May 14, 2002

MEMORANDUM

SUBJECT: Transmittal of the Meeting Minutes of the Endocrine Disruptor Methods Validation Subcommittee under the National Advisory Council for Environmental Policy and Technology (NACEPT), held December 10-12, 2001.

TO: Dorothy Bowers, Chair
National Advisory Council for Environmental Policy and Technology
Office of Cooperation and Environmental Management
and
Peter G. Redmond, Designated Federal Official
National Advisory Council for Environmental Policy and Technology
Office of Cooperation and Environmental Management

FROM: Jane Scott Smith, Designated Federal Official
NACEPT Endocrine Disruptor Methods Validation Subcommittee
Office of Science Coordination and Policy

THRU: Vanessa T. Vu, Ph.D., Chair
Endocrine Disruptor Methods Validation Subcommittee

Please find attached the minutes of the NACEPT Endocrine Disruptor Methods Validation Subcommittee second open meeting held in Washington, D.C. from December 10-12, 2001. This meeting summary covers revisions of the EDMVS mission statement and work plan, discussions on the in-utero through lactation and pubertal assays as well as the mammalian one-generation study plan.

Information about NACEPT EDMVS meetings and activities can be obtained from the website at http://www.epa.gov/scipoly/oscpendo or the OPPT Docket, OPPT 42212 at (202) 260-7099. Interested persons are invited to contact Jane Smith, EDMVS Designated Federal Official (DFO), via e-mail at smith.jane-scott@epa.gov.

cc:

Stephen Johnson
Susan Hazen
Adam Sharp
Dennis Deziel
Gordon Schisler
Sonia Altieri
OPPT Docket 42212E

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EDMVS Members in Attendance at the December 2001 Meeting

William Benson, Vice Chair
U.S. EPA

Mildred Christian
Argus Research

Theodora Emily Colborn
World Wildlife Fund

Robert D. Combes
Fund for Replacement of Animals in Medical Experiments

Rodger D. Curren
Institute for In Vitro Sciences, Inc.
(Participated by phone)

Peter L. deFur
Virginia Commonwealth University

Penelope A. Fenner-Crisp
ILSI Risk Science Institute

David Hattan
U.S. FDA

Robert J. Kavlock
U.S. EPA

William Kelce
Pharmacia Corporation

Nancy K. Kim
NY State Department of Health

Timothy Kubiak
U.S. Fish and Wildlife Service

Gerald A. LeBlanc
North Carolina State University

Ron Miller
The Dow Chemical Company

James W. "Willie" Owens
The Procter & Gamble Company

Thomas L. Potter
USDA-Agriculture Research Service

Theodore H. Schettler
Science and Environ. Health Network

Shane A. Snyder
Southern Nevada Water Authority

James T. Stevens
Syngenta

William Stokes
NIEHS

Glen Van Der Kraak
University of Guelph

Vanessa Vu, Chair
U.S. EPA

James D. Yager, Jr.
Johns Hopkins University

Facilitator
Paul De Morgan

Designated Federal Official
Jane Scott Smith

Presenters

In the Order of Their First Presentation

Jane Smith, DFO
EPA, OSCP

Gary Timm
EPA, OSCP

Dr. Earl Gray
EPA, ORD

Jim Kariya
EPA, OSCP

Dr. Ralph Cooper
EPA, ORD

Dr. Paul Foster
CIIT Centers for Health Research

PUBLIC COMMENTS

Oral Statements in the order they were made:

December 10, 2001

Rick Becker, Ph.D
American Chemistry Council

Troy Seidle
People for the Ethical Treatment of Animals

December 11, 2001

Rick Becker, Ph.D.
American Chemistry Council

December 12, 2001

Troy Seidle
People for the Ethical Treatment of Animals

Rick Becker, Ph.D.
American Chemical Council

Rochelle Tyl, Ph.D.
Research Triangle Institute

Written statements received from:

American Chemistry Council
People for the Ethical Treatment of Animals
World Wildlife Fund

NOTICE

This meeting summary has been written as part of the activities of the National Advisory Council on Environmental Policy and Technology (NACEPT), Endocrine Disruptor Methods Validation Subcommittee (EDMVS). This meeting summary has not been reviewed for approval by the United States Environmental Protection Agency (Agency) and, hence, the contents of the meeting summary do not necessarily represent the views and policies of the Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation for use.

The NACEPT EDMVS was established in partial fulfillment of a Congressional statute. When Congress amended the Federal Food Drug and Cosmetics Act (FFDCA) in the Food Quality Protection Act (FQPA) of 1996, it directed the U.S. Environmental Protection Agency (EPA) to develop a screening program to determine whether certain substances may have hormonal effects in humans. To ensure that EPA has the best and most up-to-date advice available regarding the validation of the screens and tests in the EDSP, EPA established the Endocrine Disruptor Methods Validation Subcommittee (EDMVS) under the NACEPT. The EDMVS provides independent advise and counsel to the Agency through NACEPT on scientific and technical issues related to validation of the EDSP Tier I and Tier II assays, including advice on methods for reducing animal use, refining procedures involving animals to make them less stressful, and replacing animals where scientifically appropriate. The EDMVS held their first meeting in October of 2001 and their second meeting in December 2001.

The December 10-12, 2001 open meeting of the EDMVS was announced in the Federal Register on November 21, 2001 (Volume 66, Number 225). Further information about NACEPT EDMVS meetings and activities can be obtained from its website at http://www.epa.gov/scipoly/oscpendo or the OPPT Docket at (202) 260-7099. Interested persons are invited to contact Jane Smith, EDMVS Designated Federal Official (DFO), via e-mail at smith jane-scott@epa.gov.

SUBCOMMITTEE INTERIM RECOMMENDATION

Background

On December 11, 2001 pubertal assays were presented and discussed. Dr. Ralph Cooper presented the findings of the pubertal - single dose study, noting that some results of this study differed between the Sprague-Dawley rats and the Long-Evans rats, such as the strains maturing at different ages. It was noted that these specific strain differences would not have prevented any of the endocrine active chemicals from being detected as endocrine agents.

Jim Kariya presented the design of the pubertal multi - dose and pubertal multi - chemical array study including the number of doses, the dose level(s),and the endocrine and toxicoligical systemic endpoints. He stressed the urgency that these studies proceed to the laboratory phase as soon as possible.

The EDMVS members discussed the suitability of the testing laboratory intended to perform the pubertal assays in light of the fact that the lab had recently experienced a fire. Following these considerations the members agreed on an interim recommendation.

The Interim Recommendation

- The EPA should move forward with the multi-dose and the array studies using a single appropriate strain.
- In conjunction with this activity, EPA should prepare a white paper summarizing what is known about intraspecies strains/stock similarities and differences in neuro-endocrine control of reproduction/development and in responses to endocrine active chemicals and provide the rationale for the strain/stock selection.

National Advisory Council for Environmental Policy and Technology (NACEPT)
Endocrine Disruptor Methods Validation Subcommittee (EDMVS)
Second Plenary Meeting
December 12-12, 2001
Agenda

RESOLVE
1255 23rd Street, N.W., Suite 275
Washington, D.C., 20037
(202) 944-2300

Meeting Objectives:

- Reach agreement on the EDMVS mission statement and work plan;
- Offer input and advice on:
  - The in utero through lactation assay Detailed Review Paper;
  - The pubertal assay study designs for the multi-dose and chemical array studies; and
  - The mammalian one-generation study design.

Monday, December 10, 2001

1:00 - 1:10 **Welcome and Opening Comments**
Dr. Vanessa Vu, Chair, Office of Science Coordination and Policy, (OSCP), EPA
Dr. William Benson, Vice-Chair, Office of Research and Development, (ORD), EPA

1:10 - 1:30 **Introduction, Agenda Review, and Review of Previous Meeting Summary**
Paul De Morgan, Facilitator, RESOLVE

1:30 - 3:15 **Review Revised Mission Statement and Work Plan**
Jane Smith, EDMVS Designated Federal Official, OSCP, EPA

3:15 - 3:30 **Break**

3:30 - 5:30 **Presentation and Discussion of In Utero Through Lactation Detailed Review Paper**
Gary Timm, OSCP, EPA
Dr. Earl Gray, ORD, EPA

5:30 - 6:00 **Public Comment**
Members of the public will be given an opportunity to comment on any aspect of the EDMVS work. The amount of time given to each individual will depend on the number of people wishing to provide comment.

6:00 - 6:15 **Setting the Stage for Day Two**

6:15 **Adjourn for the day**

Tuesday, December 11, 2001

9:00 - 9:15 **Settling In**

9:15 - 9:45 **Overview of Pubertal Studies**
Jim Kariya, OSCP, EPA
Dr. Ralph Cooper, ORD, EPA

9:45 - 10:45 **Presentation and Discussion of Pubertal-Single Dose Study**
Dr. Ralph Cooper, ORD, EPA

10:45 - 11:00 **Break**

11:00 - 12:30 **Presentation and Discussion of Pubertal-Multi Dose Study**
Dr. Ralph Cooper, ORD, EPA

12:30 - 1:45 **Lunch**

1:45 - 3:15 **Presentation and Discussion of Pubertal-Array Protocol** Dr. Ralph Cooper, ORD, EPA

3:15 - 3:30 **Break**

3:30 - 4:30 **Other Items Update on Assessment and Implications of RTI Lab Fire**

4:30 - 5:00 **Public Comment**
Members of the public will be given an opportunity to comment on any aspect of the EDMVS work. The amount of time given to each individual will depend on the number of people wishing to provide comment.

5:00 - 5:30 **Discussion of Information Needs and Approach to Distribution**
Paul De Morgan, Facilitator, RESOLVE

5:30 - 5:45 **Setting the Stage for Day Three**

5:45 **Adjourn for the day**

Wednesday, December 12, 2001

9:00 - 9:15 **Settling In**

9:15 - 10:45 **Presentation and Discussion of Mammalian One Generation Extension Study Associated with the Two Generation Study**
Jim Kariya, OSCP, EPA
Dr. Paul Foster, CIIT Centers for Health Research

10:45 - 11:00 **Break**

11:00 - 11:30 **Discussion of Outstanding Issues**

11:30 - 12:00 **Process Assessment**
What is working? What can be improved?

12:00 - 12:30 **Next Steps and Agenda for Third Meeting**

12:30 **Adjourn**

Introduction

The Office of Science Policy and Coordination's Endocrine Disruptor Screening Program established the Endocrine Disruptor Methods Validation Subcommittee (EDMVS). The first meeting was held in October 2001. That initial meeting was concerned with bring the members together to review the mission statement and to discuss their roles and responsibilities. This second meeting, held in December 2001, was the first time the Subcommittee members were presented with specific questions regarding assay protocols.

Endocrine Disruptor Methods Validation Subcommittee (EDMVS)
Second Meeting
December 10-12, 2001

Draft Meeting Summary

On December 10-12, 2001, the U.S. Environmental Protection Agency (EPA) convened the second meeting of the EDMVS. The meeting objectives included:

- Reach agreement on the EDMVS mission statement and work plan;
- Offer input and advice on:
  - The *in utero* through lactation assay detailed review paper;
  - The pubertal assay study designs for the multi-dose and chemical array studies; and
  - The mammalian one-generation study design.

Copies of presentation slides and other materials distributed at the meeting may be obtained by contacting Jane Smith at smith.jane-scott@epa.gov or 202/564-8476. Many of the materials also are available on the EPA website at http://www.epa.gov/scipoly/oscpendo/edmvs.htm. EPA has established an administrative record for this meeting under docket control number OPPTS-42212E. The docket is available for inspection in the TSCA Nonconfidential Information Center, North East Mall Rm. B-607, Waterside Mall, 401 M St., SW., Washington, DC. The center is open from noon to 4 p.m., Monday through Friday, excluding legal holidays. The telephone number of the center is (202) 260-7099.

Before the meeting adjourned on December 12, EPA staff presented a list of action items they had drawn from the subcommittee's discussions. In this summary the action items are listed immediately following the discussion to which they correspond.

Monday, December 10, 2001

I. Welcome and Opening Comments

Vanessa Vu, EDMVS chair and director of the EPA Office of Science Coordination and Policy (OCSP), welcomed the EDMVS members and the public to the meeting. She observed that at the first EDMVS meeting EPA presented an overview of the Endocrine Disruptor Screening Program (EDSP) and the EDMVS reviewed its mission statement and general work plan. She said that at this meeting the EDMVS would have an opportunity to review a more detailed work plan and also to provide input on several assays. She noted that a discussion of low-dose issues was not on the agenda and explained that EPA was not yet ready to formulate a policy on the issues. She said that a discussion of low-dose issues will be scheduled for the March meeting.

Bill Benson, EDMVS vice-chair and director of the Gulf Ecology Division of the EPA Office of Research and Development (ORD), also welcomed the subcommittee and thanked members for their participation.

II. Introductions, Agenda Review, and Review of Previous Meeting Summary

Paul De Morgan, senior mediator with RESOLVE, introduced himself and asked the EDMVS members to identify themselves and their organizations.

Jane Smith, designated federal official for the EDMVS, explained that the meeting was being held in accordance with the Federal Advisory Committees Act (FACA) and all materials distributed would be available through the docket. She explained that EDMVS members must meet the ethics standards for FACA committee members. Members of this subcommittee are intended to represent various stakeholders and come to the table with a bias. She invited anyone with comments on EDMVS membership or other concerns to contact her.

Maggie Rodriguez, Megatech, briefly explained travel and reimbursement logistics for members traveling under EPA invitational travel, as they will be handling EDMVS logistics from now on.

Mr. De Morgan gave an overview of the materials distributed to the members. He explained that the binder given to each member included all of the materials distributed prior to the meeting (except the appendices of *Detailed Review Paper on In Utero/ Lactational Protocol*). He listed the other materials given to the members and available to the public at the table outside the meeting room: public comments received in writing following the October meeting, an updated participant list, a revised mission statement, a clarification of the mission, and copies of presentation slides.

Mr. De Morgan then reviewed the meeting agenda. He noted that time was allotted for public comment at the end of each day and asked that commenters tailor their remarks to the day's topics as much as possible. Mr. De Morgan then reviewed the ground rules that will apply at each meeting.

Mr. De Morgan outlined the operating procedures for reviewing the meeting summaries: members will receive a draft summary four to five weeks after the meeting and will have two weeks to submit their comments; RESOLVE will work with EPA to address the comments; outstanding issues will be discussed at the following meeting; and the EDMVS chair will give final approval to the summary. He explained, however, that because of the short time period

between this meeting and the first meeting, the draft summary of the first meeting had not yet been distributed to members. Mr. De Morgan proposed emailing the draft to members on December 12. He said members would be given two weeks to review the draft and submit comments, and if necessary, unresolved concerns would be discussed at the March meeting. Members agreed with the proposed timeline.

In response to questions, Ms. Smith explained that anything submitted to the EDMVS, including public comments, becomes part of the docket and is available to the public. Ms. Smith said she also will post on the EDSP website any public comments submitted in writing. Mr. De Morgan added that the full meeting was being transcribed, and the transcript would be part of the docket.

III. Review of Work Plan and Revised Mission Statement

Work Plan

Via a PowerPoint presentation, Ms. Smith presented a draft work plan for the EDMVS outlining the timeline and milestones of the subcommittee's work through 2003. She stressed that specific dates may be revised as the program moves forward. Gary Timm, EPA, OSCP, pointed out that the work plan is for the EDMVS and includes only the steps of the EDSP in which the subcommittee is involved. While members felt that the work plan provided a good overview, they requested that more detailed plans be provided for each assay as they are introduced to the EDMVS.

Points raised during the discussion included the following:

Some members noted that the work plan did not include a review of the prevalidation program for each assay. Mr. Timm responded that each detailed review paper (DRP) will 1) provide the background to discuss endpoints of interest, 2) discuss the types of studies that need to be conducted, and 3) review the protocols that have been used to date or are likely candidates for the prevalidation protocol. He said that EPA would like EDMVS guidance on how to improve the DRPs in the future to make them as useful a tool as possible. He said the intent was for the EDMVS to provide comments and advice, based on DRPs, that EPA could use to develop a detailed work plan for prevalidation for the given assay. Several members commented that they would like to have a prevalidation study discussion for each assay so that they could review the full details of the study.

In response to questions, Dr. Vu explained that the purview of the EDMVS is the tier 1 and tier 2 assays and does not include the Quantitative Structure Activity Relationship (QSAR) models. She offered, however, that EPA could keep the EDMVS informed of the agency's work and progress on the QSAR models. A member commented that the EDMVS process should be flexible enough to allow discussion of new techniques and technologies and EPA's response to them.

Mission Statement

Building on issues and questions raised at the first meeting, Ms. Smith presented a revised mission statement and a document further clarifying the mission. She explained that in the tables

in the clarification document, a "yes" in a square indicates that the EDMVS will advise EPA on that step of the process, a "no" indicates the EDMVS will not advise EPA. She clarified that some of the assays were not included in the tables. She said she will revise the tables to include all of the assays as well as dates as in the work plan.

In response to questions, Dr. Vu explained that even for assays validated through the Organization for Economic Cooperation and Development (OECD) process, the EDMVS will be asked whether it agrees that the given assay has been validated. She said the EDMVS will also be asked to consider the utility of each assay and whether it should be included in the battery.

Noting that the use of terms varies among entities, members requested that EPA clarify what will take place at the various stages of the EPA process and what specifically is meant by prevalidation, validation, and other terms used by EPA. EPA agreed to provide clarification and address these questions at the next EDMVS meeting.

Ms. Smith reviewed the changes made to the mission statement in response to comments made at the October meeting. In response to questions she clarified that any recommendations and advice forwarded by the EDMVS to the NACEPT prior to the final EDMVS recommendation on the overall battery will be considered interim. She explained that NACEPT does not have to respond formally to the interim recommendations and advice, but they will be considered by EPA and will be part of the public record of the EDMVS.

The EDMVS agreed to accept the revised mission statement as presented by Ms. Smith.

Action Items:

EPA will develop a table merging the work plan dates and the mission clarification table.

EPA will develop timelines for the individual assays as dates become more concrete.

EPA will develop a diagram of the Endocrine Disruptor Program activities, including the validation process as it fits within the program.

EPA will distribute the final mission statement and the final operating procedures to EDMVS members.

EPA will prepare a paper to clarify the definition of and performance criteria for validation and how it translates to the Endocrine Disruptor Screening Program (EDSP).

IV. Presentation and Discussion of *In Utero* Through Lactation Detailed Review Paper

Mr. Timm began the session by noting that the DRP was still a work in progress but was being brought to the EDMVS to get input on the design of the protocol and prevalidation studies for this assay so that laboratory work could begin soon. Mr. Timm highlighted the general areas in which EPA would like guidance from the EDMVS on the *in utero*/lactational (IUL) assay:

Suggestions of revisions to improve this draft DRP or the DRPs in general;

Detailed guidance on what to include in the protocol;

Guidance on whether the assay should be considered for tier 1, "tier 1.5" or tier 2.

Earl Gray, EPA, ORD, presented information on where the IUL assay fits into the process, the

general protocol developed based on the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) report, the value and limitations of the IUL assay, and where exposure during development fits in the screening and testing process. (As indicated above, copies of slides from Dr. Gray's presentation, "OPPTS EDMVS Meeting 12/10/01," may be obtained from Ms. Smith.) Dr. Gray noted that the tier 1 screening battery as recommended by EDSTAC does not include developmental exposure. He also noted that EDSTAC recommended that EPA consider developing an *in utero* assay that included fetal exposures.

Dr. Gray listed several positive points of testing *in utero* exposure: the fetus is uniquely sensitive to some estrogen, androgen, and thyroid agents; the fetus is known to be uniquely susceptible because it is undifferentiated and effects that occur during development are frequently irreversible; and developmental exposure will detect developmental toxicants operating through non-endocrine mediated modes of action as well. He also listed potential negative points of the assay: the assay is likely to miss weak estrogen effects, the assay is labor intensive and costly**,** the assay may lack specificity and sensitivity, and the *in utero* and lactational stages are not always the most sensitive in mammals.

Dr. Gray outlined the current proposed tier 1 screening IUL protocol. He noted that the studies include a large number of endpoints to be evaluated so that all potential reproduction malformations in the males and females are included.

In response to questions, Dr. Gray commented that the assay will take 85 days and use a few less than 400 animals. He explained that the assay is different from a one-generation test in that it does not include pre-mating exposure, and it includes additional endpoints not included in the one-generation assays. He suggested that the assay may have value as a tier 1.5 assay if it were tailored based on the results of a tier 1 screen.

Points raised during the discussion included the following:

Several members expressed the opinion that the assay should not be a tier 1 screen. Some commented that the assay uses too many animals to be a tier 1 screen. Another member added that other factors such as the endpoints and histology also indicate that the IUL assay is beyond tier 1. He suggested that the protocol could be modified to use fewer animals if it were to be a tier 1 screen. Members also expressed differing opinions on whether it is necessary to have a tier 1 screen with exposure at an extremely sensitive life stage.

Other members expressed hesitancy at ruling out the IUL assay as a tier 1 screen at this point. A member commented that it is difficult to decide where the IUL assay fits before having the details and results of the various assays to compare. Another member pointed out that the assay covers two life stages of concern and may be able to replace other screens. He suggested that the dose-setting criteria should be determined before ruling it out as a tier 1 screen.

Other members pointed that there also may be negatives to including the IUL assay as a tier 2 test. One member summarized that the IUL assay should be included if it is a superior, definitive tier 2 test and obviates the need for another test.

Members acknowledged that the IUL assay may be a possible back up for assessing thyroid function if other assays do not work well for thyroid.

Based on the discussion, Mr. Timm proposed that 1) EPA develop a fairly expansive protocol that resembles a tier 2 test more than a tier 1 screen, 2) run the protocol with three dose levels plus a control and challenge it with several chemicals, and 3) bring the data back to the EDMVS for further discussion of the assay's appropriate role. Members agreed with the proposal and helped Mr. Timm identify questions for the study to address, including:

Is the IUL assay sensitive to thyroid? Is it more sensitive to thyroid than the pubertal assays?

Does the IUL protocol more effectively identify inhibitors of steroidogenesis?

Are there chemicals that would be identified by the IUL assay that would/might be missed by other tier 1 screens? (e.g., developmental exposure)

Where might the IUL assay replace other androgen and estrogen assays?

Members discussed which of the three protocols presented in the DRP EPA should use for the study. After hearing their comments, Mr. Timm proposed developing a protocol similar to protocol C in the most recent version of the DRP. He said that EPA could distribute the protocol and a study plan to the EDMVS and then hold a conference call to discuss them. Members agreed, however, one member noted that protocol C is much more complex than protocol B and asked to see a clear rationale for why some of the additional endpoints should be included.

A member commented on the importance of statistical power in this or any tier 1 assay. Noting that tier 1 screens must try to avoid false negatives, he cautioned that EPA should do a rigorous power analysis of the various endpoints. He said that it is important to determine what endpoint will be sufficient for tier 1 screening purposes and what degree of positivity will trigger tier 2 testing.

Mr. Timm requested that members submit written comments to him on the DRP so that the draft can be revised and improved and also so that the DRP format in general can be improved.

Action Items:

Prevalidation Study

EPA will develop a prevalidation study plan based on an updated version protocol C in the DRP.

The study plan will identify several chemicals and recommended dose levels with justification for choices made.

The study plan will include rationale for endpoints included.

The study plan will include statistical power analysis of various endpoints.

EPA will e-mail the study plan to the EDMVS for a 2-week review and schedule a conference call to allow members to discuss the plan.

Detailed Review Paper (DRP)

By January 4, EDMVS members will submit comments to EPA (Smith.Jane-Scott@epa.gov) regarding the *in utero* through lactational assay DRP.

The final DRP will be revised to reflect comments from EDMVS members.

The final DRP will include a table listing the endpoints of interest and predicted responses to agents of known modes of action (a qualitative prediction model).
The final DRP will include a chart comparing protocols A, B, and C.
Protocol C in the DRP will be updated to reflect the 2/23/2000 revisions to the protocol made by Dr. Earl Gray.

V. Public Comment

At the conclusion of the deliberations, members of the public attending the meeting were given the opportunity to provide comments. Mr. De Morgan indicated that each person's comments would not be captured verbatim in the meeting summary, but rather just briefly summarized. He encouraged all to submit their comments in writing to Ms. Smith for inclusion in the EPA docket and posting on the website.

Rick Becker, American Chemistry Council
Dr. Becker encouraged the EDMVS members to discuss dose selections in their deliberations on the pubertal assays, stating that use of the maximum tolerated dose should not be required. He suggested that additional prevalidation work be done on the pubertal onset assays before moving to validation. He also requested that the 15-day intact male assay be considered by EPA for validation. (As indicated above, copies of slides from Dr. Becker's presentation, "Comments on the *In Utero*/Lactational Assay, Male and Female Pubertal Onset Assays, 15-Day Intact Male Assay," may be obtained from Ms. Smith.)

Troy Seidle, People for the Ethical Treatment of Animals
Mr. Seidle noted EPA's stated commitment to reducing animal use in its testing programs and the mandate of the EDMVS to consider ways of reducing, refining, and replacing animal use. He commented that there should be a specific mechanism for the EDMVS to consider and incorporate new and, ideally, non-animal methods into the endocrine program as technology becomes available. He also requested that the EDMVS carefully consider how rat data will be compared to humans to determine the relevance of the assays for a meaningful screening program.

Tuesday, December 11, 2001

VI. Pubertal Studies

Jim Kariya, EPA, OSCP, gave a brief presentation reviewing the role and status of the pubertal assays. (As indicated above, copies of slides from Mr. Kariya's presentation, "The Pubertal Assays in the Endocrine Disruptor Screening Program," may be obtained from Ms. Smith.) He said that the pubertal assays are a high priority in the EDSP because they cover many mechanisms and because the standardization of the assays is nearly complete, with a substantial body of work showing the assays' utility. Mr. Kariya explained that a DRP *per se* had not been prepared for this assay because the review papers published in *Critical Reviews in Toxicology* cover most of the points required for a DRP. In closing he commented on the importance of starting the studies soon due to the time requirements of the studies and the time frame of the

validation program.

Ralph Cooper, EPA ORD, outlined the pubertal protocols, summarized the data that indicate the ability of the protocols to detect endocrine disrupting chemicals, and discussed some of the advantages and potential problems of the protocols. (As indicated above, copies of slides from Dr. Cooper's presentation, "Male and Female Pubertal Assays," may be obtained from Ms. Smith.) He commented that past research has led to a good understanding of the endpoints evaluated in the pubertal assays, noting that many of the endpoints are currently used in EPA testing.

Dr. Cooper listed the required and optional measures of the female and male pubertal assays and presented some of the results of single-dose studies done using the protocols. He indicated effects for which results differed between the Sprague-Dawley rats and the Long-Evans rats, including discrepancies in the ages of preputial separation. He commented that for most endpoints, the effects observed in the studies agreed with the predicted effects for the various compounds.

In summary, Dr. Cooper remarked that the pubertal protocols detect a wide variety of endocrine disrupting chemicals. He commented that the advantages of the protocols include that:

the tests are apical,
they provide dose response information, take into account metabolism since they are *in vivo* studies, and avoid the complications arising from litter effects since the individual pups are dosed,
they provide information about mode and mechanism of action,
the protocols appear to be robust across rat strains, and
the protocols involve relatively simple procedures.

He also noted the drawbacks of the protocols:

precise measures of hormones are lacking,
effects of decrease in body weight gain have not been clearly separated from direct effects on the endocrine system, although the Agency is working on this issue,
the protocols are not transplacental assays,
the protocols, especially the male, may be a bit lengthy, and
cost, in the current format, is high.

Dr. Cooper said that based on what was learned from the single dose studies, EPA intends to clarify the descriptive text in the protocols, establish performance criteria to include in the protocols, and evaluate the lower limits of detection of the protocols. He said the agency also is considering whether the protocols should recommend a rat strain.

Mr. Kariya gave another brief presentation summarizing the general design of the pubertal single-dose study, the pubertal multi-dose study, and the pubertal multi-chemical array study. (As indicated above, copies of slides from Mr. Kariya's presentation, "Pubertal Assays (female and male)," may be obtained from Ms. Smith.) For each assay Mr. Kariya outlined the general design and listed the chemicals, doses and endpoints.

Points raised during the discussion included the following:

Standardization: Members pointed out that feed, vehicle, and water can be sources of variability and contamination. They discussed the difficulties of controlling diets but in the end did not have a solution for dealing with the many problems that could arise from non-standardized feed. There was some discussion of the need to standardize the water source as well, and a suggestion to use stripped corn oil as vehicle for the test chemical rather than unspecified corn oil. Some members also commented on the standardization of techniques, particularly how the observations of vaginal opening and preputial separation are made. One member pointed out that standardizing light/dark cycles as 12 hours light and 12 hours dark rather than 14/10 would make the studies consistent with others being run in most labs. A member suggested that EPA develop a video or CD ROM to help teach specific techniques to laboratory staff. In general, the EDMVS advised EPA to standardize all factors of variability to the extent possible.

Strain Differences: Members observed that in the studies presented, differences among rat strains would not have prevented any of the compounds from being detected as endocrine agents. They noted, however, that strain differences may be more important in other studies, especially those using weaker doses. A member commented that additional data are needed before a decision on strain differences is made. Another member pointed out, in particular, that the blood-testis barrier develops at different times in different strains, which may affect results. In general, members agreed that the evidence of the effects of strain differences was not compelling enough at this point to hold up the multi-dose pubertal or multi-chemical array studies. The EDMVS reached consensus on the following interim recommendation, with the understanding that it will be forwarded to the National Advisory Council for Environmental Policy and Technology (NACEPT):

> EPA should move forward with studies (multi, array) using a single appropriate strain/stock. In conjunction with this activity, EPA should prepare a white paper summarizing what is known about intraspecies strains/stock similarities and differences in neuroendocrine control of reproduction/development and in responses to endocrine active chemicals and provide the rationale for the strain/stock selection.

Multi-dose study: Several members expressed concern that the study did not include a weak thyroid chemical. Dr. Cooper responded that EPA had not overlooked thyroid but is waiting for the results of some special studies currently underway. Members also commented on the dose levels of the chemicals. Dr. Gray pointed out that determining dose response is not the key purpose of the study; rather, the question to be answered is whether the assay works for this mechanism with an environmental chemical and the absence of an effect on body weight.

Multi-chemical study: The EDMVS discussed the chemicals to use in this study and suggested EPA consider some modifications to the list as indicated below. They also suggested that any endpoints included in the study should be required rather than listing some as required and some as optional.

Suggested Chemicals for Study on Females:	Atrazine
	Propylthiouracil
	Fadrozole
	Fenarimol

Bisphenol A
Ketoconazole
Methoxychlor *(Added to replace Octylphenol)*
Perchlorate (or other weak thyroid) *(Added)*

Suggested Chemicals for Study on Males:

Atrazine
Propylthiouracil
Ketoconazole
Linuron
p,p'-DDE
Finasteride *(Added)*
Methoxychlor *(Added)*
Vinclozolin *(Added to replace Procymidone)*
Perchlorate (or other weak thyroid) *(Added)*

Other points raised during the discussion included:
Take small organ weights in the multi-chemical array study,
Consider multiple labs for prevalidation as validating animal tests is a new endeavor,
Test a weak estrogen in the males to determine whether the same answer can be obtained from males and females,
Take study as an opportunity to carry some animals out further and possibly obtain some of the data the tier 2 assays will seek, and
Include more negative compounds to test for false positives.

Action Items:

Standardization

EPA will consider standardizing: vehicle; diet; water; dose-setting; light cycle; and definitions and techniques, including how vaginal opening and preputial separation measurements are to be made.
EPA will consider developing a training video for techniques of concern.

Strain Differences

EPA will consider doing other studies on 1) strain differences in onset of puberty and 2) strain differences in sensitivity (e.g., adding Long-Evans rats to multi-dose study), but will proceed using Sprague-Dawley rats at this time.
The interim recommendation will be forwarded to the NACEPT.

Multi-dose study

EPA will consider adding a weak thyroid chemical.
EPA will consider negative controls.

Multi-chemical study

EPA will consider including small organ weights.
EPA will consider using the chemicals listed above.
EPA will consider negative controls.

Literature Review
EPA will check the literature for postnatal day of formation of blood-testis barrier in rats.

VII. Other Items

RTI Lab Fire

Greg Schweer, EPA, OSCP, explained that information on the RTI laboratory fire was being shared with the EDMVS in the interest of transparency. He noted that representatives from RTI briefed the EDMVS on the fire at the last meeting and members received copies of the RTI report on the fire, the investigation, and the post-fire improvements. Mr. Schweer then introduced Dr. Rochelle Tyl, study director for life sciences and toxicology at RTI.

Dr. Tyl briefly described what happened during and immediately following the fire and then summarized the facility remediation, collections, and biological evaluations conducted in response to the fire. (As indicated above, copies of Dr. Tyl's slides may be obtained from Ms. Smith.) She reported that extensive testing of animals in rooms adjacent to the room with the fire showed no clinical effects. She said it has not yet been determined whether the fire was started by an employee or by someone else, but she is confident that the security measures now in place are sufficient to dissuade employees from similar actions in the future.

Dr. Vu said that at this juncture, EPA believes the report from RTI is adequate and the agency intends to proceed with the tests scheduled to be conducted at the laboratory. She asked that EDMVS members let EPA know if they see any scientific issues related to the fire that may compromise the tests.

EPA Settlement Agreement with the Natural Resources Defense Council (NRDC)

Rob Wing, EPA Office of General Council, spoke briefly about the NRDC settlement to help clarify some points for members. He said that NRDC brought a suit against EPA claiming that the agency had failed to meet the 1999 deadline to implement the endocrine disruptor screening program. He explained that the suit resulted in a private settlement agreement that is not overseen by the court; if NRDC believes EPA is not meeting its obligations, NRDC must file another suit in order to take legal action. Mr. Wing said that under the settlement, EPA agrees to use best efforts to meet various deadlines. He explained that if EPA does not meet the deadlines it must file semi-annual validation status reports with NRDC.

General Issues

During the day's discussions, several general issues were raised. Comments on them are summarized below.

- Members requested that EPA articulate its dose-setting procedures. Mr. Kariya pointed out that EPA would like the advice of the EDMVS on dose setting generally for all the assays, but he requested that members hold their comments until a later meeting when more time could be devoted to the issue.

- Members commented that without consistency of chemicals across assays, it will be difficult to compare them for the battery decision. They advised EPA to develop a core set of chemicals to be included in all the studies. Mr. Timm commented that EPA would like input from the EDMVS on what this set of chemical should include.
- Members expressed concern that the protocols require culling by decapitation **and requested** that EPA find a method of culling that is better for animal and operator welfare.
- Members raised the issue of the relevancy of animal tests to human health. The subcommittee considered an interim recommendation requesting that EPA develop a white paper on the issue of relevance. One member observed, however, that the data are not available to prepare such a paper. Dr. Vu commented that the question of relevancy is larger than the EDSP and involves a host of issues for the agency to examine. She suggested that no interim recommendation be made at this time. She said instead, EPA staff will take some time to summarize the agency's activities on relevancy, and then the EDMVS can determine what aspect the EDMVS should address.

Action Items

- EPA will update the table of chemicals used in EDSP studies to include:
- ER/AR binding study chemicals,
- adult male chemicals, and
- corrections.
- EPA will consult with EDMVS to choose a core set of chemicals to include across assays.
- EPA will consider including systemic toxicants that are expected to be non-endocrine-active in assays.
- EPA will code chemicals in prevalidation studies, if possible.
- EPA will provide EDMVS with rationale for doses when presenting studies.
- EPA will find a more humane way for sacrifice of culls, if possible.
- EPA will provide members a status report/update on agency activities addressing animal relevancy to human health.

VIII. Public Comment

At the conclusion of the presentations and discussion, members of the public attending the meeting were given the opportunity to provide comments.

Rick Becker, American Chemistry Council

Dr. Becker shared his comments on the mammalian multigenerational reproduction study. He presented a comparison of three studies and concluded that the data do not suggest that the design of the current mammalian multigenerational reproduction study is deficient. He asked EDMVS members to keep these data in mind as they discuss the multigen study. He also offered several specific suggestions for further review and research. (As indicated above, copies of slides from Dr. Becker's presentation, "Perspectives on the Mammalian Multigenerational Reproduction Study," may be obtained from Ms. Smith.)

IX. Information Needs and Approach to Distribution

Mr. De Morgan asked members for their input on how best to manage the large amount of

information members need in order to prepare for each meeting. After some discussion, members agreed with the following suggestions:

Distribute the documents in electronic format whenever possible.

Distribute materials as soon as possible and provide a schedule of when various documents are expected to be sent.

Develop a coding system to help organize the documents and provide an index.

Prepare a binder for each meeting containing the relevant documents. Note which materials were sent out but not included in the binder and which are in the binder but were not sent out

After the meeting, mail the binders to members who travel from out of town.

Wednesday, December 12, 2001

X. Mammalian One Generation Extension Study

Paul Foster, Chemical Industry Institute of Toxicology (CIIT) Centers for Health Research, presented background information and outlined the rationale for the one-generation extension study. (As indicated above, copies of slides from Dr. Foster's presentation, "Background and Rationale for 'Extended One-generation' Study," may be obtained from Ms. Smith.) Introducing the presentation, Mr. Kariya commented that the study is only to investigate a potential modification of another assay (viz., the 2-generation study) that may result in more information.

Dr. Foster began by outlining the basic test protocols that provide information on endocrine active chemicals (EACs). He pointed out that the stated purpose of a tier 2 test is to provide definitive information on hazard characterization of EACs, noting that the ability to measure effects at different doses is critical. After describing the general multigenerational protocol Dr. Foster noted that the multigeneration reproduction study covers the critical developmental windows for sexual differentiation and should detect potent estrogens and antiandrogens. He also noted some shortcomings of the study:

- Only one animal per sex per litter from the first generation of pups (F_1 generation) is examined at adulthood;
- A number of developmental endpoints are not included or are triggered only in the second generation (F_2 generation), which stops at weaning; and
- Gross necropsy at weaning is helpful but not sufficient.

Dr. Foster said that EPA is considering an alternative assay for potential use based on a transgenerational study design that uses fewer litters but examines more offspring from each and includes more endpoints related to EACs.

Dr. Foster explained that the multigeneration reproduction study was originally designed to provide significant information on reproductive toxicity and, to a more limited extent, postnatal development. He said the study is now being used for hazard characterization of EACs, where postnatal development is a key indicator of adverse response, and posed two questions to be addressed:

- Should the same degree of scientific rigor applied in prenatal development studies be used in postnatal evaluations?
- Should consideration be given to the design of a transgenerational test protocol to

specifically meet the needs of hazard characterization of EACs?

Dr. Foster commented that a transgeneration study might be used as a substitute for the costly, labor intensive multigeneration study. He suggested it might also be considered as a "tier 1.5" assay to obtain information about whether or not a compound really does have a potential adverse outcome, which would then trigger a definitive study containing more animals or endpoints. He commented that he considers the one-generation extension study protocol to be hypothesis-led, seeking to answer some of these questions.

Dr. Foster then outlined the objectives for the proposed one generation extension study:

- Determine whether some of the effects from perinatal exposure to well characterized antiandrogens that can be readily detected after puberty are missed in weanling animals of the F_1 generation.
- Determine whether some of these effects occur at an incidence that would go undetected if only one male per litter is retained past puberty and examined at adulthood.

Dr. Foster also outlined attributes of the study that need to be incorporated:

- Select dose levels of characterized agents that will produce clear effects and approximate a Lowest Observed Adverse Effect Level (LOAEL).
- Use numbers of litters comparable to that used in a standard multigeneration/prenatal toxicology study.
- Since the exposure period is limited, use a route of exposure that maximizes control of administered dose on a milligram per kilogram per day basis.

Following the presentation, there was some confusion among members as to the role and purpose of the one-generation extension study. Mr. Kariya clarified that EPA is not proposing it as a separate, additional assay but as a potential modification to the existing mammalian two-generation assay. Dr. Benson explained that Dr. Foster's proposal is an experiment to explore how the two-generation assay might be improved. He said that the question posed to the EDMVS is whether this experiment will be useful in obtaining information on how to modify the two-generation study. Dr. Kariya said the data from Dr. Foster's work would be brought to the EDMVS for its advice on what the data indicate, whether it is an appropriate modification for the two-generation assay, or whether there might be other uses for it.

Points raised during the discussion included the following:

Members commented on the importance of testing females as well as males. Dr. Gray commented that EPA is considering a separate study on females.

One member noted the lack of scientists with sufficient background and training to make educated decisions about study design and triggers on a case-by-case basis. She advised, therefore, that the triggers be built into the protocol. She also noted a lack of expertise for examining fetal and immature tissue.

Members expressed support for the idea of using all the pups from a litter, as Dr. Foster suggested, rather than culling.

A member noted that if the one-generation extension study described by Dr. Foster replaced the current multigenerational study the functional evaluation of the F_1 progeny would be lost because they are not mated. He suggested that it should be considered whether the breeding

of the F_1 progeny produced much information that had an impact on risk assessment or dose settings.

A member echoed Dr. Foster's point that the increase in cost and logistics of a modified multigenerational study will be significant.

Members commented on the importance of international harmonization on the multigenerational study. They noted that through extensive effort, guidelines for the study were accepted internationally in 1998. A member commented that given this recent effort, the international community may resist another modification.

A member commented that to evaluate the adequacy of the current protocol, a point-blank comparison is needed between the existing design and the proposed design.

A member commented that gavage is not an acceptable route of exposure for a multigenerational reproduction study for standard risk assessment. He questioned how information from such a study could be extrapolated to human risk. Dr. Foster disagreed, expressing a doubt that feeding patterns of rodents are more similar to human exposure than gavage is. He said that the kinetics must be known before the most appropriate route of exposure can be determined. Other members echoed the importance of considering route of exposure and kinetics. One suggested that the study be designed somehow to measure exposure in terms of the real, absorbed dose.

A member suggested that tissues from the animals in this study be used to start to examine other problems of concern such as cardiac birth defects and pancreatic effects.

Following the discussion Mr. Kariya summarized the overall direction he had heard from the members. He said he heard general support for the study but also heard that EPA must be sure of the purpose of the study and must be clear that it is not geared toward regulatory implementation at this point. He also noted the suggestions to test females at some point, though not necessarily in this study, and to gather other tissues such as the heart and pancreas for evaluation.

Action Items:

EPA will proceed with the one-generation extension study.

EPA will consider doing a separate one-generation extension study involving females.

XI. Public Comment

At the conclusion of the presentation and discussion, members of the public attending the meeting were given the opportunity to provide comments.

Troy Seidle, People for the Ethical Treatment of Animals

Mr. Seidle asked the EDMVS members to consider the question of who makes the final determination that a test method is or is not valid or has not been validated. He pointed out that the EDSTAC recommended that the task be performed by the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM). He pointed out that the EDMVS is under tremendous time pressure and suggested that EPA should not overburden the EDMVS by giving them this task that is the function ICCVAM was intended to perform.

Rick Becker, American Chemistry Council

Dr. Becker commented that, to the best of his knowledge, the multigeneration study design modifications have not been discussed at the OECD in any formal way. He said that in his opinion, the multigeneration guidelines are a long way from being reconsidered at the international level.

Dr. Becker also commented that he was still unclear on the design and hypothesis of the transgeneration assay. He said that if the question being tested is whether there are reproductive track effects from known agents that would not be detected in weanlings but would in adults, the answer is already known. He said it is also known that increasing the number of animals in any experiment will increase the probability of detecting effects. He suggested that the real hypothesis to be tested is whether the revised Office of Prevention, Pesticides, and Toxic Substances (OPPTS) 1998 guidelines for the multigeneration study are sufficient. He also made specific suggestions of how EPA could test that hypothesis.

Rochelle Tyl, RTI

Dr. Tyl, using overheads, shared data from a recently completed multigeneration study on butylbenzylphthalate done according to the OPPTS 1998 guidelines. (As indicated above, copies of Dr. Tyl's slides may be obtained from Ms. Smith.) She said that the client for the study agreed to track endpoints beyond what the guidelines required. Based on what she found, Dr. Tyl commented that the one-generation extension study is an excellent tool for hypothesis testing. She explained that by examining all offspring males as adults and then randomly deleting the data on some of them, the researchers will be able to determine whether all reproductive malformations detected in the full group would be detected in the smaller group. She clarified that because the study is *in utero* lactational exposure it is a litter-based analysis. She explained that, therefore, increasing the number of pups examined per litter will not increase the "n," but it will give a better characterization of the litter response.

XII. Process Assessment

Mr. De Morgan asked members to share their comments and suggestions on the process and how it might be improved. Comments made by members included the following:

- Distribute the presentations prior to the meeting.
- When distributing the materials, clearly delineate the issues EPA would like the EDMVS to address, the actions to be taken, the questions to be answered, etc.
- When asking for comments or approval of something, provide a clear idea of what the data will be used for.
- Clarify how and when consensus is reached or separate comments are conveyed.
- List the subcommittee's decisions.
- Clarify the EPA vision of validation in regard to the roles of other entities.
- Clarify the "big picture" (e.g., suite of chemicals common to all studies, EPA's definition of validation).
- The questions prepared by EPA on the materials discussed at this meeting were helpful.
- Provide a detailed protocol to review in the prevalidation stage for each assay.
- Show the validation procedure for each assay/study.
- Provide a standard presentation of each assay, including basic components such as the

rationale for the study design, the list of chemicals, and the rationale for the choice of chemicals.
- Revise the chemical table.
- Establish a way to add chemicals to the list as the process continues.
- Be aware of international discussion on these topics.
- Display and summarize data in tables or other formats that make them easy to compare.
- Form a small group to begin considering the triggering process.

Members also asked how to share comments that go beyond the level of detail covered during the meetings. Ms. Smith said that they should submit comments to her and she will pass them to the appropriate EPA staff and make them available to the other members.

Mr. De Morgan commented that he had spoken with EPA staff about preparing a "next steps memo" to distribute to members within a week or two after each meeting. He explained that EPA would prepare and distribute the memo to summarize some of the key advice heard at the meeting from individual members and the group. He said the memo would outline next steps and action items for members and EPA. He explained that the memo will serve as a very brief summary of meeting highlights while the full summary is being prepared.

XIII. Next Steps and Agenda for Third Meeting

Members discussed a suggestion to hold the March meeting in Gulf Breeze, FL, where they could tour a non-mammalian, aquatic facility. They decided, however, to meet again in Washington, DC. The group also discussed the meeting site and decided to continue to meet at the RESOLVE offices. Some of the members who travel for the meetings requested that mid-week dates be considered for future meetings to prevent having to travel on Sunday evening.

Future Meeting Dates
- The third EDMVS meeting will be March 25-27, 2002.
- The fourth EDMVS meeting will be June 10-12, 2002.
- RESOLVE will email schedule-availability forms to members to determine the best date**s** for the fifth and sixth meetings.

March Agenda Items
Ms. Smith presented a list of items tentatively scheduled to be on the agenda for the March 25-27 meeting. Mr. De Morgan noted that items 1 through 6 were previously slated for the March meeting, and items 7 through 9 were added based on discussion at this meeting.

1. Steroidogenesis DRP
2. Fish Reproduction DRP
3. Aromatase DRP
4. Avian 2-gen DRP
5. Fish Chronic DRP
6. Low dose
7. Dose-setting for pubertals
8. Overview of current efforts to address relevancy issues

9. Validation – definition, performance criteria, and how it translates to the EDSP

First Meeting Summary

- RESOLVE will email the October meeting draft summary to EDMVS members immediately following this meeting.
- By January 4, EDMVS members will review the October meeting draft summary and submit comments to RESOLVE.

XIV. Closing Remarks

Dr. Vu thanked the EDMVS members and the public for attending and thanked the speakers for their presentations. She said that EPA will seriously consider the comments heard at the meeting and will try to make the EDSP as transparent as possible for the public. Dr. Benson thanked members and the public for their patience and dedication.

MEETING SUMMARY
OF
ENDOCRINE DISRUPTOR METHODS VALIDATION SUBCOMMITTEE
ON
DECEMBER 10-12, 2001
AT
RESOLVE, 1255 23rd STREET, N.W. SUITE 275
WASHINGTON, D.C.

This meeting covered revisions of the EDMVS mission statement and work plan, discussions on the in-utero through lactation and pubertal assays as well as the mammalian one-generation study plan.

/s/ **/s/**

Ms. Jane Scott Smith
Designated Federal Official
NACEPT EDMVS
Date: 5/14/2002

Vanessa T. Vu, Ph.D. Director
Office of Science Coordination and Policy
NACEPT EDMVS Chair
Date: 5/14/2002