites into Lake Apopka. By 1986, juvenile alligator populations in the lake were reduced to 10 percent of their 1980 level.

Male and female alligators raised in captivity from eggs collected from Lake Apopka in 1992 displayed modified gonadal morphology when compared to alligators raised from eggs collected elsewhere. Abnormalities were noted in reproductive organs and germ cells of both sexes. Two alligators identified as females due to the lack of a penis, were later shown to have testes. Two other alligators had penis-like structures but were later identified as females because of the presence of ovarian tissue. Studies showed abnormally low testosterone levels in males and elevated estradiol levels in females.

It has been difficult to assign the endocrine alterations seen in Lake Apopka's alligators to any single compound since the eggs were found to contain a mixture of chemicals that exhibit ED effects (e.g., p,p'-DDD and p,p'-DDE). Additionally, the Lake's sources of contamination included agricultural activity, sewage discharge, municipal runoff, and the 1980 spill.

In ecological risk assessment, it remains difficult to identify a pattern of endocrine disruption for a particular waste chemical and to identify a causative agent from among the many stressors affecting hormonal patterns and developmental patterns. Likewise, it is difficult to rule out a suspected chemical as a source of endocrine effects in wildlife populations.

Major science needs discussed were:

- Expansion of the wildlife models currently in use;
- Better understanding of normal (baseline or unexposed) hormone levels in wildlife to serve as a reference;
- Research into the mechanisms by which steroids and endocrine-disrupting chemicals elicit organizational effects on reproductive, immune, and neurological systems in animals, especially non-mammals;
- Ability to link assays developed for specific biomarkers for a receptor-related mechanism to symptoms of contaminant exposure; and
- Data to determine how current test protocols using steroid receptors obtained from mammalian, fish, amphibian, or avian model species, can be applied to reptiles.

Conclusions and Next Steps

Region 4 is working with ORD's Las Vegas and Cincinnati laboratories to identify chemicals at the Lake Apopka Site and determine their toxicity. ORD's Las Vegas laboratory examined raw data from GC/MS analyses of two ground water samples for evaluation and identification of tentatively identified and unknown compounds (TICs) from the Tower Chemical Site. ORD was able to identify several dichlorobenzophenones that were mis-identified as another compound by the CLP laboratory. However, ORD was unable to identify all of the unknown compounds. Recommendations for alternative analytical methods or modifications to existing analytical methods to improve the identification and quantification of the dichlorobenzophenones and unknown compounds are forthcoming.

ORD Cincinnati has been asked to examine the Site data and research papers on Lake Apopka to determine if effects observed in alligators could be caused by chemicals related to the Site.

Questions and Responses

Question: Is anyone doing something on the northeast side of the Lake?

Response: The [ORD] laboratory in Athens is working on that. Contact Ann Keller.

Question: Is there pre-spill information on alligator populations near the spill Site?

Response: Not that we know about. The Lake was already undergoing changes due to other stresses (e.g., agricultural, residential development).

Question: Have you looked at invertebrates, fish, or other receptors?

Response: No, we had some fish tissue residue data, but we are unsure if it is useable. There has been some work done on turtles and mosquito fish.

Question: Have you collected core samples from the sediment?

Response: We did not collect [sediment] core samples.

Question: It appears that more mechanistic data would be helpful. Has there been any fractionation studies done to identify the active chemical?

Answer: I am not aware of any that have been done. That might be interesting – the original thought was that the chemical was a mixture of DDT and dicofol. We are now finding out that it was not dicofol, but rather dichlorobenzophenone.

Bioassays for Identification of Endocrine Disrupting Chemicals in Fish - Tala Henry (ORD/NHEERL)

Fish are important organisms to consider in screening chemicals for endocrine disrupting chemical (EDC) activity. Field observations indicate that effects due to EDCs could be affecting this class of organisms. For example, feminization and vitellogen production in male fish are associated with exposure to municipal sewage treatment effluents and EDCs in pulp and paper mill effluents produce reproductive and endocrine effects in fish. In addition, fish possess unique life history and physiological characteristics that may not be captured by mammalian screening and testing assays, i.e., the production of the yolk protein, vitellogen, by females.

Several *in vitro* and *in vivo* bioassays being developed by NHEERL in support of OPPTS screening and testing activities were presented, including:

- Rainbow trout estrogen receptor competitive binding assay;
- Rainbow trout liver slice vitellogenesis assay; and
- Fathead minnow short-term fish reproduction assay;

The scientific basis and general experimental design for each assay was discussed. The rainbow trout assays are designed to provide data for the development of quantitative structure-activity relationships (QSARs) to be used for chemical ranking and prioritization activities. The fathead minnow reproduction bioassay is one of the Tier 1 Screening Assays recommended by the EDSTAC (Endocrine Disruptor Screening and Testing Advisory Committee). Assay performance with a variety of known mammalian endocrine disruptors (e.g., estradiol, methoxychlor, methyltestosterone, vinclozolin, flutamide, and fadrozole) was summarized. It was noted that NHEERL is also evaluating the impact of EDCs on reproductive endpoints in the estuarine fish, the cunner.

Conclusions and Next Steps

A variety of assays are being developed with the goal of understanding endocrine disruption in fish species and providing tools for assessing the potential risk of chemicals via endocrine disrupting mechanism(s). Data from the different assays will be useful for various ecological risk assessment activities, including hazard identification (i.e., ranking and prioritizing chemicals for further testing) and effects characterization (i.e., screening and testing chemicals for potential endocrine disrupting activity).

Questions and Responses

Question: The texture of liver tissue makes it difficult to obtain thin slices. How did you slice the liver samples?

Response: We used an established technique, similar to that used in mammalian research. A special 'tissue-slice' microtome was used and the sample was continuously flushed with a cold buffer solution.

Question: Is induced vitellogen production a true change, or will it go away once exposure ceases?

Response: Unlike female fish, male fish do not have a 'clearance' mechanism for vitellogen. In the fathead minnow study, a single bolus exposure to estradiol resulted in elevated vitellogen levels for at least 18 days, when the test was terminated. The duration of vitellogen production will however, depend on the duration of exposure to estrogenic compound(s).

Question: Is the inducibility of vitellogen sufficiently long to be a useful measurement?

Response: Induction of vitellogen mRNA occurred in fathead minnows within eight hours and was elevated until at least 72 hours after exposure. In a laboratory test, this time course provides us with sufficient time to conduct the analysis and show a response to the dosing. However, the transient nature of the mRNA induction could be problematic if one were using it as a biomarker of exposure in the field.

Question: In the research you discussed, the fathead minnow was one of the species used as a model. This species is typically not a very sensitive indicator organism, does it follow that it would be a good *in vivo* model?

Response: The fathead minnow seems to be amply sensitive to this type of endpoint. We are seeing the types of responses we expect in males and in egg production in females.

Reproductive Consequences of EDCs in Birds: What do Laboratory Effects Mean to Field Species? - Mary Ann Ottinger (ORD Grantee – University of Maryland)

Current research is focusing on the impact of estrogenic pesticides and attempting to determine if they alter normal development and affect neuroendocrine and behavioral responses in Japanese quail. Lifetime reproductive patterns in the endocrine and behavioral components of sexuality are also being investigated. The Japanese quail presents an advantageous model system because they are a rapidly maturing and aging species; the embryos are accessible and easily dosed without concurrent maternal effects; and the endocrine, neuroendocrine, and behavioral responses are well studied.

It was noted that the sexual differentiation in birds is not the same as in mammals. We normally think of testosterone being critical for the differentiation of the male mammal. For birds, it appears that sex is determined by the relative exposure to estradiol and androgens. With potential EDCs interacting in such a “relative” sex determinant system and competing with or adding to either estrogenic, antiestrogenic, androgenic, or antiandrogenic effects, the relative exposures to levels of hormones, and thus sexual determination, would be thrown off. It was also noted that high steroidal hormone concentrations are present in the yolk in early embryonic development. The yolk serves as a depot or reservoir since the steroid can pass easily from the embryonic circulatory system into the yolk and then back again. Maternal exposure to estradiol results in increased levels of estradiol in the yolk. There is an extremely critical exposure period to steroidal hormones that alters sexual behavior in adult males. When eggs are injected with either estradiol or testosterone on day 10 or 12 of incubation, resulting males showed no sexual behavior as adults. Exposure beyond day 12 (i.e., day 14 on) does not affect sexual behavior.

The experimental design and resulting data for a two generation test using methoxychlor were presented. Offspring (F1) of low-dosed parents were exposed to the same levels of methoxychlor as the parents. Resulting F2 offspring were not dosed with methoxychlor. Results indicated that there appeared to be some “carry over effect” in the F2 generation.

Conclusions and Next Steps

Potential impacts of exposure in the field may be important to various behavioral, endocrine, and neuroendocrine endpoints. There has to be some combination of sensitive and less sensitive endpoints used in terms of developing information about the impact of a chemical or combination of chemicals on the endocrine system of animals because some of the very sensitive endpoints may not be reflected in later endpoints. These have to be examined carefully, placed in the proper context, and then integrated (laboratory and field).

Questions and Responses

Question: Have you done any work where you varied the exposure early in the development vs. late in the development?

Response: The only kind of studies that we've done with that involved the steroid hormones. Basically, in the Japanese quail once you go past that critical period, the reproductive system is not necessarily going to be altered. However, other systems (e.g., the thyroid system) can be altered later. In terms of long-term effects on reproduction, they are well organized by the time they reach 14 days of incubation.

Question: For methoxychlor, what are the population impacts at these low doses?

Response: If they are chronically exposed, as they are in the laboratory, and if it's a precocial species (e.g., quail), I would say there would be some effects especially on a delay of reproductive development and that might impact their ability to reproduce. In terms of passiformes (i.e., perching birds), exposure might effect them later on more than it would a precocial species. We haven't really begun to research altricial species very much at all.

Question: These are social animals, and I know that sound and visual cues have a lot to do with background development. Have you tried to tweak the sensitivity of this aspect – remove or reduce the cues? Have you tried to test them in isolation?

Response: Yes. It didn't really affect things.

Question: You said that you test fecal hormone levels. How many days can you wait after collection before they start to degrade? How frequently do you have to collect the specimens to get meaningful results?

Response: These studies have been done in the crane. We haven't analyzed samples using quail yet. In the crane, the steroids do not degrade; they are around for a long time. We have collected and frozen quail fecal samples, but have not yet analyzed them.

Development of an Amphibian Metamorphosis Model for Thyroid Axis - Joe Tiege (ORD/NHEERL)

Amphibian metamorphosis provides a good model for thyroid disruption for several reasons:

- Amphibian metamorphosis is a thyroid hormone-dependent event;
- Metamorphosis transforms an aquatic larval life stage to an adult tetrapod; and
- Metamorphosis is characterized by the development of new tissue, the resorption of tissue, and the remodeling of tissues.

The three primary reasons that the EDSTAC-proposed Tier 1 assay is not ready for use as a screening tool (the developmental stage 60 is relatively insensitive; tail tissue is relatively insensitive; and changes in tail resorption rates are not diagnostic) were discussed in detail. Although the proposed assay may not be viable, the *Xenopus*-based (African clawed frog) model shows promise because it provides an elaborate thyroid hormone-dependent process on which a screening method can be based and there is considerable information in the literature on the morphological development of all of the major systems, the biochemical pathways for thyroid hormone synthesis, and the best information on critical gene expression for any amphibian, because of its popularity as a model system.

A research approach strategy for gene expression analysis was discussed. Typical and novel analytical methods were presented.

Several ways that this research can benefit the Agency were presented:

- This research can serve as a basis for a thyroid disruption screening tool;
- It can establish the utility of novel analytical methods; and
- It can allow researchers to develop applications for existing environmental problems.

Data were presented to support using perchlorate as a model chemical for evaluating the amphibian methods due to its known endocrine effects on the thyroid.

Conclusions and Next Steps

Information from the literature shows that perchlorate is responsible for the inhibition of thyroid hormone production. A current research proposal is to take the existing methods and develop dose/response data for perchlorate, then compare those results against that obtained using the newer methods to define the effects of perchlorate exposure on gene expression. Also, there are plans to conduct research using water collected from contaminated sites and compare the results with single-chemical studies.

Questions and Responses

Question: Since the method is just liquid chromatography and mass spectrometry, why has it taken so long to develop the new method for this particular application?

Response: There was a capillary electrophoresis method that preceded it. There is a desire on the clinical side to “get a handle” on these other precursors or degradation products. Chromatography patterns of the compounds have already been defined, the problem is the detection method.

Question: When would you propose to begin dosing – when do you do the analysis if you don’t do it at stage 60 ?

Response: We could take some guesses. I think the transition between pre-metamorphosis and pro-metamorphosis, when the thyroid becomes functional, is a good place to start. We know that as soon as there is thyroid secreted at very low levels we have external morphological indicators that are easy to see. However, we recently started a baseline study and we’re collecting organisms at stage 50, 52, 54, 56, 58, 60, etc. and we are going to subject them to all three of the analyses. We will use that information and what we already know to make that determination.

Endocrine Disruption in Invertebrates with an Emphasis on Assays for Estuarine Crustaceans - Chuck McKenney (ORD/NHEERL)

Invertebrates present an important resource for future EDC research given their value as indicators of effects at the ecosystem level. Although crustaceans have a critical role in energy transfer through the ecosystem, they have received much less attention in EDC research than have vertebrates. The few examples of EDC investigations with these organisms suggest the importance of investigating other examples of invertebrate endocrine disruption using complementary laboratory and field studies.

The conclusions reached by several workgroups (Endocrinology, Testing, and Field Assessment) were presented and discussed. The conclusions included:

- More information on the hormonal systems of insects and crustaceans is readily available than on other invertebrate groups;
- *In vitro* assays (e.g., receptor binding) should be developed to better understand invertebrate endocrinology;
- Vertebrate hormones are found in some invertebrate phyla (e.g., echinoderms);
- Many processes/functions (e.g., metamorphosis, regeneration) are unique to invertebrates and are under hormonal control. Vertebrate studies can not be used to model these processes/functions;
- Specific elements of the endocrine process need to be integrated into existing assays using reproduction, metamorphosis, and growth as endpoints. Additional endpoints (e.g., molting, pigmentation, morphological abnormalities) should be considered;
- EDC screening and testing assays for invertebrates need to be validated and test methods need to be standardized;
- Additional systematic studies of field populations of invertebrates need to be conducted and reported; and
- EDC research in invertebrates will be most cost-effective if the focus is on those groups that are well known, ecologically important, or economically significant.

Endocrine regulation of metamorphosis in insects and crustaceans was compared. The comparative effects of two insect growth regulators (methoprene and fenoxycarb) on three crustaceans were discussed. The effects on female reproduction in fiddler crabs by the application of Altosid (contains methoprene) in a salt marsh were presented.

Conclusions and Next Steps

None presented

Questions and Responses

Question: Were the impacts of the growth regulators on other organisms, for example daphnia [water flea], looked at?

Response: Yes, in daphnids we noticed a bit of intersexuality or mixed sexuality. We were also not sure of the sexes of some copepods commonly found around wastewater treatment plants.

Question: [Could not hear question – it was not repeated]

Response: There is some evidence in the literature showing that reproduction in crustacea is controlled by steroid hormones.

Question: In today's environment, could you register methoprene?

Response: Yes, remember there have been some 30,000 chemicals taken out of production since 1988. We have seen a shift to less broad spectrum pesticides and many of the newer pesticides have lower application rates.

Question: Is anyone looking at the estrogen and androgen studies that were conducted approximately 20 years ago?

Response: Yes, there were some done with echinoderms.

SESSION III: STATE AND APPLICATION OF TOXICOLOGIC DATA

Michel Stevens (ORD/NCEA), Co-Chair
Solomon Pollard (Region 4), Co-Chair
Tom Baugh (Region 4), Facilitator

Endocrine Disruptor Screening Program: Male and Female Pubertal Assays - Ralph Cooper (ORD/NHEERL)

The purpose of this presentation was to review the pubertal protocols proposed in the EDSTAC report for the male (alternate) and female (recommended) rat, to discuss available data that indicate the ability of the protocols to detect EDCs, and to discuss the advantages and potential problems of these protocols.

The pubertal assays are designed to detect compounds that display thyroidogenic or anti-thyroidogenic activity, estrogenic or anti-estrogenic, androgenic, or antiandrogenic activity. These assays also are assumed to be sensitive to compounds that alter hypothalamic-pituitary control of the gonads and/or thyroid.

The endpoints for the assays were discussed. One of the key endpoints measured was the age of the animal at the onset of puberty as indicated by vaginal opening in the female and preputial separation in the male. A variety of chemicals were selected to test. Both protocols demonstrated that the onset of puberty could be significantly modified (advanced or delayed by days) by a variety of environmental compounds. Furthermore, in instances where a thorough dose-response evaluation has been conducted, these protocols proved to be most sensitive. Importantly, the majority of data available indicates that any alterations in body weight, that might be considered to be an important confounding factor in such studies, can be controlled by appropriate protocol design and statistical analysis. For example, atrazine, delays in the onset of puberty at a dose as low as 12.5 mg. per kg, yet there was no alteration in body weight until the dose reached 150 mg. to 200 mg. per kg. Addressing the sensitivity of these assays their ability to detect relatively weak EDCs, the atrazine dose-response study revealed a lower NOAEL and LOAEL than previously published (i.e., the dose required to induce mammary gland tumors, 22.5 mg/kg/day for >6 months)

Concerns and Next Steps

This study indicates that pubertal assays can be used to detect a wide variety of EDCs. These assays have a number of advantages and disadvantages:

Advantages:

- They are apical tests;
- Produce good dose response data, the issue of metabolism is addressed because it is an *in vivo* test, and you can dose individual rats;

- Provide information for mode of action and mechanistic studies;
- Appear to be robust across several strains of rats; and
- Protocols involve relatively simple procedures.

Disadvantages

- Value of some of the hormone measures is still unclear;
- Body weight issue appears to be resolved but still have some concerns that a compound may exist that shows delay in puberty in male simply because it affected body weight;
- Dosing is not done during organogenesis – this is not a transplacental assay; and
- Cost might be a factor

Questions

Question: How would you interpret the results of the battery run to classify a substance as an EDC, which of the measurements or how many do you need in order to classify the chemical?

Response: Any of them.

Question: How do you determine the dose selection for chemicals that we know little about?

Response: The dose selection is usually based on the MTD [Maximum Tolerated Dose - which is traditionally set as a 10% reduction in body weight in a subchronic feeding]. Most studies would use a dose just below the MTD, then maybe 50% of the MTD and then a lower dose; so there are three doses plus the control. My concern is that when you have a steep dose/response curve [this approach] gets messy. I should think that you could be able to answer most of your concerns if you use levels just below the MTD. The problem with [this approach] is that if you are giving estrogen, the MTD is going to be extremely low I would probably have to keep going lower than I could see. The animal is very sensitive to the appetite depressant effects of estradiol and I don't know if you would ever see an estrogenic effect with estrogen. So these are the body weight issues that exist.

Comment: When we've looked at estrogen with methoxychlor in the multi-generation studies with the estrogenic toxic substances, you do see an acceleration of vaginal opening and when you are accelerating a landmark I don't think that body weight is a big concern. And that is an important point.

Which endpoints should be used to call a chemical an endocrine disruptor? I think the point made from the screening assays is that these results from these assays were not to label things as EDCs, it was to identify them as endocrine active and trigger them into Tier 2 testing. A lot of the controversy was over prematurely labeling chemicals as endocrine disruptors.

Question: What is meant by prepubital separation? And my other question is since you have such a battery of tests, how do you handle conflicting information; doesn't this whole question boil down to one or two assays that would answer the question for you simply whether or not puberty has developed?

Response: By prepubital separation, we mean that the prepuce (foreskin) on the penis separates from the glan penis. This is an androgen dependent event.. Your

second question was a little confusing – [are you asking]: can we just take one of these measures and use it as opposed to all of the others we included? And, what if something changes in the opposite direction? To answer the second question: We've run enough of these to know now; [that] if we're measuring all of the androgen dependent endpoints in the male [it would be unlikely] that we see something that went in the opposite direction – I couldn't envision that happening. There is redundancy in this protocol and that helps us see things that come out in a cluster – we're still looking for the most sensitive endpoint. It provides assurance that we are covering all bases at first and this is a proposed assay. When you have developed a certain level of comfort for these markers, you may say that these are redundant and you only have to do one.

Identifying Environmental Chemicals with Estrogenic Activity - Susan Laws (ORD/NHEERL)

Laws discussed the classical pathway for estrogenic activity (direct gene induction). She identified synthetic and naturally occurring chemicals found in the environment that exhibit estrogenic activity.

The Tier 1 screening battery includes four assays designed to measure estrogenic activity: the *in vitro* estrogen receptor (ER) binding assay, the *in vitro* ER reporter gene assay, the *in vivo* uterotrophic assay, and the *in vivo* female pubertal assay.

The purpose of the ER binding assay is to identify chemicals that have the ability to bind to the estrogen receptor. Its advantages are: it is a rapid, well-documented technique; it can use estrogen receptors from diverse species and tissues; and the data have been used as training sets for QSAR models. The assay's limitations are that: it requires radioactivity and animals; receptor binding affinity does not always parallel activity; it does not consider pharmacokinetics; it does not distinguish between ER agonist and antagonist; and it provides no distinction between ER subtypes.

The purpose of the ER reporter gene assay is to identify chemicals that induce ER gene transcriptional activation. The advantages of this assay are: 1. it provides large-scale screening, 2. radioactivity and animals are not required; 3. mammalian or yeast cells can be used; and 4. there is some ability to distinguish between ER agonists and antagonists. There are also limitations to the ER reporter gene assay: 1. there is no single standardized assay with a historical data base, 2. *in vivo* pharmacokinetics are not presented, 3. yeast cell walls can affect the uptake of test chemicals, and 4. this assay is more technically difficult than some of the Tier 1 assays.

The uterotrophic assay detects the ability of a chemical to stimulate or inhibit an estrogenic response in the rodent uterus. It has been used as a bioassay for estrogenic activity since the 1930s. The advantages of this assay are: 1. reproducibility; 2. specificity; 3. the limited number of animals required; 4. availability of historical data; and 5. a positive response can be readily correlated with reproductive and developmental effects. The purpose of the female pubertal protocol is to identify chemicals that alter pubertal development and thyroid function through steroid-mediated mechanisms of actions.

Conclusions and Next Steps

Law summarized the data from the *in vitro* and *in vivo* assays she conducted and described how the assays can be used, together, in evaluating estrogenic activity.

Questions and Responses

None

Environmental Androgens and Antiandrogens: Current Issues - Earl Gray (ORD/NHEERL)

Gray described the Tier 1 screening battery as it relates to assays for androgen and antiandrogen activity. He discussed two *in vitro* and two *in vivo* assays, and also identified two alternative assays.

- AR (androgen receptor) binding and/or transcriptional activation (*in vitro*);
- Steroidogenesis (*in vitro*);
- AR agonists and antagonists in the Hershberger assay (*in vivo*);
- Pubertal female rat assay (*in vivo*);
- Pubertal male assay (alternative); and
- *In utero*/lactational protocol (alternative).

The androgenic effects of feedlot effluent potentially contaminated with Trenbolone were discussed. Trenbolone is an anabolic growth promoter. It is a potent androgenic compound with a half-life of 6 months in manure piles. Exposed female mosquito fish found in the Fenholloway River demonstrated masculinization – approximately 80% are at least partially masculinized as demonstrated by changes in sex-related physical characteristics (anal fin shape/size alterations).

The mechanisms of action of chemicals that alter the development of androgen-dependent tissues was discussed. Human exposure estimates were compared to those estimated to produce adverse effects in laboratory animals.

Gray discussed the Tier 2 testing for androgens and antiandrogens. He provided proposed enhancements to the existing protocols. He suggested that the current Agency multigenerational guidelines for addressing “low dose” effects are not always adequate. The limitations of the current assays focusing on antiandrogens include false negatives in standard teratology and multigenerational studies. In addition to insufficient sample size, difficulties exist in the collection, analysis, and interpretation of data relating to the endpoints. Gray proposed a tailored Tier 2 testing scheme using a transgenerational protocol.

The results of a male rat study assessing AR antagonists were presented. The study demonstrated reproductive tract malformations and a delay in puberty.

Conclusions and Next Steps

Data from androgen and antiandrogen studies indicate the following:

- Some humans are exposed to EDCs at levels that produce adverse effects in animals;
- Androgens and antiandrogens do induce adverse reproductive tract malformations that may elude detection because an inadequate number of F1 individuals are examined after maturation in the existing protocol;
- Developmental toxicology studies fail to detect reproductive tract malformations induced by androgens and antiandrogens, even though they use a sufficient number of animals;
- Many malformations and functional alterations in various reproductive tissues are not routinely assessed in multigenerational studies and thus are missed;

- U-shaped dose-response curves and adverse responses (other than endocrine effects) are displayed by some EDCs;
- Some androgen and antiandrogen-induced effects do not display an apparent threshold level;
- Mixtures of antiandrogens produce adverse effects that are additive even though they act through different mechanisms; and
- Information in IRIS and REDs is inaccurate for some EDCs.

Questions and Responses

None

New Technologies and Implications for Risk Assessment - Timothy Zacharewski (ORD Grantee – Michigan State University)

Zacharewski presented a genomic approach to assessing the risk of chronic and subchronic exposure to EDCs. He stated that the approach allows a more comprehensive understanding of the physiological, cellular and molecular effects within the whole organism. He outlined findings and techniques developed for the Human Genome Project and discussed their implications for risk assessment. Zacharewski presented and defined the term “toxicogenomics” which integrates toxicology, the “omic” technologies (genomics - gene expression, transcriptomics - gene expression, proteomics - protein synthesis, metabonomics – metabolite function), with bioinformatics, and statistics.

Three current toxicogenomic projects were identified:

- Effects of gestational (*in utero* exposure through the pregnant female) and lactational (exposure through the nursing female) exposures to estrogenic substances on male reproductive development and fertility;
- Examination of the effects of estrogenic chemicals on *in vivo* human brain cell differentiation; and
- Establishing gene expression profiles for various class of chemicals and mixtures using *in vitro* and *in vivo* models.

Zacharewski used the Sharpe-Skakkebaek hypothesis regarding sexual development in male mammals as it applies to potential pathways for estrogenic EDC effects as the basis for the design of the assessment strategy of diethylstilbestrol and genistein.. His research included the construction and use of cDNA microarrays to analyses gene expression and supplement traditional histopathological analysis of test animals. He presented data on the effects on testicular gene expression of gestation and lactation exposure to diethylstilbestrol and genistein in three-week old male rats.

Conclusions and Next Steps

Several limitations were presented. This approach presents an extremely large amount of data and, therefore, is resource intensive. Researchers may be dealing with thousands of endpoints and this raises questions on how to deal with that statistically. At this time, correlations/ associations between global gene expression data and toxicity do not exist. Additionally, inaccuracies in databases and clone collections were present. With the completion of the Human Genome Project, most of these inaccuracies should be corrected.

Zacharewski presented some final thoughts:

- It is more likely more questions will be raised in the near future than answered;
- Array technology has much more potential than just identifying changes in gene expression, protein expression, or metabolite profiles;
- Robust statistical analysis strategies are required that consider replicate data sets;

- Changes in gene expression, protein expression, or metabolite profiles are not evidence of toxicity, unless correlated to an adverse effect; and
- These technologies are in their infancy and will undergo significant changes as they improve.

Questions and Responses

Question: This seems to generate uninterpretable data. Have you looked at anything that you can interpret?

Response: Yes, there have been a couple of chemicals that we have looked and confirmed what is already known. There has been some study on dioxin and estrogen. Thirty years of research have been replicated and confirmed in a single microarray experiment.

Comment: I'm not convinced.

Question: Biomics. How can it be used in screening assays?

Response: We hope to be able to identify specific genes that are going to respond to estrogenic compounds and the corresponding tissue specific responses that can be used as biomarkers of exposure or for screening purposes.

Risk Characterization of Dioxins - Linda Birnbaum (ORD/NHEERL)

Birnbaum discussed the different “classes” of dioxins and dioxin-like compounds. She stated that the polychlorinated ones are the only group that we have given risk factors to; other similar halogenated compounds are found in the environment and need to be evaluated. The PCDDs and PCDFs were never produced intentionally, but are byproducts resulting from industrial and combustion processes. It was noted that burning household waste may account for as much as 50% of new air emissions of dioxin, and the burning of livestock carcasses in Britain has been producing high dioxin emissions.

Major sources of dioxins, past and present, were identified. Dioxin transport, deposition, and human/wildlife exposure were discussed. It was noted that adverse effects of exposure to dioxin have been identified in wildlife and domestic animals as well as in laboratory animals.

Birnbaum further noted that 1% of the human population show three times the exposure level as that found in the general population. She identified the biochemical and toxic effects of dioxins, and listed the following recent findings on human effects due to dioxin exposure including:

- Cardiovascular disease;
- Diabetes;
- Cancer;
- Porphyria;
- Endometriosis;
- Decreased testosterone; and
- Various developmental, reproductive, and immune effects.

The system regulatory role of the Ah receptor was presented. Dioxin exposure may cause inappropriate activation of Ah receptor; the receptor may no longer be available to perform its normal modulating role in signaling and response pathways; and it may both directly and indirectly result in altered gene expression.

Research shows that dioxin is absorbed through the oral and inhalation routes. Studies indicate that deposition through dermal exposure is limited. Body fat is the major repository of dioxin in humans; in laboratory animals, it is found primarily in body fat and the liver at high doses. The loss of stored dioxin through metabolic processes is slow in humans – its half life is 5 - 15 years and is a function of body fat.

Birnbaum stated that nearly all vertebrate animals studied respond to dioxin. The question concerning human exposure is not whether there is a response, but rather at what doses they respond. She summarized data associating body burdens with adverse biochemical, and function effects and compared the body burdens associated with cancer and non-cancer (including endocrine disruptor) effects.

Conclusions and Next Steps

It was concluded that body burdens in the general population are at or near the concentration where effects might be expected to occur. It was found that environmental concentrations and body burdens have been decreasing over the past decade and efforts to reduce emissions and exposure should be continued.

Questions and Responses

Question: Circadian cycles are sensitive to gravitational, magnetic, and other outside forces. Are there any studies on the effects of dioxins on those?

Response: Not that I know, although we have shown that the Ah receptor has a circadian rhythm. It would be an interesting study.

Question: Body burden: where does the value come from, serum or tissue?

Response: In people, it is often based on lipid-adjusted serum levels.

Question: What is the oxidative stress associated with?

Response: We have some idea as to the cause, but don't really know. We looked at a lot of measures (including DNA damage), but we see different responses and can't tie them to an endpoint.

Question: Is breast feeding still recommended? How does body burden even it out?

Response: Yes, although the mother may be transferring the chemical, the baby is growing fast. The body burden in the infant only goes to three-to-five times that of the mother and not the 100 plus times we originally thought. The benefits of breast feeding are still thought to outweigh the risks.

An Overview of the Ecotoxicity of Alkylphenols, with Emphasis on Nonylphenols - Donald Rodier (OPPTS/OPPT)

Alkylphenols are chemicals used to synthesize industrial compounds such as surfactants and pesticide formulations. Rodier reviewed their uses, and the several product categories they are used to synthesize. Of all the alkylphenol products manufactured, nonylphenol ethoxylates are the most widespread. Rodier discussed the toxicity of nonylphenol ethoxylates and its metabolites to fish, invertebrates, and algae. Although nonylphenol has a low to moderate bioconcentration potential, questions remain on the bioconcentration potential for some of the nonylphenol ethoxylate metabolites such as the 1 & 2 ethoxylates and ether carboxylates.

Research indicates that alkylphenols are estrogen mimics. They:

- Bind to the estrogen receptor;
- Induce growth in estrogen-deprived MCF-7;
- Induce vitellogenin in male fish;
- Induce testicular inhibition in fish; and
- Induce testis-ova in fish.

Rodier summarized the endocrine disrupting effects found in the literature. He discussed the findings of multi-generation tests on fish and mysid shrimp and also a field study. The multi-generation test on mysid shrimp was compared to a population model. The most sensitive endpoints were found to be the mean number of young per available reproductive day, and growth. He also discussed a littoral zone study assessing the toxicity of nonylphenol on fish, various zooplankton and macroinvertebrates.

Conclusions and Next Steps

The following conclusions were presented:

- The alkylphenols, nonylphenol (NP), and octylphenol (OP) are highly toxic to aquatic life;
- The relative toxicity of the parent surfactant and the metabolites appear to be $NP > NPEO_{1\&2} > NPEC_{1\&2} > NPEO_n$;
- Studies have shown effects as low as $\leq 1 \mu\text{g/l}$ but clear dose/responses are often lacking, inconsistent, or statistically insignificant;
- A multi-generational fish study seems to show effects as low as $2 \mu\text{g/l}$;
- An OPPT risk assessment for nonylphenol indicated that there does not appear to be a widespread risk nation-wide, but there are areas that could be impacted;
- The European Union completed their risk assessment of nonylphenol and recommended banning “down the drain” uses; and
- It was anticipated that Sweden and Denmark would phase-out nonylphenol by 2000.

Questions and Responses

None

SESSION IV: COMMUNICATING THE SCIENCE TO EPA MANAGERS AND THE SCIENCE COMMUNITY

Jim Cogliano (ORD/NCEA), Co-Chair
Daniel, Stralka (Region 9), Co-Chair
(Gary Timm, Tony Maciorowski, OPPTS/OPPT)

Bobbye Smith (Region 9), Facilitator

Breakout Session: How has our science policy evolved?

See APPENDIX D: Breakout Group Summary

Facilitated Discussion

Tony Marcioroski (OPPTS/OPPT) led a facilitated discussion on key issues surrounding endocrine disruptors. Participants were asked, “If you work in Eco Risk Assessment, what are the issues important to you regarding Endocrine Disruptors?” Several issues were identified by participants including: how to model exposure, timing of exposures, and effects (e.g., classic tests – apical not histopathologic). Other areas of interest to the group relating to endocrine disruptors included applicability of lab studies to field situations; retrospective, chemical and forensic toxicology; and “field” vs. “new” (retrospective vs. prospective risk assessments).

Questions and Responses

Question: In the Lake Apopka Case Study, what happened to tracing adverse effects back to exposure?

Response: OPP is starting to look at probabilistic risk assessment [as is used in Superfund risk assessments], specifically [considering] additional routes of exposure and residues [of pesticides]. OPPTS has completed one probabilistic risk assessment study. The chemical selected had a known [toxic] outcome. We tried the process.

General Comments

- What does probabilistic risk assessment mean?
 - ▶ Lab data exposure [using lab data rather than only field exposures]
 - ▶ Range of Prospective Risk [using multiple values of exposure parameters]

-
- Human health impacts [consider only one species, but ecological risk assessments should evaluate impacts] on multiple species and higher trophic levels.
 - Eco folks do problem formulation [but is that approached the same way in human health risk assessment?]. Is human health [risk assessment] more intuitive?
 - Eco risk assessors use an individual-based toxicity study.
 - When is something “significant?” We don’t want to lose sight of the effects on individuals [especially in the case of endangered or special status species].
 - Need to do Superfund-like field surveys for effects - Lab work is good for a causal link (i.e., if you don’t know what is going on in the field, you are not going to see its causal relationships).
 - Andy Avel’s group is doing an effluent survey.
 - Is anyone doing metapopulation work?
 - Is a remote effect ever going to affect a site-specific effect?
 - At what level is protection - population? Are individual-based toxicity tests used?
 - ▶ Trying to take the step to populations
 - ▶ EDC work helps because EDC effects are related to reproductive effects.
 - National monitoring strategy for PCBs is in the planning stage. We need feedback on what to measure.
 - ▶ Should the focus be on known effects (e.g., from dioxin, PCBs, mercury), or on newly emerged chemicals?
 - Are there EDC bioassays to include [for monitoring or assessment]?
 - ORD is looking for biomarkers for exposure.
 - Looking at the Fathead Minnow model (vitellogenin).
 - Mixtures - How to deal with them? What to look for?
 - Eco Forensic Issues:
 - ▶ Toxicity Identification Evaluation (TIE) procedures/approach. Could use EDC *in vitro* assays as tools.
 - Seeing a shift in endpoint from acute to more subtle [or chronic] effects (e.g., species used to die [due to acute effects from pollution] – now they are living longer and we are seeing reproductive disorders).
 - For every terrestrial risk assessment there are approximately twenty aquatic risk assessments.
 - Superfund has not yet used an ED endpoint, *per se*, but can do a biological study
 - ▶ Have not used it as a basis yet, but we are moving in that direction.
 - ▶ Use the ED-mediated effects to select endpoints.
 - What about the interplay between [fish] ingestion [and human or higher trophic levels] ?

Use of Effects on Endocrine Function in Risk Assessment of PCBs - Deborah Rice (ORD/NCEA)

The goals of the PCB non-cancer assessment are to identify the most sensitive organ systems, endpoints, and existing studies; draw possible conclusions concerning the relative toxicity of different congener classes; and provide guidance relevant to specific situations identified above.

Rice identified four major health effects in body burdens that are not unusual in the general human population (reproductive, immune deficiency, neurotoxicity, and thyroid effects). She discussed the TEQ approach and compared Ah receptor activation and ryanodine receptor activation.

Rice summarized some conclusions regarding congener-specific toxicity:

- For immunotoxicity, reproductive effects, and physical development of offspring, the TEQ (Toxicity EQuivalency) approach will probably provide protection for all PCBs;
- For developmental neurotoxicity, both dioxin- and non-dioxin-like congeners are active, thus the TEQ approach is not appropriate;
- For thyroid effects, both congener classes are active; and
- For all endpoints, available evidence does not suggest differential toxicity for lightly vs. highly chlorinated congeners.

Rice noted that PCBs can affect the balance in the endocrine system by changing the pharmacokinetics of hormones; by impersonating a hormone; by changing the number of hormone receptors; and by indirect effects on hormone interactions. She summarized the major endocrine effects of PCBs which include:

- Estrogenic and antiestrogenic, androgenic and antiandrogenic, any of which would affect reproductive function and fetal development;
- Thyroid function; and
- Glucocorticoid metabolism.

Several non-reproductive targets of PCBs were also identified.

Rice affirmed that all biological systems are in intimate and constant communication with each other. As an example, she described the effect of PCBs on the hypothalamus - pituitary axis and indicated that they affect neurotransmitters, especially dopamine, a major neurotransmitter in the hypothalamus. This may be a primary effect of PCBs, which would then be reflected in downstream changes in endocrine function in numerous tissues.

Conclusions and Next Steps

The developmental effects of exposure are timing-related. Because of timing of exposure, some effects noted in fetal development will not be evident in adults and some effects will continue to sexual maturity. Rice raised the question "are the endocrine effects of exposure special?" and stated that:

- Endocrine disruption is a mechanism by which adverse effects are produced, not something completely different;

- Endocrine disruptors should be treated the same as chemicals that act by other means;
- Toxicity in any organ system may be caused by multiple mechanisms, endocrine or otherwise;
- One can not assume, *a priori*, that endocrine-mediated effects are more sensitive than any others; and
- It is important to continue looking at other endpoints even after an endocrine-mediated effect is identified.

Questions and Responses

Question: How do we make nice, clean lines in our decisions?

Response: We need to take things apart. We need to be able to keep in mind that we have to put things back together. Endocrine effects of exposure may not be the primary effect. We need to keep looking.

Question: How do we communicate what our risk assessment and characteristics really mean?

Response: People don't want to hear what you don't know. They want to know what everything you are saying means to them. There needs to be an awareness, and it has to be communicated, that we don't have data for certain organ systems. Maybe we have to write a statement around the risk assessment that the RfD [Reference Dose] is based on the data we now have.

The 1997 Special Report on Endocrine Disruptors Then and Now - Donald Rodier (OPPTS/OPPT) and Ralph Cooper (ORD/NHEERL)

The 1997 report, “Special Report on Environmental Endocrine Disruption: An Effects Assessment and Analysis” (EPA/630/R-96/012) was commissioned because several incidences/studies suggested that endocrine disrupting effects were occurring. The following examples were discussed:

- 1) Cultured estrogen-dependent cancer cells were found growing in a supposedly estrogen-free medium;
- 2) Routine nest surveys found demasculinized alligators;
- 3) Studies alleging that current sperm counts were lower in human males than historic values;
- 4) The use of diethylstilbestrol to increase fertility in humans resulted in severe EDC effects to offspring; and
- 5) Public concerns about the environmental impact of birth control pills (i.e., composition of effluent) were raised further by the presence of hermaphroditic fish below sewage treatment plants.

Finally, the publication of the book *Our Stolen Future* and the production of the documentary *Assault on the Male* were also factors in stressing a need for a report which summarized the current literature and analyzed the veracity of the findings and conclusions of key studies regarding endocrine disruption.

The purpose of the document was discussed and the topics it covered were presented. The report provided a very broad definition for the term “endocrine disruptor” and highlighted what was known and unknown about suspected EDC ecological effects, effects on male and female reproduction, endometriosis, breast cancer, prostate cancer and effects on the immune system.

As a result of the report’s conclusions, the Science Policy Council prepared the following Interim Position:

“Based on the current state of the science, the Agency does not consider endocrine disruption to be an adverse endpoint per se, but rather to be a mode or mechanism of action potentially leading to other outcomes, for example carcinogenic, reproductive or developmental effects, ...”

Conclusions and Next Steps

None.

Questions and Responses

The following three questions were presented for discussion, however, insufficient time remained:

- 1) What is the current thinking about a decline in sperm counts and endometriosis?
- 2) How have assays changed? What progress has been made in correlating assay results with data from tests of longer duration?
- 3) What is our current (2001) thinking about long-term tests?

Use of Screening and Testing Results in Assessment and Decision-Making - Gary Timm (OPPTS/OPPT)

Timm restated the EPA policy on EDCs and indicated that the assessment of testing results could lead to regulatory action if adverse effects are shown to occur. He posed the question “is mode of action important?” He answered the question by stating that there is a legal requirement under the FQPA (Food Quality Protection Act) to address endocrine disruptors, the cumulative risks must be considered, and modes of action may be useful in understanding the risk to receptors from pesticides.

Three modes of action were presented: (natural, generalized) hormone modes of action, estrogen receptor mode of action (as one class of hormones), and endocrine disruptor modes of action. The purpose of Tier 1 screening and the proposed battery of Tier 1 tests for endocrine disruptor modes of action were discussed. Timm indicated that life stage sensitivity is a particular consideration in developing screening tests. In general, the adult organism is the least sensitive life stage to endocrine disruption. The *in utero* life stage is the most sensitive stage and many effects are irreversible and will become permanent. The pubertal life stage is of intermediate sensitivity, but some effects of *in utero* exposure will not become evident until onset of puberty.

Timm presented a proposed Tier 2 testing battery and stated that the tests should:

- Determine if effects are a primary or secondary disturbance of endocrine function;
- Establish exposure/concentrations/timing and effects relationships;
- Be sensitive and specific;
- Assess relevant endpoints;
- Include the life cycles of live-bearing and egg-laying species;
- Include a dose range for full characterization of effects;
- Be conducted in accordance with GLP; and
- Be validated.

Timm assessed “where we are” in terms of the types of tests used for chemical registration and their usefulness in evaluating endocrine effects: 1. existing test protocols for chemical registration do not adequately account for ED effects; 2. ED is a mode of action that produces some effects that are unfamiliar or for which we have not previously tested; 3. the current chemical risk assessment guidelines may need to incorporate new endpoints; and 4. the existing chemical risk assessment approach should apply to ED effects.

Conclusions and Next Steps

The following conclusions were presented:

- Test protocols must be sensitive to endocrine effects;
- (Chemical) Risk assessment will be conducted as chemicals come through the regulatory system -- during the registration process;
- (Chemical) Risk assessment guidelines will be developed based upon experience;
- Defaults will be used for low dose extrapolation when mode or mechanism of action data are unavailable; and

- Current defaults will continue to be used until compelling evidence indicates they should be changed.

Questions and Responses

Question: What is the timeframe for Tier 1 and Tier 2 [testing]?
Response: December 2002 for proposed Tier 1 chemicals;
 December 2003 for published Tier 1 chemicals; and
 December 2005 for report on Tier 2 data.

Discussion on Ecological Risk Assessment - Tony Maciorowski (OPPTS/OSCP)

Maciorowski briefly presented background information on the early history of ecological risk assessments growing out of the development of stream sanitation efforts (1890's), water pollution assessment (through the 1950's), water quality criteria development (1970's and 80's), and chemical product registration (1980's). Until the 1990s, there was no standard approach to ecological risk assessments, and they were conducted in a variety of ways. Additionally, human health and ecological risk assessors seldom shared data or other information, or assessment criteria. There were also differences in how each evaluated data (e.g., laboratory data vs. field data).

He then asked, "Where are we today?", and "How have we done?" Regulatory actions have dramatically improved the acute toxicity problems in the nations waterways, and "open cesspools" (river) are largely a thing of the past. The really "bad" places of the 1960's (e.g., Kanawha River, WV and Cuyahoga River, OH) have gotten better, but the water quality of pristine areas of that time (e.g., Upper Clinch and New Rivers, VA) has declined. To a large extent, we have learned how to assess and regulate the harshest water pollution problems that affect aquatic life. However, we are still learning how to deal with more subtle, but equally problematic, issues of chronic problems that may affect populations and communities. Major questions remain on: how to best deal with local, regional and ecosystem spatial and temporal scales; the extrapolation of laboratory testing to field situations; and validation or "ground truthing" of prospective ecological risk assessments in the field.

EDCs provide an area where these questions come together. It is clear that some animal populations, including birds, fish, reptiles, amphibians and invertebrates, have been affected by chemicals through endocrine mechanisms. However, the scope and scale of these impacts is only beginning to be studied. The EDC arena represents a forum to ask a number of questions.

Discussion

What are some key issues in ecological assessment and EDCs?

- Exposure: timing of exposure during critical periods of development.
- Conventional (apical) vs. mechanistic (endocrine-specific) endpoints in field and laboratory studies.
- Where do we go when we have demonstrable effects, but are missing satisfactory [chemical-specific] exposure information? What are some good examples? There has been criticism of field studies that focus on effects without adequate chemical analyses and exposure considerations.
- OPP is starting to employ probabilistic risk assessments that include additional routes of exposure and residue concentrations.

- What are probabilistic risk assessments? The use of laboratory-derived fate-and-effects data and modeling to estimate the potential risk to receptors under different field situations.
- When is an impact “significant” and at what spatial, temporal [and organizational] scale (i.e., individual, population, community, or ecosystem)?
- Field work (site-specific risk assessments) provides measurements of exposure and effects *in situ*. Laboratory work can link effects with specific concentrations under controlled conditions. Relatively little [work] is being done at the population level. Many [managers] are uncomfortable making decisions on the effects at locations remote from the site;
- Guidance on the level of protection is not currently available from the Agency. Is it appropriate to use individual-based toxicological tests?
- EDC research will help ecological risk assessment because many of the EDC effects are related to reproduction.
- A national monitoring strategy for PCBs is in the planning stage. We would like some feedback from the EDC community on things that should be considered. Right now, the focus is on the Great Lakes and known chemicals (e.g., dioxin, PCBs). What are the newly emerged EDCs? Are any bioassays available?
- ORD is looking for exposure biomarkers.
- We are looking at the immediacy of exposure to estrogenic compounds using the fathead minnow and vitellogenesis as a model.
- Mixtures and the inability to identify causal relationships because we don’t have the analytical tools yet. Fractionation methods have their limitations. If you do not know what chemical you are looking for in the field, you probably won’t see it. We need to sort through mixtures and come up with methods that have endpoints based on “sort” findings.
- Several approaches/questions concerning forensic ecology, and *in vitro* assays of effluents (mixtures) have been raised. What is being discussed in terms of chemicals and analytical methods?
- Concerning the effluent toxicity test program [where TIE approaches are used]– if you have a point source and also effluent input, you need to continue fractionating until you can identify the toxic moieties. This approach assumes that there is not a cumulative effect.
- You might be able to use EDC *in vitro* [tests] as a tool.
- Perhaps we need to shift from acute to more chronic [endpoints]. Today there are [tests that can detect] more subtle responses than those for the old [test] endpoints.
- Terrestrial assessments are not as mature as aquatic assessments – there are many more aquatic assessments.
- Talking from a Superfund viewpoint, we haven’t used EDCs as an ecological endpoint, but we may be taking those into account [by using population endpoints]. We are moving in the direction of using EDCs as a basis in an ecological risk assessment.
- Some sites pose a significant human health concern – one reason is that fish that are contaminated are consumed.
- What about the interplay between fish consumption or the consumption of other wildlife, and human health?

SESSION V: COMMUNICATING THE SCIENCE TO THE PUBLIC

Kevin Garrahan (ORD/NCEA), Co-Chair
Sophia Serda (Region 9), Co-Chair
Kelly Leovic (ORD/NERL), Co-Chair
Ross Highsmith (ORD/NERL), Co-Chair
Bobby Smith (Region 9), Facilitator

Mock Public Meeting: PCBs in Landscape, AL - Elmer Akin (Region 4)

Role Play: Group 1 Response Evaluation

1. Alabama Department of Environmental Management

Question: *“How is EPA going to address endocrine effects in the risk assessment for the ABC Chemical site?”*

Response: EPA does not regulate EDCs.

Discussion Issues:

- EPA does not know all endpoints at this time.
- General toxicity numbers are protective of endocrine endpoints.
- There are large uncertainties in the area of neurological effects. They are aware of the possibility of learning disability effects. This is an emerging area of science.
- We do not know everything about endocrine disruption:
 - The science is evolving; and
 - Some things we just don't know.
- Site data, blood data, chronic disease data - can we link those?
- We have a lot of data on PCBs and more is forthcoming.
- Endocrine effects are just one effect; there are others.
- Because we have so much data on PCBs, uncertainties and questions increase.
- Focus on the question; emphasize what you do know.
- Explain blood levels and what/where are possible exposures (clusters).
- Communities can get assistance interpreting results from the State Health Department.
- At your first meeting, do a lot of listening; learn and build credibility and trust.
- Response:
 - We don't know everything.
 - We need your input/opinion [in order] to [conduct] follow-up [activities] with your community.
- Beware of telling just [the results of] an assessment and not telling what you are going to do about it.

1. Joseph Jones, Resident of Landscape (wife has breast cancer)

Question: *“My wife is dying of breast cancer. What has all of EPA’s research told you about whether humans are currently being exposed to EDCs at levels that cause cancer?”*

Response: It is possible that your wife has cancer [due to exposure to high levels of EDCs]. We are working with EPA, and are removing the soil.

Question: When are you going to address the landfill and the off-gassing? My lawyer has data that shows 10% higher [level of cancer effects in people near the landfill].

Response: Step-up the study to determine the source of gases?

Discussion Issues

- Get data/information from his lawyer.
- Express sympathy about the length of time it has taken to evaluate and cleanup the site.
- There is scientific disagreement over whether PCBs cause breast cancer.
- Beware of being too specific to one incidence.
- Not in position to determine cause and effect.
- Possibility of other resources and agencies (e.g., ATSDR).
- Admit that studies on cancer clusters take a long time.
- One out of eight women gets breast cancer. It is a complicated disease, and the causes are still under investigation.
- Are basic [cancer rates] in the community enough [information]?
- Admit cancer is a terrible disease. As much as we would like to determine one case link, we can not. Show empathy.
- With scientists, indicate that EPA is putting more attention on EDCs.
- To EPA: Let’s look at morbidity and mortality [statistics] here; consider human tissue sampling; cancer tracking agencies.

3. Dr. James Mason, Resident of Landscape (Reverend - concerned about test scores in his small charter school)

Question: *“I run a school in the Landscape area and my students’ test scores are down. It’s the ABC Chemical plant’s fault and EPA, you need to do your job and clean up this mess!”*

Response: In terms of this chemical, there is scientific evidence of transgenerational effects.

Comment: Some of my children were not in the testing group.

Response: I would like to follow-up with you later on.

Discussion Issues

- Direct to state and other officials.
- To EPA: Do checks [for information concerning] school performance, poverty indicators [prior to the meeting].
- To EPA: Have data on other chemicals (e.g., lead, etc.).
- Emphasize exposure reduction/minimization (i.e., “what you as a community can do”).
- EPA: Part of the message is that we can not address past [or non-Superfund] exposure.
- EPA: Describe what risk assessment can and can not do.
- EPA: Learning disabilities are associated with a number of factors (e.g., demographics, socioeconomic, other).
 - ▶ More general certainty regarding EDCs and learning disabilities vs. cancer effects
 - ▶ Work with state and local departments of education.
 - ▶ Form “Response Team” format with ORD and other experts to have discussion. forums. [ORD] used to interact and engage each other in response teams more.

Role Play: Group 2 Response Evaluation

1. Joseph Jones, Resident of Landscape (wife has breast cancer)

Question: *“I come to find out that where I fish in Chocolate Creek and Clean Lake, the fish have been contaminated with PCBs and other chemicals for years. My kids played in areas where PCBs were dumped. Now many of my relatives and friends are showing up with thyroid diseases. I want to know if the PCBs and those EDCs caused these effects? Am I going to pass something to my kids?”*

Response: There is a possibility that some children will have learning disabilities as a result of the mother eating contaminated fish during pregnancy.

Discussion Suggestions

- Hand out business card and offer to meet and discuss privately.
- Credibility issue: the question was not answered. Also, the ABC Chemical plant representative was passing notes to EPA officials before the meeting.

Positives:

- Levels in fish;
- Validating concerns; and
- Acknowledge that she did not address the question initially.

Negatives:

- Credibility; and
- Didn’t acknowledge that it has been a long time [in getting from discovery of the problem to investigating it].

2. Dr. James Mason, Resident of Landscape (Reverend - concerned about test scores in his small charter school)

Question: *"I run a school in the Landscape area and my students' test scores are down. It's the ABC Chemical plant's fault and EPA, you need to do your job and clean up this mess!"*

Response: In terms of this chemical there is scientific evidence of transgenerational effects.

Comment: Some of my children were not in the testing group.

Response: I would like to follow-up with you later on.

Positives:

- More resources (not realistic);
- Started with position (levels are going down);
- Thank you (i.e., showing empathy); and
- Tests so far haven't shown elevated blood levels.

Negatives:

- Transgenerational (expanding the question, big word).

How to improve the response?

- Give practical advice (e.g., "Don't eat the fish").
- Offer to discuss issue in private.
- Educate local doctors on the issue (Can ORD help here?).
- Know beforehand what you can do (e.g., asked company to voluntarily provide funds where EPA can not).
- Focus on the present time and what can be done.
- EPA case team work out strategy/message (each answer should address):
 - 1) We are doing something now;
 - 2) This is not the only site; and
 - 3) Don't eat the fish.
- Look into/consider EPA (or other organization, e.g., ATSDR) offering blood tests?
 - ▶ Negative (slippery slope).

3. Mary White, Resident of Landscape (has contaminated soil, has elevated PCB levels in blood, is nursing a 15 month old girl, and is pregnant with her second child).

Question: *"I've read I pass these chemicals to my baby when I nurse. How can you tell me it's safe to nurse my baby!"*

Response: This is an issue to be concerned about. PCBs are passed through breast milk to the baby. However, the benefits from breast feeding (bonding, nutrition) outweigh the risk. There is concern about the pregnancy, but I don't know what to tell you about [the risk to the fetus].

Positives:

- Maintained good eye contact;
- Addressed the question and offered that breast milk was good for the baby;
- Showed empathy;
- Right person - a woman; and
- Acknowledged that it was not the mom's fault.

Negatives:

- Brought up pregnancy concerns, but did not provide an answer (e.g., offer to work with her physician). It is not what you say, but how you say it.
- So, what are the problems with pregnancy?:
 - ▶ [PCB exposure can have] subtle changes [neuromuscular effects], e.g., eye blinking in infants;
 - ▶ This isn't the first time we are taking action;
 - ▶ High levels are unacceptable;
 - ▶ Avoid scientific terms; and
 - ▶ Need to put risk [from breast feeding] in context:
 - ◆ Personal choice; and
 - ◆ At what point do the benefits outweigh the risk?

Role Play Summary Recommendations:

How to improve:

- Project Manager should help with credibility issue, "this is why we brought these experts;"
- Respond to emotion (not just the science);
- Have realistic expectations; allow the public to vent;
- Build trust with the public before the meeting; and
- Have the facts right from ORD (work the science into your empathetic response).

Themes

- Credibility, factual;
- Concern, empathy;
- Facts - Be succinct;
- Need level of risk; and
- Suggestions for practical ways to reduce exposure.

SESSION VI: SOURCES OF EXPOSURE AND THEIR MANAGEMENT

Gregory Sayles (ORD/NRMRL), Co-Chair
Mark Johnson (Region 5), Co-Chair
Patti Lynne Tyler (Region 8), Facilitator

Region 5 Endocrine Disruptor Efforts – Impact on Sewage Effluent Dominated Streams - Larry Zintek (Region 5)

Region 5 efforts are focused on looking at the alkylphenols, nonylphenols, octylphenol, bisphenol A, estrogens and androgens, PCBs, DDT, and other endocrine disruptors. Several efforts underway in Region 5 were discussed.

Zintek presented data from several studies demonstrating that effluent concentrations of chemicals of interest are above the effect level in water bodies receiving effluent from Publicly Owned Treatment Works (POTWs). Water and sediment samples (per the protocol for the specific project discussed) were taken and analyzed by the Central Regional Laboratory (CRL) using methods similar to those practiced by environmental laboratories. The samples were analyzed for the chemicals of concern using gas chromatograph/mass spectrometer (GC/MS) methods. Commercially available standards of known purity were used. Data showing elevated levels of nonylphenol and nonylphenol ethoxylates in carp taken from the North Branch of the Chicago River were presented. The sample results were compared to distances from POTW discharge locations.

Zintek discussed the Region 5 CRL Methods Initiative Proposal, which is a cooperative effort with ORD in Cincinnati and Region 3. The project is to be incorporated into 40 CFR 136. Three objectives have been completed to date:

- Completed the sediment Standard Operating Procedure (SOP) for NP, NP1EO, NP2EO, octylphenol, and bisphenol A for analysis by GC/MS/FS;
- Completed the draft water SOP for NP and OP analysis by GC/MS/SIM; and
- Completed the synthesis and commercial availability of standards with known purity.

Conclusions and Next Steps

Zintek discussed current studies and proposed work including:

- Fathead minnow study for toxicity identification evaluation (serum vitellogen concentrations; testosterone/estrogen ratios, and gonad histopathology);
- Investigation of the fate of natural and synthetic sex hormones in POTW effluents, determination of the fate in the receiving stream, and determination of the major contributors to contamination in fish; and

- Combined Great Lakes National Program Office/USDA fish study.

Work is continuing in an effort to complete the GS/MS/SIM Water SOP, improving the methodologies currently in use, continued sediment and water monitoring, and investigating other compounds of interest (e.g., pharmaceuticals).

Key findings of the studies discussed indicate that:

- The chemicals of concern are present in effluent, sediment, and fish many miles downstream from the POTW discharge point;
- NP1EO and NP2EO should be included with NP to assess the total exposure to biota;
- Bioaccumulation seems to be direct rather than via the food chain;
- Sediments are important reservoirs for the contaminants; and
- Researchers must look at the exact chemistry of toxic components and not generalize to mixtures.

Questions and Responses

Question: Will you sample drinking water?

Response: We have plans to sample drinking water, but the time frame has not yet been established.

Endocrine Disruptor Source-to-Dose Exposure Research - Chuck Steen (ORD/NERL)

Steen summarized the scientific elements of risk assessment. They included: source/stressor formation; transport, transformation, and fate; environmental characterization; exposure; dose; and effects. How each of the ORD laboratories contribute to research on EDCs is based on the risk assessment paradigm.

The approach to human exposure research used by NERL was presented as:

- Developing of the model;
- Identifying data and scientific gaps through the application of the model;
- Prioritizing data needs;
- Conducting measurement studies;
- Using data to test and refine models;
- Applying the refined model to identify gaps; and
- Repeating the process.

NERL's EDC research program was begun in 1996 with a literature review of the EDCs that are most important from an exposure standpoint. It was noted that several of the chemicals were listed as Persistent Bioaccumulative and Toxic (PBTs) chemicals. NERL researchers co-authored the EDC research plan in 1996/1997 and the peer review was completed in 1999.

Steen described the development of NERL's EDC exposure method and discussed the Near Laboratory Initiative. The EDC component of the Near Laboratory Initiative was presented.

The efforts to build a collaborative research framework were identified. This framework should include both federal agency (EPA, USGS, FWS) and state participants.

Steen discussed NERL's efforts to build a database platform and to incorporate the database into compartmental and multimedia model development and use. The Middle Neuse Basin Model was used as an example of this effort.

Current human health research efforts were discussed and included the:

- Children's Total Exposure to Persistent Pesticides program;
- Agricultural Health Study; and
- Pesticide Exposure Study.

Conclusions and Next Steps

It was noted that funding for EDC exposure research is not currently available. NERL plans to continue to address source-to-dose research on hormonally active agents as part of other research programs. Modeling and methods research will be incorporated into their efforts.

Questions and Response

- Question: The risk equation uses an average daily dose. If we assume some exposure today, no exposure tomorrow, and so forth. At some point it seems that the assumed risk breaks down when exposure only comes a few days a year. How is the risk measured?
- Response: We have very little data on intermittent exposure, or low level chronic exposure. We are trying to get those data now. We would like to follow exposure over 20 years by participating in a 10,000 cohort study beginning at age 2. We still do not have a lot of answers.

Biological Indicators of EDC Exposure or Whatever - David Lattier (ORD/NERL)

Lattier presented ORD's EDC strategy and the role that NERL/EERD plays in that strategy. He discussed EDC exposure and the research into developing new indicators as well as investigations concerning bioavailability. The role of advances in molecular biological technology and its value in identifying and closing research gaps was presented.

Single and multiple gene expression studies were discussed. The results of a single (vitellogenin) gene expression study using fathead minnows exposed to field collected source water were presented. Data from the following study sites were presented and compared: Pecan Creek, TX; Canadian Department of Fisheries and Oceans Experimental Lakes area; Nelson Experimental Studies Area of the University of Kansas; Miami University's Little Scioto River (OH) study; and the Lake Hartwell Sediment Natural Attenuation Study (NRMRL). Results showed the efficacy of this type of a test to detect the effects of EDCs using this technique.

Multiple gene expression utilizes a differential display similar to DNA fingerprinting. It was noted that mixtures are a complex issue and that there can be many different types of effects expressed, including abiotic factors, in addition to effects from chemical exposure. Microarrays will be the future technology for such types of analyses. DNA expression is valuable in providing patterns that may lead researchers to understand the mechanisms associated with exposure to mixtures.

Conclusions and Next Steps

Current and planned future activities include:

- Refining microarray and display technology;
- Applying new indicators to regional studies;
- Promoting technology transfer to the Regions;
- Assessing the effectiveness of risk management; and
- Linking exposure to effects.

Questions and Responses

None

Risk Management of EDCs - Andy Avel (ORD/NRMRL)

Avel discussed NRMRL's purpose and efforts. The NRMRL conducts research into the prevention and reduction of risks from pollution that threatens human health and the environment. Research was identified in the areas of drinking water regulations research, bioremediation, and pollution prevention technologies. Based on the Risk Assessment paradigm, he described ORD's integrated approach to EDC research, emphasizing the need for the early involvement of the risk management research component. This component is useful in identifying research tools that are currently available, initiating the development of new tools, and building EPA's ability to respond to issues sooner.

Avel summarized the EDC presentations to follow and NRMRL's risk management research goals.

Conclusions and Next Steps

None

Questions and Responses

None

Risk Management Evaluation and Research - Greg Sayles (ORD/NRMRL)

Sayles described the development and contents of the publication *Risk Management Evaluation of Endocrine Disrupting Chemicals*, which is currently under development. The document is based on an approach to risk management research that incorporates several key components: stakeholder input; risk management evaluation; planning the research; conducting research projects; disseminating the results; and incorporating the risk information into the risk management evaluation step.

The purpose of the document is to describe the current knowledge of risk management for likely EDCs. The developers received input from ORD laboratories, EPA program offices, EPA regions, and stakeholders. The document summarizes the current knowledge concerning known health and ecological effects, significant sources to the environment, significant exposures and environmental sinks, and risk management tools.

Sayles discussed the document's design and contents of various proposed chapters. Additional information is available at the NRMRL website (www.epa.gov/ORD/NRMRL/EDC).

Conclusions and Next Steps

None

Questions and Responses

Question: Do you anticipate addressing specific questions, e.g., if the focus is on risk management for EDCs, as I implement those strategies, do I simultaneously manage other pollutants, or do I exacerbate the management of one or the other?

Response: We are trying to do that ... the document is organized by individual chemical but it is recognized that contaminants exist as mixtures in the environment. We do need discussion on chemicals that are co-contaminants and if remediation of one attenuates the other.

Concentrated Animal Feed Operations - Andy Avel (ORD/NRMRL)

Avel defined concentrated animal feed operations (CAFOs) as those operations that:

- Maintain animals in confinement and are animal feed operations (AFOs);
- Contain 1,000 or more animal units; or
- Contain 300 to 1,000 animal units if discharge is made through manmade structure or to waterways; or
- Are a significant source of water pollution; and
- Are NPDES point sources of pollution.

He explained that CAFOs are of concern because runoff enters water bodies from lagoons as a result of storm events, improper design and construction, spills and leaks of manure-laden water, and over application of manure to agricultural land. Additionally, the lagoons are often abandoned and CAFOs are often located in nonagricultural areas with inadequate land to accommodate the manure load.

Examples of CAFOs were presented. Avel discussed proposed regulations addressing CAFOs under the Clean Water Act. The regulations would be based on nutrient loading and bacterial contributions to surface water. He pointed out that the proposed regulations would not necessarily be protective of EDC effects.

Avel presented and discussed several research questions that are under investigation:

- Are EDCs present in animal wastes?
- Does the typical operation result in EDC effect risk?
- Do typical waste management practices effectively treat EDCs?
- Do risk management tools exist that will treat EDCs?
- What new risk management tools are needed?

An ongoing study on EDC fate and transport with CAFO waste field application was presented. The approach for the study being conducted at a swine operation in Indiana was described. The field plot configuration, water collection system, and instrumentation were summarized. The study is expected to assist in identifying risk management needs to reduce EDC effluent from CAFOs; and to provide a better understanding of:

- EDCs in swine and cattle wastes;
- The fate and transport of EDCs through soils; and
- The efficacy of CAFO waste management.

Conclusions and Next Steps

The next steps are to review and evaluate the data from the swine operation study and use its results to refine research needs.

Questions and Responses

Comment: We see the problems on Maryland's Eastern Shore. We have a situation that combines intensive poultry operations and a high water table. Alum is mixed with the manure prior to land application. It ties the steroids to the soil and impacts on the native steroids in the surface waters. Additionally, pesticides are applied over each layer of manure to control flies.

Question: Is the mix of EDCs in CAFOs different than what we see in municipal waste?

Response: Yes, particularly in the area of antibiotics. Municipal waste treatment may provide some ideas about what works and what does not work in the treatment and management of EDC-containing wastes.

An Engineering Approach to Estrogenic EDCs in Wastewater - Paul McCauley (ORD/NRMRL)

The research problem was identified as: POTW discharges appear to have endocrine disruptor effects, including estrogenic effects on several fish species, where the effects appear to be mediated through the estrogen receptor. Model chemicals, including estrogen and metabolites, and alkyl phenols and metabolites, will be tested. The long term objectives of the study are to determine the fate of the EDCs during wastewater treatment, and to design an engineering solution that will reduce EDC discharge in wastewater and sludge.

McCauley described the basic research approach. Chemical analysis using solid phase extraction and GC/MS will be paired with bioassays: 1. fathead minnow using estrogen receptor mediated induction of vitellogenin by measuring messenger RNA; and 2. recombinant DNA yeast assay. This approach will enable the association between EDCs and their effects.

Two pilot scale wastewater treatment systems, one with aerobic sludge digestion and the other with anaerobic sludge digestion, will be tested to evaluate the effectiveness of the two systems in removing EDCs from wastewater.

Conclusions and Next Steps

Research plans are to analyze the results of the pilot scale systems and modify them as needed. The results from the pilot plants will be compared with field data from POTWs. The data will be used to recommend procedures for improved estrogenic EDC removal by POTWs.

Questions and Responses

Question: Is there a way to estimate the rate of reaction or to estimate the level of metabolites at each stage of the process?

Response: The process is not that well defined in POTWs. We are just trying to establish the effectiveness of the process and will not be able to conduct metabolite studies.

Question: Are you considering that the milk protein casein in the makeup of artificial wastewater may have estrogenic effects?

Response: The mixture of artificial sewage is still being developed – I am unaware of any estrogenic activity of casein. I would like to hear from you concerning possible estrogenic effects from casein.

Removal of Endocrine Disrupting Chemicals (EDCs) by Drinking Water Treatment Processes - Dr. John L. Cicmanec (ORD/NRMRL)

The information contained in this presentation is a summary of EPA Document 625/R-00/0-15 of the same title. The document is a user-friendly guidance manual for drinking water treatment plant operators and it provides public relations personnel with a brief update on the status of EDCs in the environment. The presentation identified DDT, PCBs, dioxins, phthalates, bisphenol A, alkylphenols, and the pesticides endosulfan and methoxychlor as the principal EDCs that are presently known. Results from ecological studies, laboratory investigations, and actual human exposure were used in making this assessment.

Information was presented on the conventional drinking water treatment processes of coagulation, sedimentation, filtration, and disinfection and it was shown that these processes demonstrate variable performance with respect to the removal of EDCs. Among the five specialized water treatment processes, only granular activated carbon (GAC) and powdered activated carbon (PAC), show high efficiency and low operation costs for the removal of many EDCs. The principal features that affect performance of GAC and PAC are contact time, temperature, carbon dose, and multi-component absorption. Since GAC is exothermic, it operates more efficiently at low temperature; eventually, GAC must be reactivated for continued use over time.

Cicmanec discussed drinking water treatment in Cincinnati, Ohio. He stated that when filtration was initiated in 1907, the human death rate from typhoid fever dropped from 400 per 100,000 to 55 per 100,000. When chlorination was introduced in 1915, a further reduction in the drinking water-related death rate to 15 per 100,000, was noted. The granular activated carbon system was introduced in 1993 at a cost of \$60 million, which created an overall cost to the consumers of only two cents per 1000 gallons of water.

Conclusions and Next Steps

It was concluded that: 1. conventional water treatment can not be expected to remove EDCs; 2. most higher molecular weight EDCs can be removed by GAC and PAC; and 3. PAC can remove most EDCs inexpensively and is particularly useful for seasonal contamination.

Questions and Responses

Question: Is Cincinnati monitoring for steroids or EDCs in their finished water?

Response: No, except for phthalates, but their [phthalate] presence is thought to be due to laboratory error.

Comment: The regeneration of GAC may release dioxins

Response: The temperatures used in Cincinnati are furnace level and not high enough to result in the release of dioxin.

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Comment: It does not take much heat to produce dioxin, so the furnace in Cincinnati is still probably producing dioxin.

Question: What is your opinion on the point of using filters for domestic drinking water?

Response: For them to be effective, you have to replace them very frequently and you can not regenerate the activated charcoal at the tap. Breakthrough [of contaminants] may occur rapidly and you can go from a non-effective dose of chlorinated compounds to an effective dose before you expect it.

Question: Are you looking at the physical characteristics of [feed lot] soils? Have you looked at the various types and the presence of soil organisms?

Response: Our initial goal is to look at fate and transport through leaching. What you suggest is a very fair avenue to pursue in the next phase.

Question: In the Cincinnati study, was there any consideration given to EDCs [effects] other than estrogenic?

Response: The process removes some of the precursors. We haven't tried to analyze to see if you can capture some of the smaller molecular weight chemicals.

Comment: In terms of drinking water treatment research, we are currently looking at seven steroids and trying to develop testing to get at detection limits in the nanogram per liter range using MS. The University of Cincinnati is trying to set up a bench scale estrogenic study and compare conventional treatment, GAC, PAC, and nanofiltration. Then, if time and funding are available, they might move to a pilot scale project. Some pilot scale facilities are available locally. Currently they are trying to find out how to detect the compounds and that [detection limit issue] is holding the process up. The equipment has been ordered.

SESSION VII: NEXT STEPS

David Klauder (ORD/OSP), Co-Chair
Bobbie Smith (Region 9), Co-Chair and Facilitator

The Workshop participants brainstormed a number of ideas that could be part of the follow-on activities of the group and which would benefit Regions, Program Offices, and ORD. Four major products or activities were identified:

1. Responses to Frequently Asked Questions on EDCs. A cross-Agency workgroup will develop draft responses to the questions that were raised during the Mock Public Meeting. The product will be a document that provides Agency-reviewed example responses to these frequently asked questions. This document can be used by the Regions at public meetings where EDCs are an issue of concern.

2. Advising Nursing Mothers and Mothers-To-Be who are Harboring Elevated Levels of EDCs. There was interest at the Workshop for involving ORD, other Agency scientists, and scientists from other federal agencies in interactive discussions with physicians on the following two questions:

- a. Are there body burden levels of PCBs and other endocrine disrupting chemicals, as determined by blood or milk analysis, in mothers of infants above which physicians should recommend that mothers not breast feed their children?
- b. Are there body burden levels of PCBs and other endocrine disruption chemicals in women of child-bearing age above which physicians should warn, prior to a pregnancy, of the potential for adverse effects to the fetus?

OSP/ORD committed to looking for opportunities to engage the appropriate scientists and physicians in discussions of these questions.

3. Input to OPPTS on Tier 1 Screening. The OPPTS representatives at the Workshop agreed to invite Regional participation on cross-Agency workgroups to:

- a. Develop the criteria for prioritizing the list of chemicals identified for Tier 1 screening.
- b. Determine the best methods to use in evaluating specific endpoints and next steps for some of the bioassays that were presented at the Workshop.
- c. Peer review background documents to be prepared on mammalian, fish, and avian methods.
- d. Develop a communication strategy for the results of the Tier 1 tests, including how to or how not to use the resulting test data.

4. Listing of Regional Risk Assessments Considering EDCs. Workshop participants identified the need to accumulate existing case studies and risk assessments on chemicals that may causing toxicity via an endocrine disrupting mechanism. This information could be used to inform ongoing and future risk assessments at sites throughout the Regions. A

Regional workgroup will be formed to compile a bibliography of these case studies and risk assessments.

APPENDIX A: AGENDA

APPENDIX B: LIST OF PARTICIPANTS

APPENDIX C: SLIDES FROM PRESENTATIONS

APPENDIX D: BREAKOUT GROUP SUMMARY

APPENDIX E: EDC WORKSHOP PARTICIPANT EVALUATION SUMMARY