

AGENDA
NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS

May 14, 1997

Building 101, Conference Rooms A & B, South Campus
National Institute of Environmental Health Sciences (NIEHS)
Research Triangle Park, North Carolina

8:45 - 9:30 a. m.	Reports of the Director, NIEHS and NTP, and Director, ETP	Dr. K. Olden, NIEHS Dr. G. Lucier, NIEHS
9:30 - 10:00 a. m.	Concept Review: Molecular Oncology Support	Dr. R. Maronpot, NIEHS
10:00 - 10:15 a. m.	<i>Break</i>	
	<u>Endocrine Disruptor Initiatives</u>	
10:15 - 10:35 a. m.	Introduction	Dr. G. Lucier
10:35 - 11:00 a. m.	Nomination and Selection of Agents for Reproductive/Developmental Toxicology	Dr. M. Shelby, NIEHS
	NIEHS Activities on Endocrine Disruptors	
11:00 - 11:30 a. m.	- Toxicology	Dr. R. Chapin, NIEHS
11:30 - 12:30 p. m.	<i>Lunch</i>	
12:30 - 1:00 p. m.	- Mechanistic Studies	Dr. J. Lindzey, NIEHS
1:00 - 1:30 p. m.	- Risk Assessment Methodologies	Dr. C. Portier, NIEHS
1:30 - 2:00 p. m.	- Extramural Activities	Dr. J. Heindel, NIEHS
2:00 - 2:20 p. m.	<i>Break</i>	
	Other Federal Agency Activities	
2:20 - 2:40 p. m.	NCEH/CDC	Dr. T. Sinks, NCEH
2:40 - 3:00 p. m.	FDA/NCTR	Dr. B. Schwetz, FDA
3:00 - 3:20 p. m.	EPA	Mr. G. Timm, EPA
3:20 - 3:40 p. m.	NSTC (National Science and Technology Council)	Dr. R. Melnick, OSTP/NIEHS
3:40 - 4:15 p. m.	<u>Discussion</u> -Linkage of fundamental knowledge -- Toxicology and risk assessment	The Board
4:15 - 4:30 p. m.	Major agenda topics for future Board meetings	Dr. G. Lucier

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**NATIONAL TOXICOLOGY PROGRAM
BOARD OF SCIENTIFIC COUNSELORS**

May 14, 1997

Summary Minutes

SUMMARY MINUTES
NTP BOARD OF SCIENTIFIC COUNSELORS MEETING
May 14, 1997

The National Toxicology Program (NTP) Board of Scientific Counselors (the Board) met on May 14, 1997, at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. (*Attachment 1: Federal Register meeting announcement; Attachment 2: Agenda and Roster of Members.*) Members of the Board are Drs. John Stegeman (Chairman), Eula Bingham, Clay Frederick, George Friedman-Jimenez, Carol Henry, Kim Hooper, Meryl Karol, Franklin Mirer, John Mulvihill, Curtis Parker, Richard Peterson, and Patricia Rodier. Expert consultant to the Board is Dr. Hiroshi Yamasaki. All were present except Drs. Friedman-Jimenez, Mulvihill, and Yamasaki.

I. Report of the Director, NIEHS & NTP: Dr. Kenneth Olden, Director, reported that the budget process was moving forward, testimony having been given in the House and due to be given yet this month in the Senate. The NIH would receive about a seven and a half percent increase, while the NIEHS would receive the third highest percentage increase of all the institutes, permitting the NIEHS to do the exciting science that it wants to do. In his testimony, he described four critical priority areas for NIEHS research, these being Mixtures, Models, Susceptibility and Exposures. Dr. Olden spoke of the Environmental Genome project, which will provide data on the genetic basis for individual susceptibility to environmental insults. Funds have been raised from various sources including other NIH institutes, and with NIH Director Dr. Harold Varmus's enthusiastic participation, funds will be made available from the NIH Director's one percent transfer account. This funding will amount to \$8-10 million which is enough to fund the first year of the project. Dr. Olden said that through collaborations with the National Center for Environmental Health (NCEH)/Centers for Disease Control and Prevention (CDC), and with the Environmental Protection Agency (EPA), data assessing human exposures to chemicals would be obtained. He reported that the Institute may receive monies from Veterans Affairs in the Department of Defense pertaining to Gulf War illnesses to evaluate multiple chemical sensitivity, mixtures and measures of susceptibility. Dr. Olden commented that interactions with constituency groups are going well as are partnerships with Federal agencies such as CDC, the National Institute for Occupational Safety and Health (NIOSH), EPA, and the Food and Drug Administration (FDA). Dr. Henry congratulated Dr. Olden on carrying forward with the external review of the NIEHS, noting that the NIEHS was the first NIH institute to initiate such an indepth review. She requested that the NTP Board receive copies of the final report when available. Dr. Olden thanked Board members, including Dr. Henry, who had appeared before the external review committee. He said the review should give an assessment of where the Institute stands and what the challenges and knowledge gaps are, and he expected a report by the end of the year.

Dr. Olden presented a certificate and acknowledged the contributions of a retiring member of the Board, Dr. Karol.

II. Report of the Director, Environmental Toxicology Program (ETP), NIEHS:

- Dr. George Lucier, Director, ETP, also welcomed the members, especially the five new members, Drs. Frederick, Hooper, Parker, Peterson, and Rodier, and thanked them for giving their time and expertise. He also commented on the external review, providing

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the Board with information on this while noting that he had twice made presentations before the committee. Board members recently had received copies of a briefing book on the ETP and NTP assembled for the external review and he asked for any comments they had on the book. In his remarks to the review committee, Dr. Lucier had stressed not only the interagency, but also the NIEHS integration of research activities.

- Dr. Lucier commented on a recent guest perspective on human studies that he had written for the EPA Risk Policy Report which stressed the intent to incorporate human studies into our toxicological evaluations and priority setting. He said a staff epidemiologist was being recruited who would be located in the Epidemiology Branch but with primary responsibility to the toxicology program. A person with exposure assessment expertise will be hired on a temporary basis to work on the interagency agreements with CDC and EPA. He noted that Dr. Thomas Sinks, Associate Director for Science, NCEH, would be describing the exposure assessments on endocrine disrupting chemicals to be done under their interagency agreement with NIEHS.
- Dr. Lucier told the Board that the final report of the *ad hoc* Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) was out and a copy was provided with their meeting folders. A major recommendation to establish a permanent ICCVAM and a concept proposal to provide support for this, an external advisory board, and review panels, had been approved at the last Board meeting. The permanent ICCVAM would provide peer reviewed recommendations to appropriate regulatory agencies for their use. He said there had been much support from the NTP's various stakeholders for this endeavor.
- Dr. Lucier commented on the NTP's collaboration with EPA to set priorities for the toxicological evaluation of drinking water disinfectant byproducts. Priorities will be set for which of these chemicals will be entered into 2-year bioassays, which will be studied in transgenic mouse models, and which will undergo evaluation for reproductive and developmental toxicity, neurobehavioral, or immunotoxicity.
- Dr. Lucier commented on the status of studies mandated by Congress on the health effects assessment of electric and magnetic fields (EMF) about which Dr. Olden will have to report to Congress in 1998. This assessment is funded equally by the Federal government and the electric power industry. A significant portion of the NIEHS funding is allocated to the grants program for support of basic studies. He said there will be three workshops, one already held in late March dealing with *in vitro* and mechanistic studies, with a second to take place in January 1998 dealing with human studies, and the third to be in late March 1998 dealing with animal studies including findings from rodent bioassays which will have been peer reviewed by the Technical Reports Review Subcommittee of the Board earlier in March 1998. A final working group will meet in May 1998 to integrate all the data into a health assessment document which should provide the basis for Dr. Olden's report to Congress.
- Dr. Lucier reported that a contract for support of the NIEHS/NTP Center for the Evaluation of Risks to Human Reproduction was now in the award phase. The Center, as had been previously described for the Board, will evaluate the potential for adverse effects of chemicals on reproduction and development using external expert review leading to narrative documents assessing risk under various exposure scenarios.
- Dr. Lucier announced that the next meeting of the Board's BRC Subcommittee will be on October 30-31, 1997, and the next meeting of the Technical Reports Review Subcommittee will be on December 9-10, 1997.

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- January 1998 dates are being sought for the next meeting of the Board which will deal primarily with strategies for validating transgenic animals for use as toxicity testing systems. A number of validation studies are ongoing at the NIEHS, and in collaboration with ILSI and other organizations comparing carcinogenicity findings in transgenic mouse models with findings in long-term bioassays.

Discussion: Dr. Bernard Schwetz, NCTR/FDA, commented that the FDA, and other agencies such as EPA, were pushing for development of a process to allow the use of new testing methodology in regulation. No such process is currently in place. He said a positive aspect of the ICCVAM process has been the bringing together of agency staff to discuss alternative methods and how they might be incorporated into the regulatory process. Dr. Mirer stated that the Board and BRC Subcommittee at their last meeting had expressed a strong consensus on the need for help in interpreting epidemiological data in reviews for the BRC. Dr. Lucier reiterated again the Program's commitment to recruiting a staff epidemiologist and an expert in exposure assessment as well as to add such expertise to the Board. Dr. Olden said that there had been a recent review of how well the Epidemiology Branch interacts with other parts of the intramural program, and it seemed likely that there needed to be an enhancement of collaborations between epidemiology and toxicology. Dr. Henry affirmed the importance of there being coordination between the NIEHS/NTP and the CDC, and expressed concern about duplication of effort. Dr. Lucier responded that there were frequent interactions between the NIEHS and NCEH/CDC. Dr. Sinks noted that Dr. Olden and Dr. Samuel Wilson, NIEHS Deputy Director, had been meeting with him and others at NCEH about every two months to move the relationship forward. Dr. Frederick noted that epidemiology is very expensive and expressed concern about available resources being spread too thin. Dr. Lucier agreed but said that an epidemiologist associated with the toxicology program would leverage with other programs such as at CDC.

III. Concept Review:

Molecular Oncology Support -- (Attachment 3) Dr. Robert Maronpot, NIEHS, presented the concept, and Dr. Clay Frederick, Board Member, served as principal reviewer. Dr. Maronpot gave some background noting that the original contract had been awarded about 11 years ago and was focused on oncogene analysis, oncogene activation, and molecular dosimetry. About three to four years ago, the contract was recompeted and made available to any qualified investigator with an approved protocol to be used in support of mechanistic studies. He said the scope is broadened in the present proposal to provide an NIEHS testing/research resource to rapidly perform *in vivo* pilot and risk characterization studies for investigations of toxicity and carcinogenicity by generating samples for analysis of oncogenes, tumor suppressor genes, cell proliferation, apoptosis, and pathogenesis of organ-specific toxicity and carcinogenicity.

Dr. Frederick said that based on review of publications associated with the existing contract and the goals of the Institute as described in the briefing book members had received, this project was well in line with the goals of the Institute. As measured by quality and quantity of publications including peer reviewed journals, the productivity had been good. He said the peer review system described for review of proposed studies seemed quite adequate. Based on past performance, the output from this contract should provide good data for quantitative modeling. Dr. Frederick said he strongly supported the proposal. Dr. Karol asked for some idea of cost. Dr. Maronpot said that in recent years

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the annual cost had been between \$ 300,000 and \$400,000. Dr. Henry asked how a possible expansion of the effort might affect the Institute's budget, and also, wondered if there were human studies planned. Dr. Lucier said that there should be no effect on other programs, and no human studies were planned. In response to Dr. Hooper, Dr. Lucier said that material from human studies might be utilized in the future. Dr. Frederick moved that the concept be approved. Dr. Karol seconded the motion, which was approved unanimously by the Board.

IV. Endocrine Disruptor Initiatives:

A. Introduction -- Dr. Lucier said his purpose was to introduce a series of presentations on NIEHS and NTP agency activities in the area of endocrine disruptors. He noted that the NIEHS has had research activity in this area for 25 years or more. The first NIEHS sponsored conference on estrogens in the environment was held in 1979, while the fourth conference will be held July 20-23, 1997, in Arlington, Virginia, and the preliminary program was included in members meeting folder. The area of endocrine disruptors recently has become highly visible, perhaps in part catalyzed by the book Our Stolen Future, resulting in legislative mandates such as that associated with the Safe Drinking Water Act. Dr. Lucier listed what is known about endocrine disruptors:

- There are chemicals in the environment that possess hormonal activity - however the magnitude of human exposure is not well quantified (Dr. Sinks will address);
- Environmental exposures to endocrine disrupting chemicals have been shown to damage wildlife;
- Hormone receptor systems are similar in animals and people so wildlife effects raise concern for human health (there is a dosimetry issue here);
- Children are likely to be more sensitive to the adverse effects of endocrine disruptors than are adults;
- Diseases and dysfunctions at some hormonally sensitive sites are reported to be increasing, such as breast cancer, testicular cancer, and sperm counts. However, these trends have not been clearly linked to endocrine disrupting chemicals. (Dietary factors are involved to some extent.); and
- Plant and fungal estrogens may pose health threats although some plant estrogens may be beneficial.

Dr. Lucier said the NIEHS focus is on the connection between biology and risk assessment models. He said that Dr. Robert Chapin will describe animal toxicity studies. One aspect of mechanistic studies at NIEHS will be presented by Dr. Jonathan Lindzey in his discussion of the development and use of an estrogen receptor knockout (ERKO) mouse. Dr. Lucier briefly enumerated types of epidemiological studies in progress and referred reviewers to the briefing book for more detailed descriptions. He said that Dr. Christopher Portier would talk about receptor-mediated events and how information obtained was used in development of biologically-based dose-response models. Dr. Lucier stated that specific questions the Board was being asked to address were:

- Is the scope of NIEHS/NTP research on endocrine disruptors appropriate?
- Are we missing opportunities?
- Taken together, are we strengthening the links between fundamental knowledge, toxicology, human studies and risk assessment?

There are a number of cooperative or collaborative activities are going on through interagency agreements. Dr. Lucier commented on significant contributions to the field by the extramural program, noting that 15 grants were recently awarded for an RFA on endocrine disruptors.

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Discussion: Dr. Henry asked what level of staff and resources are devoted to endocrine disruptor studies. Dr. Lucier responded that the proportions haven't changed much over the past 10 or so years. Dr. Hooper observed that the results in animals could help in design and interpretation of epidemiological studies. He wondered how NIOSH might be better involved with its access to worker populations. Dr. Lucier said this is why the Program needs a coordinator for human studies. He stated that a closer relationship has developed with NIOSH over the last two years, and recently, an interagency agreement has been established with NIOSH for the evaluation of complex mixtures found in the workplace, beginning with asphalt fumes. Dr. Bingham said that in looking at workplace situations she had seen a health hazard evaluation (HHE) by NIOSH on optical brighteners where there were observed estrogenic effects in detergent industry workers, and undoubtedly, even babies would be exposed through percutaneous absorption from detergent in their clothes. She thought we needed to make better connections through examining structure-activity-relationships of estrogenic chemicals. Dr. Chapin, NIEHS, said that all of the 90-day toxicity studies have reproductive endpoints built into the end of them, and positive findings are passed on to Dr. Stephen Schrader at NIOSH for evaluation of potential effects in humans.

B. Nomination and Selection of Agents for Reproductive/Developmental Toxicology -- Dr. Michael Shelby, NIEHS, said there are actually three areas that they address, first being (1) the effects that a chemical may have on the genetic integrity of germ cells, i.e., sperm or eggs, with the second being (2) the effects of a chemical on the capacity of an individual bearing germ cells to reproduce, i.e., fertility, and the third being (3) the effects of a chemical on development of the conceptus. Dr. Shelby described the types of studies used by the NTP under these areas. Under reproductive effects are two fairly standard assays -- reproductive assessment by continuous breeding, and a newer 35-day assay developed by Dr. Chapin that can assess reproductive, developmental and general toxicity of a chemical. Specialized assays are developed when needed, e.g., recently, for tamoxifen and nonylphenol. Under developmental effects are assays for morphological malformations (terata), while specialized studies may look at the effects, e.g., of perinatal exposures. Dr. Shelby said that under genetic effects, there are four standard assays -- (1) the dominant lethal test, which is the assay first performed on a suspect chemical to determine whether it is a germ cell mutagen, (2) measurement of total reproductive capacity in females, (3) the heritable translocation test and (4) the morphological specific locus test, which are more involved assays intended to characterize the nature of the genetic damage. There are also specialized studies including exposure of zygotes and induction of aneuploidy. Dr. Shelby listed the sources of chemical nominations for reproductive and developmental toxicity studies which include labor unions, industry groups, public interest groups, NTP member agencies, other government agencies, and private citizens. Chemical selection for a chemical is guided first by internal NIEHS review including examination of the rationale and a thorough literature search. Advice is sought from the NTP Board and an *ad hoc* advisory panel, as well as from members of the scientific community at large. Dr. Shelby stated that among the primary criteria for selection are (1) plausibility of test exposure or availability of chemical, (2) extent of human exposure, including numbers of people, their ages, and levels of exposure, (3) availability of test data, (4) existence of conflicting results or poor quality studies, (5) the possibility of hypothesis testing, (6) known effects from 90-day toxicity studies, and (7) evidence of effects in humans. He concluded by saying that attention to public health needs must be foremost in selecting chemicals for study.

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Discussion: Dr. Rodier asked why there were no assays for functional central nervous system (CNS) effects since that is an area of great public health concern. Dr. Shelby replied that these effects are included in standard developmental toxicology protocols but CNS studies may be added when indicated. Dr. Jean Harry, NIEHS, reported that the NTP is trying to look at more sophisticated methods for assessing effects in the developing nervous system. Collaborations are ongoing with the NCTR in the area of endocrine disruptors and with EPA on functional evaluations. She said that we are trying to draw on clinical observations to aid in discerning effects to look for in animal studies. Dr. Karol encouraged looking at mixtures and not just pure chemicals. Dr. Mirer commented that solvent exposures (5-10 ppm) were major hazards for reproductive toxicity in the workplace. Dr. Hooper stated that while there was a long list of chemicals listed in California as potential carcinogens, there was a much shorter list of potential reproductive and developmental toxins, indicating a strong need for much more data for chemicals at this endpoint. Dr. Henry noted the separate processes for selection of chemicals for carcinogenicity studies and for reproductive toxicity studies and wondered why, and asked how the Board could help increase emphasis on studying some of the high volume occupational chemicals for which there is a lack of reproductive and developmental toxicity data. Dr. Shelby said the processes historically had been separate perhaps because of high and emotional public concerns about cancer but more recently, as exemplified by the endocrine disruptor issue, the public has become more sensitive to concerns about reproductive effects of chemicals. Dr. Peterson said the greatest concern is for chemical effects on early development and the greatest information gap is on the effects of exposures to women during pregnancy. He suggested that effects in fish and wildlife, eg., with endocrine disruptors, should be triggering NTP studies. Dr. Shelby responded that they were with studies on deformed frogs in Minnesota being a recent example. Dr. Bingham reiterated the need for more studies on workplace chemicals while suggesting that the recent emphasis on children's health and their greater sensitivity to chemical effects pointed to the need for increased resources for reproductive and developmental toxicity studies and methods to detect more subtle effects. Dr. Lucier noted that the NTP was developing molecular screens for hormonal activity to aid in the priority setting process. Dr. Rodier said that these screens would not have detected teratogenic effects of ethanol, lead or mercury. Dr. Henry inquired as to what specific questions was input sought from the Board. Dr. Lucier replied that with regard to the nomination/selection process there were two: (1) is the way that the NTP is seeking nominations appropriate and are the right people being involved?, and (2) are the criteria being used to set priorities in the selection process appropriate and are the right issues being considered in priority setting, such as should there be more import given to occupational chemicals? Dr. Karol hoped that recent SAR models for reproductive toxicity were being considered and Dr. Lucier said they were. Dr. Hooper urged a future review of the adequacy of the screening systems in use.

C. NIEHS Activities on Endocrine Disruptors -- Toxicology: Dr. Chapin described studies assessing environmental chemicals for estrogenic activity at three different levels of action: competitive binding with the estrogen receptor *in vitro*, transcriptional activation of estrogen responsive genes *in vitro*, and effects on an estrogen-responsive tissue (uterotropic assay) *in vivo*. Ten different chemicals of diverse structure were studied, and he noted the difficulty of discerning structure-activity relationships. Two of the chemicals were positive controls -- 17 β -estradiol and diethylstilbestrol (DES). Dr. Chapin displayed the results of the measures of competitive binding, transcriptional

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activation, and the uterotrophic assay for the 10 chemicals. This three-test combination offers a systematic and mechanistically informative approach to assessing estrogenicity. Recent work has focused on phytoestrogens and some industrial chemicals. Dr. Chapin said the program was committed to the concept of detecting effects *in vivo* and he described two studies. One study, being conducted by Dr. Barry Delclos through an interagency agreement with NCTR, tests the premise that neonatal exposure to endocrine disrupting chemicals may result in decreased sperm counts in mature males. Three questions were posed: (1) are there effects occurring at lower doses than expected over multiple generations?; (2) are there changes in carcinogenicity of a chemical between *in utero* exposure only and *in utero* plus continuing postnatal exposure?; and (3) do any of the toxic effects disappear in a subsequent unexposed generation? The first three chemicals being studied are the naturally occurring phytoestrogen, genistein, the synthetic pesticide, methoxychlor, and the detergent breakdown product and spermicide, nonylphenol. Dr. Chapin described the first detailed multigeneration study on nonylphenol performed by NIEHS to provide data on the reproductive/developmental effects of the chemical *in vivo* in rodents. Prior to this study, there were few *in vivo* data available for this chemical. Primary effects observed were accelerations in vaginal opening, changes in kidney weights and structure, and an unexpected finding that effects on sperm count were not noted until the F2 generation. Dr. Chapin concluded that sensitivity as demonstrated through life stage exposures was an important issue in the study of endocrine disruptors. With regard to human exposure, Dr. Chapin alluded to the interagency agreement by NIEHS with the National Center for Environmental Health/CDC under which human blood and urine will be analyzed for a lengthy list of natural and synthetic endocrine disruptor chemicals. These results will aid in setting priorities for which chemicals to study.

Discussion: Dr. Bingham commented that humans would probably not be exposed to nonylphenol by itself but as a component of a mixture, and urged that mixture studies be considered. Dr. Chapin agreed and said that a bottom up approach would be used. Dr. Frederick noted the large amounts of human hormones excreted in urine, including metabolites of birth control pills, and wondered if studies were considered with the hormones and metabolites. Ms. Retha Newbold, NIEHS, said that 17 β -ethinyl estradiol would be the fourth compound to be studied under the interagency agreement with NCTR. Dr. Peterson expressed concern as to there being adequate resources and a critical mass of scientists with appropriate expertise for carrying out the initiative on endocrine disruptors. Dr. Lucier responded that increased resources would come primarily from redirecting funding from other projects. He said that existing staff along with recent key recruitments would be sufficient.

Mechanistic Studies: Dr. Jonathan Lindzey, NIEHS, said that one of the major activities of Dr. Kenneth Korach's laboratory over the years has been to study the biological effects of endogenous and exogenous estrogens, both naturally occurring and synthetic, and attempting to dissect the molecular mechanisms of action. Dr. Lindzey discussed the relationships among the estrogenic substance, the estrogen receptors (ER α and β), and estrogen response elements (ERE) leading to alterations of transcription in target genes. He described an *in vitro* yeast-gene activation system in which they are looking at the ability of estrogenic chemicals to activate an artificial reporter construct. This confers the capability for screening many compounds. In this system, DES was shown to be most potent in activating EREs, although zearalenone and a trichlorinated biphenyl displayed

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similar potency, and genistein somewhat less. Dr. Lindzey said that experiments corroborating these findings in a mammalian cell system were underway. He then spoke of *in vivo* studies in a mouse model where they have knocked out the ER α gene. These studies are designed to determine if the biological effects of estrogen compounds are mediated exclusively through the nuclear ER α receptor or through the ER β and/or membrane receptors. Dr. Lindzey described data obtained on genistein using the estrogen receptor knockout (ERKO) mouse.

Discussion: Dr. Stegeman asked whether these types of studies feed into the NTP. Dr. Lucier responded that they did and said there were considerable collaborations as to the kinds of chemicals that might be looked at in these systems to determine which act through the estrogen receptor and which don't with many implications for priority setting. Dr. Peterson commented that these types of studies were valuable in uncovering the role of estrogen in sperm production. In response to a question from Dr. Henry, Dr. Lucier thought the real use of the ERKO mouse model would not be as a screen for chemicals but rather in characterization of *in vivo* molecular mechanisms of action..

Risk Assessment Methodologies: Dr. Christopher Portier, NIEHS, said his group is involved in physiological issues around endocrine disruption and in studies of multiple toxicity endpoints. Some of the chemicals studied include tamoxifen, water disinfection byproducts, pesticides, TCDD, and melatonin. Among the studies they have produced include those looking at the mechanisms of estrogen action and the molecular epidemiology of dioxins. These fall under the overall umbrella of mechanistic modeling and risk assessment. With regard to endocrine disruptors, projects include statistical analysis of receptor assays such as the yeast model described by Dr. Lindzey, and mathematical models for receptor-mediated toxicants. Dr. Portier said the three main components to be determined are the maximal effect, shape of the dose-response curve, and the EC50 (measure of potency). From these and other measures, estimates of linearity vs. possible thresholds of effect can be assessed. An aim is to look at all the data and provide tools to the regulatory community to look at a broad spectrum of information and try to make decisions as to what the information is telling them. Dr. Portier described the components of the basic endocrine signalling model and how this could be used. He talked about characterizing a model for dioxin distribution and metabolism, a physiologically-based pharmacokinetic (PBPK) model, which is usually the first step in looking at a chemical. The next level is to characterize known molecular and biochemical events where there is dose-response information for a chemical. Another model was created for dioxin's effects on dysregulation of thyroxine. Dr. Portier said that the findings for dioxin were provided to EPA to aid them in risk assessment. He noted that considerable work had been done on distribution, metabolism and secretion of melatonin as part of the effort to evaluate toxicity of electric and magnetic fields (EMF). Dr. Portier described recent studies creating a pharmacodynamic model for gap junctions, and other studies on environment estrogens. In summary, he said their research was important in development of methods for analysis of different levels - ranging from the empirical to the highly theoretical - to help mechanistic understanding and linking broad arrays of data sets. The aim is to develop and apply models to aid in moving NIEHS research into the regulatory arena.

Discussion: Dr. Mirer asked whether the studies described were NIEHS or NTP funded. Dr. Portier said both and for example, a part of his laboratory does PBPK models for the NTP. Drs. Frederick and Peterson stressed the importance of interactions with other

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parts of the intramural program and even other agencies, such as EPA, for obtaining data to validate their models.

Extramural Activities: Dr. Jerrold Heindel, NIEHS, said there were approximately 100 NIEHS funded grants in the current portfolio that had involvement with endocrine disruptors. Of these, 46 have to do with TCDD, 34 with estrogenic chemicals, 14 with PCBs and PBBs, four with lead, and one with an antiandrogen. He said there were many more grants involving lead but the four he noted had to do with lead and alterations in hormone levels. About 33 of the grants are in the reproductive and developmental area, with seven in immune system effects, very few in neurobehavioral, and 10 grants in cancer. About one-quarter of the grants are in human/epidemiologic studies, 40% in animal studies, and 37% in *in vitro* models or mechanistic studies. Dr. Heindel commented that NIEHS is the primary institute funding research on dioxins and over the past 20 years most of the seminal discoveries on this class have been NIEHS conducted or supported. He described the types of studies being funded on estrogenic chemicals and noted that perhaps underserved were studies on phytoestrogens and on development of biomarkers. Dr. Heindel reported that a recent Request for Applications (RFA) intended to stimulate research on endocrine disrupting chemicals and women's health outcomes resulted in 77 applications leading to award of 15 grants. He reviewed types of studies being conducted under this RFA on basic mechanisms, reproductive toxicity, and human studies. Dr. Heindel said that the 15 grantees came to NIEHS last November, made presentations, and met with intramural scientists doing research on endocrine disruptors. He concluded by mentioning areas where there are gaps or more emphasis needs be given, being: molecular mechanisms; *in utero* exposures; immune and nervous system effects; mixture studies; genetic susceptibility; diseases; and extrapolation/human risk.

Discussion: Dr. Karol asked how applications would be stimulated in these future emphasis areas without an RFA. Dr. Heindel said these areas are highlighted on the NIEHS Home Page on the World Wide Web, and, of course, an RFA could be issued if need be. Dr. Rodier said there needed to be more stimulus for applications concerned with the developing nervous system. Dr. Henry noted that the Department of Energy has two large programs concerned with endocrine disruptors and asked that scientists from them be considered for future interagency workshops.

D. Other Federal Agency Activities on Endocrine Disruptors -- National Center for Environmental Health/Centers for Disease Control and Prevention (NCEH/CDC): Dr. Thomas Sinks, NCEH, briefly described the NCEH, noting there was a large group studying the epidemiology of birth defects. There are several groups associated with endocrine disruptors including a study on breast cancer rates in Alaska and a cohort from the polybrominated biphenyl exposed people in Michigan. He said a ripe area for exploring as health outcomes for endocrine disruptors is the NCEH program in developmental disabilities. Dr. Sinks reported that his own laboratory had three components: characterization of concentrations of chemicals in human urine and blood; a nutritional biochemistry group that is looking at assays for phytoestrogens and is also responsible for the NHANES survey; and a national resource for quality assurance for newborn blood spot testing. Dr. Sinks said the rest of his presentation would focus on his laboratory's studies of chemical concentrations and identity in human samples which is also the subject of the interagency agreement (IAG) between CDC and the NIEHS, and titled "Assessment of Human Exposure to Environmental Toxicants to Support the NTP".

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He thought that the key parameter for NTP's purposes was exposure. Dr. Sinks reviewed the key steps in exposure assessment: location of exposure- nearness to source, which can be measured or modeled; external dose; route of entrance to body, e.g., ingestion; internal dose; and biologically effective dose. He spoke of how biomonitoring helps prevent disease by measuring: what toxicants get into people?; how much gets in?; does disease result from exposure?; what populations are at increased risk?; and are preventions effective?; with the first two of most importance to the NTP. Dr. Sinks said that he wanted to demonstrate the value of exposure assessment leading to environmental regulation with an example, that being blood lead levels in U.S. young children. He compared blood lead levels taken during NHANES II (1976-1980) where 80% were at or above the current level of concern, 10 µg/dL, while levels taken during the first phase of NHANES III (1988-1991) were dramatically shifted downward to an average level below 10 µg/dL. During the period of NHANES II, lead levels in gasoline declined about 60 % and correspondingly, blood lead levels in people declined. Dr. Sinks displayed charts giving urine concentrations of nonpersistent pesticides and blood levels of volatile organic compounds obtained from 1000 subjects each during NHANES III, and said this exemplified the type of information that could be obtained on endocrine disruptors for the NTP. He explained that NHANES III is the Third National Health and Nutrition Examination Survey, and is the only national survey in the U.S. that does clinical workups, collects blood and urine as well as performing several measures of health assessment. Under the terms of the IAG, CDC will measure levels of selected environmental toxicants (or their metabolites) -- primarily suspected endocrine disruptor compounds -- in serum and/or urine from the general population of the U.S. Dr. Sinks showed the listing of suspected endocrine disrupting compounds to be measured in human serum or urine and which include insecticides, herbicides, fungicides, industrial chemicals, and phytoestrogens.

Discussion: Dr. Hooper inquired as to how the chemicals were selected. Dr. Sinks said that first chemicals were selected for which they had analytical capabilities, and then there were negotiations between NIEHS and CDC as to other chemicals to be looked for. For information on NHANES including the upcoming NHANES IV, Dr. Sinks said that CDC had a Web page on the Internet.

NCTR/FDA: Dr. Bernard Schwetz, Director of NCTR and Associate Commissioner for Science at FDA, said a detailed listing of FDA initiatives on endocrine disruptors could be found in the Federal Research Project Inventory maintained by the EPA on the World Wide Web, so he would focus on just a few of note. First, he noted that although the FDA has dealt with regulation of hormones for decades, the current level of awareness about potential endocrine disruptor issues in the product centers is low. As a result, he has formed a committee of representatives from the centers for foods, drugs, devices, biologics and veterinary drugs to examine the questions about the agents that may fall under the jurisdiction of these centers. Questions to be examined in a workshop include determination of regulatory authority, basis and approaches for the risk assessment/management processes, toxicological testing requirements, critical toxicological and epidemiological endpoints, examples of regulatory decisions on endocrine disruptors, and unmet research needs. Dr. Schwetz described the development of an estrogen knowledge base at the NCTR. This is a database combined with appropriate computational tools to provide information, either categorical or predictive, on other related but untested compounds, perhaps obviating testing, and drawing on

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experts in estrogen biology/toxicology, computational chemistry, and computer systems to provide a resource for reviewers within FDA concerned with new applications of chemicals or new chemicals. Dr. Schwetz commented also on the multigeneration and other toxicity studies underway or planned under the IAG with NIEHS. These chemicals are: genistein, a phytoestrogen; vinclozolin, a fungicide with antiandrogenic properties; nonylphenol, an environmental estrogen found in surfactants and detergents; ethinyl estradiol; and methoxychlor as a positive control. He reported that additional reproductive and developmental toxicity endpoints were being added to better define endocrine disrupting activity. Dr. Schwetz noted the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) of which he is a member. He said this committee was driven by EPA legislation and would be discussed by Mr. Gary Timm. Dr. Schwetz cautioned against defining endocrine disruption only as estrogenic or anti-estrogenic activity because there are important compounds that act as endocrine disruptors through other mechanisms, citing sulfamethazine as an example. Dr. Schwetz summarized his remarks by stating that endocrine disruptor questions within the FDA (1) go across all product centers, (2) raise issues of risk assessment, and (3) involve numerous hormones, while commenting that research is primarily directed at (1) methods development, (2) agent-specific data, and (3) aims to develop an estrogen knowledge base as a predictive system prototype.

Discussion: Dr. Hooper thought that based on the diverse structures of estrogenic compounds prediction of estrogenic activity would be difficult. Dr. Schwetz responded that receptor binding activity was the most accurate measure. Dr. Peterson asked about the degree of interaction between NIEHS and NCTR under the IAG. Dr. Schwetz said there were meetings of scientists from both agencies to discuss design and protocol questions, considerable telephone communications, quarterly reports, and final reports of findings will pass through the NTP peer review process.

EPA: Mr. Gary Timm, EPA, reported that endocrine disruptors are one of the six risk-based high priorities. He pointed to the published report of the EPA-sponsored workshop on research needs for the risk assessment of health and environmental effects of endocrine disruptors contained in member's briefing books. Mr. Timm spoke of the Office of Research and Development's endocrine disruptor research plan divided primarily among biological effect studies, exposure studies and studies linking them with funding increases from the last fiscal year to the current one in both extramural grants and the intramural program. Among types of intramural studies are development of new *in vitro* assays, enhancement of long-term bioassays for reproductive and developmental effects to provide more sensitive endpoints, better characterization of dose-response relationships, and a better definition of endocrine effects in nonmammalian species/wildlife. Mr. Timm said that more work was planned in looking at mixtures and how to factor findings into the risk assessment process, and there was a need to develop models for predicting environmental concentrations of chemicals. Another way to classify the intramural activities would be under methods, models or measurements. Among external grant projects being funded in the current year are those on - short-term screening systems - new measurements and methods - modes and mechanisms of action - QSAR and animal models - "baseline" endocrine status in wildlife and laboratory surrogates - role of hormones in sexual differentiation and reproductive development of non-mammalian species- and, sites/systems with problems suspected to be related to endocrine disruptors.

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Mr. Timm turned to discussing recent legislation addressing endocrine disruptors that was enacted in 1996. The Food Quality Protection Act (FQPA) was passed first and the Safe Drinking Water Act (SDWA) Amendments followed. The FQPA mandated that EPA must (1) develop an endocrine disruptor screening and testing plan by August 1998, (2) implement this program by August 1999, (3) report to Congress by August 2000, and (4) test pesticides for estrogenic effects that may affect human health. Under FQPA and SDWA authority, EPA can test other drinking water contaminants, test for other endocrine effects, test for environmental effects, and test anything on the TSCA inventory. Mr. Timm stated that in implementation of the legislation, there were some strategies that EPA thought necessary including involving outside experts in identification and resolution of scientific issues, identifying, involving and obtaining consensus of all major stakeholders in developing a screening and testing strategy, and recognizing the efforts of ongoing scientific workshops and building upon their efforts. To implement the FQPA's endocrine testing mandate, the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) was established. He said that the EDSTAC mission was to develop a process for identifying new and existing screening tests and mechanisms for validation, agree on a set of available screens for early application, develop a strategy for selecting and prioritizing chemicals for screening, and determine when to test beyond screening. EDSTAC is comprised of about 45 members representing industry, environmental and public health groups, Federal and state agencies, labor and academia. The Committee at its initial meeting in December 1996 defined "endocrine disruptor" and agreed that "adverse effects" should be included. To get the work done, the Committee was broken into working groups on principles, prioritization, screening and testing, and communication and outreach. A draft final report from the Committee is expected in January 1998. Mr. Timm concluded with a brief description of international activities on endocrine disruptors including recent workshops.

National Science and Technology Council (NSTC): Dr. Ronald Melnick, NIEHS, said the NSTC was established by the President in 1993 to set national goals and coordinate science and technology research among Federal agencies with respect to policies and budgets, and is comprised of Cabinet secretaries, heads of White House offices and heads of agencies. The NSTC has nine committees which provide input on research and development priorities and budgetary issues related to the total Federal effort in science and technology. The pertinent committee for purposes of this presentation is the Committee on Environment and Natural Resources (CENR). Of five priority areas established by the CENR, one is the health and ecological effects of endocrine disrupting chemicals. This effort began in 1995 with a working group co-chaired by Dr. Larry Reiter, EPA. Dr. Michael Mac, Department of Interior, co-chairs the effort on ecological effects, and Dr. Lucier co-chairs a group on human aspects. Three objectives were formulated: (1) to develop a planning framework for research, (2) to conduct an inventory of ongoing research, and (3) to identify research gaps and coordinate a research plan for the high priority needs. The first two have been completed. Dr. Melnick said the working definition of an endocrine disruptor was "an exogenous agent that interferes or probably alters production, release, transport, metabolism, binding or elimination of blood borne hormones." The framework document, included in the member's briefing books, is divided into three sections. One is on the current state of scientific knowledge and is divided into health and ecological effects and exposure assessment; a second is on underlying uncertainties; and the third and largest section deals with a research needs approach for planning and dealing with the endocrine disruptor issues. The approach taken in the

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planning framework follows somewhat of a risk assessment paradigm. Under research needs on methods would be research to develop and validate cost effective methods to detect and/or characterize effects or exposure in human and wildlife populations including development of biomarkers of exposure, response and susceptibility. Next is research on development of models predictive of effects in target media or species. There is a large basic research component, which includes most of the mechanistic research on endocrine disruptors. Research needs in this category involve risk models of dosimetry, exposure, and biologically-based dose-response, and research pertaining to mixtures, particularly as it relates to synergistic or antagonistic effects. Under research needs on measurements, there would be research on quantifying the magnitude of effects on exposures. Under exposure determinations/followup would come epidemiological and ecological monitoring of human and wildlife populations. Multidisciplinary research would combine laboratory and field studies. Research on sentinel species would involve use of sensitive animal populations as early warning systems. Dr. Melnick turned to talking about the inventory of ongoing research where inclusionary criteria were used in deciding which basic and applied research projects should be included. He described types of studies that would or would not be included, and enumerated the various categories and subcategories that might be found under a chemical entry in the inventory. Dr. Melnick said that under primary focus, human health accounts for 70 % of the entries, ecology about 17 %, and exposure assessment 13 %. Looking at the inventory as it pertains to methods, models and measures subcategories, the largest number of entries have to do with basic research. Looking at experimental endpoints, reproduction and development comprised the largest number of entries with carcinogenesis second. PCBs and dioxins constitute the largest number of chemicals in the inventory but many other chemicals termed endocrine disruptors are not well represented in the database. Among living organisms, mammalian have the largest representation with rodents the most and humans next, while fish are cited most often under ecology. Dr. Melnick said that in summary, there seems to be a large effort going on in the Federal government focused heavily on the human health aspects but one needs to look carefully to see whether what is needed for risk assessment and environmental policy is being done well. Few studies directly address effects of exposure to endocrine disruptors and human risk. Also, studies on mixtures need more effort, as well as emphasis on additional agents and multidisciplinary studies. Dr. Melnick reported that the website for the endocrine disruptor initiative, which contains the inventory, is www.epa.gov/endocrine.