National Toxicology Program
Board of Scientific Counselors

December 6, 2007

National Institute of Environmental Health Sciences
Research Triangle Park, NC

Summary Minutes

I. Attendees

Members in attendance:
Christopher Bradfield, University of Wisconsin
Tracie Bunton, Eicarte LLC (via telephone)
Edward Carney, Dow Chemical Company
Russell Cattley, Amgen
Kenny Crump, Louisiana Tech University
Prescott Deininger, Tulane University
William Janzen, Amphora Corporation
Nancy Kerkvliet, Oregon State University
Gail McCarver, Medical College of Wisconsin (Chair)
Jon Mirsalis, SRI International
Jim Riviere, North Carolina State University
Harish Sikka, State University of New York at Buffalo
Keith Soper, Merck Research Laboratories
Vernon Walker, Lovelace Respiratory Institute

Members not in attendance:
Germaine Buck Louis, National Institute of Child Health and Human Development
Katharine Hammond, University of California at Berkeley

***Ad Hoc* Members**
Michael Collins, University of California at Los Angeles (via videoconferencing)
Tomas Guilarte, Johns Hopkins University (via videoconferencing)
Eve Mylchreest, Wyeth Laboratories (via videoconferencing)
James Popp, Statoxon LLC
Stephen Roberts, University of Florida (via videoconferencing)
Russell Thomas, The Hamner Institute

NIEHS Staff
Jack Bishop
John Bucher
Rajendra Chhabra
Paul Foster
Mary Gant
Veronica Godfrey
Grace Kissling
Ruth Lunn
Robin Mackar
Dori Germolec
Michelle Hooth
C.W. Jameson
Dave Malarkey
Michael Sanders
William T. Schrader
Barbara Shane
Michael Shelby
Robert Sills
Diane Spencer
William Stokes
William Suk
Kristina Thayer
Raymond Tice
Molly Vallant
Nigel Walker
Samuel Wilson
Kristine Witt
Mary Wolfe
Michael Wyde

Other Federal Agency Staff
Mark Toraason, National Institute for Occupational Safety and Health (NIOSH)
Paul Howard, National Center for Toxicological Research (NCTR)

Public
Andrew Ballard, BNA, Inc.
Reshan Fernando, RTI International
Diane Gerkin, Battelle
Milton Hejmancik, Battelle
Kenneth Hudnell, Solar Bee Inc.
William Kelly, Jr., Center for Regulatory Effectiveness
Bobbie Peterson, RTI
Catherine Price, RTI
Ivan Rusyn, University of North Carolina
Ritchie Shoemaker, Center for Research on Biotoxin Associated Illnesses
Rosalind Volpe, ILZRO

II. Introductions and Welcome

The National Toxicology Program (NTP) Board of Scientific Counselors (BSC) met on December 6, 2007, at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. Dr. Gail McCarver, Chair, welcomed everyone to the meeting and asked the BSC members and attendees to introduce themselves. Dr. John Bucher, NTP Associate Director, NIEHS, welcomed and thanked the BSC members for their attendance and service to the NTP.

Dr. Gail McCarver extended a special welcome to the new board members including Dr. Bunton who could not attend. She thanked Drs. Sikka, Deininger and Walker, who were rotating off the BSC, for their service. Dr. Barbara Shane made a few announcements and read the conflict of interest statement. She noted that none of the *ad hoc* reviewers could vote and no conflicts of interest were identified.

Dr. Samuel Wilson, Acting Director of the NIEHS and NTP, welcomed the BSC members and expressed his gratitude to them for attending the meeting to advise the NTP. He welcomed the new BSC members and hoped their interaction would be valuable and enlightening. Dr. Wilson congratulated Dr. William Suk on his appointment as the Acting Deputy Director of NIEHS and NTP. Dr. Suk was in the extramural division at NIEHS where he led the Superfund Basic Research Program and he was instrumental in its founding in the late 1980s. Dr. Wilson introduced Ms. Robin Mackar from the NIEHS Communications Office who provides news from the media relating to environmental health sciences. He also introduced Ms. Mary Gant, an NIEHS staff located at NIH, Bethesda, who serves as liaison for ongoing Congressional activities in Washington, DC. He spoke about the NIEHS budget and said the NTP is supported primarily by NIEHS funding. NIH and other agencies are on a continuing resolution through mid-December and are looking forward to a favorable budget for 2008. He welcomed the federal agency representatives and thanked them for their continued participation in NTP.

III. Update

Dr. John Bucher, NTP Associate Director, said the realignment of NTP within the Division of Intramural Research was accomplished on October 28, 2007. The NTP is now composed of five offices and five branches. He congratulated Dr. Mary Wolfe who has been appointed as the Deputy Program Director for Policy and Dr. Nigel Walker as the Deputy Director for Science.

The Program Office has the following groups:

- Office of Liaison, Policy and Review, Dr. Wolfe, Director
- Office of Nomination and Selection, Dr. Scott Masten, Director
- Center for the Evaluation of Risks to Human Reproduction (CERHR), Dr. Michael Shelby, Director.
- Report on Carcinogens Group, Dr. C.W. Jameson, Director
- NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), Dr. William Stokes, Director

The five branches are:

- Bimolecular Screening Branch, Dr. Raymond Tice, Acting Branch Chief.
- Cellular and Molecular Pathology Branch, Dr. Robert Sills, Chief
- Host Susceptibility Branch, Dr. Jef French, Acting Branch Chief
- Program Operations Branch, Dr. Cynthia Smith, Acting Branch Chief
- Toxicology Branch, Dr. Paul Foster, Acting Branch Chief

Dr. Bucher congratulated Dr. Ronald Melnick for receiving the David P. Rall Award for Advocacy in Public Health from the American Public Health Association in November 2007 and Dr. Frank Johnson who co-authored a paper reporting the outcome of the sequencing of 15 strains of mice, which was published in Nature in 2007.

Some significant activities include interagency agreements with NCTR and NIOSH. Dr. Nigel Walker will be responsible for coordinating NTP studies with NCTR and Dr. Dori Germolec with NIOSH. Dr. Bucher described the progress that has been made in installing the exposure equipment for the NTP studies on radiofrequency radiation from cellular phone devices. This project has been an extraordinarily complicated and difficult; pilot studies will begin in early 2008 followed by the prechronic and chronic studies. The studies will be performed at the Illinois Institute of Technology in Chicago.

a. NTP Branches
   Dr. Bucher summarized the main activities of each branch.

- The Toxicology Branch coordinates the toxicology efforts within the program. The branch directs the scientists involved in designing the general toxicology, carcinogenesis, immunotoxicology, reproduction and development, and genetic toxicology studies. The branch also oversees preparation of NTP reports and activities in toxicogenomics.

- The Program Operations Branch coordinates the activities related to the initial aspects of studies including chemistry, ADME, quality assurance, maintenance and development of data capture and retrieval systems, central data repository, and the NTP website. This branch also administers contracts for procurement of goods or services in support of NTP and NIEHS scientific activities.

- The Cellular and Molecular Pathology Branch was previously named the Laboratory of Experimental Pathology. Staff within this branch manage, evaluate, review, and report pathology data for NTP toxicity and carcinogenicity studies and provide laboratory animal medicine diagnostic support for intramural investigators. Two workshops, one on immunopathology and a second on reproductive and developmental pathology, are planned for 2008. Dr. Susan Elmore, a member of the branch, was the senior author on a paper that won the Best Paper Award from the Society of Toxicologic Pathology that was published in Toxicologic Pathology in 2006.

- The Host Susceptibility Branch will be responsible for planning, conducting and analyzing toxicity studies in multiple strains of rodents and for developing partnerships with intramural and/or extramural scientists, the NTP, and private companies to examine the relationship between the genetic underpinnings of response to diseases and phenotypic outcomes. Dr. French issued a request for information from the academic community on how to best structure a program of this type. The NTP has received 29 responses from existing NIH grantees in support of this type of program. The NTP recently met with NIH staff involved in the RAID (Rapid Access to Intervention Development) Program to learn about this model for government/private partnerships in drug evaluation. NTP may form a BSC subcommittee to advise on this program.

- The Biomolecular Screening Branch is responsible for developing and implementing high and medium throughput screening activities within NTP that can be used for rapid detection of biological activities relevant for toxicity and/or carcinogenicity studies resulting from environmental exposures. The NTP is collaborating with the EPA ToxCast Program and the NIH Chemical Genomics Center (NCGC) on these high throughput screening (HTS) activities. This branch is also responsible for NTP development of automated screening assays with *C. elegans* in Dr. Jonathan Freedman's laboratory. The branch is also charged with developing analysis tools to assess the integration of HTS endpoints with the findings from NTP's standard toxicology studies. These activities are in-line with recommendations proposed in the recent National Research Council (NRC) report, "Toxicity Testing in the 21st Century, a Vision and a Strategy." The NTP established an IAG with the NCGC and provided $1 million in support in 2007 and plans to provide similar support for 2008. The NTP is also developing a Memorandum of Understanding (MOU) with the EPA and the NCGC to more clearly define each group's role in this endeavor.

b. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM).

The NTP Interagency Center for the Evaluation of Toxicological Methods (NICEATM), provides scientific and administrative support for ICCVAM. ICCVAM will celebrate its 10-year anniversary at a symposium in Bethesda, MD in February 2008. Topics at the meeting will include the ICCVAM/NICEATM five-year plan, a presentation by Dr. Bernard Schwetz on the evolution and future of toxicity testing, and a discussion by Dr. Daniel Krewski on the NRC report mentioned above. A roundtable discussion on "Future Directions in Test Method Research, Development, Translation, and Validation" by ICCVAM-member agencies will also be included.

NICEATM/ICCVAM will hold the workshop "Acute Chemical Safety Testing: Advancing *In vitro* Approaches and Humane End points for Systemic Toxicity Evaluations" on February 6 and 7. The objective is to investigate the pathway(s) involved in acute systemic toxicity to identify knowledge that can be applied to the development of new methods and humane end points that will further reduce, refine, and/or replace animals in chemical safety testing.

In October, ICCVAM forwarded recommendations on the utility of four *in vitro* methods as replacements for the *in vivo* rabbit eye test (Draize eye test) to federal agencies. This is a significant achievement because if a chemical tests positive in one of these *in vitro* tests, there is no need to test it *in vivo*.

c. Center for the Evaluation of Risk to Human Reproduction (CERHR)

CERHR evaluates the potential effects of environmental exposures on human reproduction and the development of children. It publishes the outcome of these evaluations in monographs. One section of a monograph is the NTP Brief, which provides the NTP's opinion as to whether a chemical causes reproductive and/or developmental effects in humans based on scientific support. CERHR has completed 20 monographs. Presently, CERHR is evaluating bisphenol A (BPA). As a first step in this evaluation, an external expert panel meeting was held in March 2007 and in April an interim draft expert panel report was released for public comment. There was a second expert panel meeting in August 2007, and the final expert panel report was released for public comment on November 26. Comments are due at the end of January 2008. The NTP is currently developing the draft NTP Brief on BPA and will release the draft for public comment and bring it to the BSC for peer review at its next meeting. The NTP will supplement the BSC review on BPA with several *ad hoc* experts. Following the BSC meeting, the NTP will finalize the NTP Brief and publish the NTP CERHR Monograph on BPA.

Dr. Bucher noted that the NTP responded to concerns about conflicts of interest (COI) relating to the contractor that prepared the background information to the first BPA expert panel report by reviewing all NTP contracts for potential COI. In concert with the Office of General Counsel and the NIH Division of Acquisition Policy and Evaluation in the Office of the Director, NIEHS developed language on COI that has been incorporated into its contracts.

The NTP conducted an audit of the two key activities carried out by the contractor. The first task was to conduct an independent survey of the literature on BPA to identify references and compare them with the list selected by the contractor for inclusion in the August 2006 draft expert panel report and with the total bibliography considered by the expert panel during development of the first three drafts (August 2006, December 2006, March 2007) of the expert panel report. The second task was to audit the fidelity with which changes requested by expert panel members were incorporated into the December 2006 and March 2007 draft expert panel reports by the contractual staff. The audit provided assurance that the draft BPA expert panel reports considered all the relevant references and included changes that were requested by the expert panel members.

Future NTP Expectations

Dr. Bucher reiterated the NTP's expectations, which were outlined at the last BSC meeting to:

- continue to provide basic toxicology information for public health protection
- increase the emphasis on the understanding of exposure relationships and genetic determinants in exposure
- integrate results from the new data-rich techniques of genomics, proteomics, and HTS with existing NTP testing information
- develop new methods for toxicological assessment
- provide guidance on the proper use of toxicological data for hazard identification, characterization, and regulation
- be the leading toxicology program in the world

BSC Discussion

Dr. Popp was delighted with the progress of the radiofrequency program and asked when studies would begin. Dr. Bucher replied that the pilot thermal studies would begin in January 2008 and the pre-chronic perinatal studies in February.

IV. Mold

a. Background

Dr. Dori Germolec, NIEHS, introduced the topic, provided background on the development of NTP's initiative, and outlined proposed studies on mold. A private individual nominated molds to the NTP for study. In 2004, both the BSC and the NTP Executive Committee endorsed the study of molds and suggested that the program study organisms commonly found indoors (i.e. *Aspergillus* spp. and *Penicillium spp.*) as well as *Stachybotrys spp.,* and in 2006, a study concept was presented to the NTP BSC. The BSC suggested that the NTP solicit expert input (1) on how to conduct large-scale, "real-life" exposure, rodent toxicology studies on the toxicological endpoints that should be measured, and (2) on how to mimic the exposure conditions found in damp or water-damaged buildings.

Exposure to elevated levels of indoor mold has been associated with numerous symptoms including cognitive deficits, pulmonary effects such as allergies, asthma, and immunological effects such as dermatitis, skin rashes, hypersensitivity, itching, amongst

others. The Institute of Medicine report, "Damp Indoor Spaces and Health," stated that there is evidence for an association between exposure to damp indoor environments and some respiratory health outcomes, especially asthma in sensitized individuals. The report identified many data gaps including the effects of mold on neurotoxicity, rheumatic diseases, reproduction, and cardiopulmonary deficits. The measurement of exposure to fungal allergens has been restricted to the spores of a selected number of fungi and the potential for different fragments of fungi to cause adverse health effects and clinical outcomes is unclear.

NTP held an informational meeting with experts on March 5, 2007, to receive input on the design of toxicity studies on mold that would mimic "real-life" exposures and to discuss how best to study fungi because their physical and chemical properties vary by life stage and growing conditions. They discussed the exposure of whole organisms versus isolated fractions or toxins; growing conditions such as temperature, humidity, and substrate, life stages, and effects of the age of the culture, which can influence the type of toxin produced.

The experts recommended that the NTP use single organisms as well as molds co-cultured on different building materials grown under high humidity conditions. They proposed that fresh isolates of single species rather than archived cultures be studied. Organisms, whose growth stage is known, could then be harvested and dried for use in the entire study. Exposure via inhalation would be appropriate. They pointed out that biomarkers specific for the fungal strain need to be evaluated as well as host antibodies, fungal products, protein adducts, metabolites in host tissues, tissue burden, and distribution in the test organism. Ancillary studies to address susceptible populations or specific endpoints relating to autoimmune disease should also be included. All experts felt that exposing animals to "real-life" moldy environments would be a relevant strategy. They suggested that the NTP partner its rodent toxicology studies with groups that could undertake clinical studies to obtain as comprehensive an understanding as possible of the health effects of mold.

The specific aims of the rodent study are:

- To assess organ system toxicity following inhalation exposure to molds
- Evaluate available biomarkers of exposure and effect (both general and specific for the organisms to be studied)
- Evaluate the contribution of different organisms to overall health effects by studying individual isolates as well as co-cultured molds.

The NTP will conduct subchronic studies in rodents via inhalation. Exposure will be to two mixtures of molds; one will be a mixed culture of molds from a water-damaged building from New Orleans, Louisiana and the second will be a mixed culture of molds from a damp building with reported health effects (sick-building syndrome). Four isolates of individual organisms will be studied: *Stachybotrys chartarum* isolate 1, *Stachybotrys chartarum* isolate 2, *Aspergillus versicolor, and Alternaria alternata*. The two isolates of *Stachybotrys* produce two different types of mycotoxins. Each sample will be characterized with respect to its mycotoxins, glucans, allergens, particle size,

protease activity, colony forming units, and spores. Neurotoxicity will be evaluated using a functional observation battery, and impacts on the cardiovascular, respiratory, gastrointestinal, and immune systems will be monitored.

These studies will provide important information on which fungi may cause human health effects and identify potential target organs for toxicity, dose-dependent effects, and the utility of biomarkers other than IgE as measures of exposure. NTP hopes that the rodent studies will provide information on additional clinical measures or outcomes that could be examined in epidemiological studies.

Dr. Germolec next discussed NTP's role in the "Heading off Environmental Asthma in Louisiana (HEAL)" project, a collaboration between NIEHS and Tulane University in Louisiana. The primary objective of HEAL is to implement an asthma counselor (AC) program that addresses the multidimensional impact of hurricane Katrina on children with asthma in New Orleans as (1) to whether there is an increase in allergens due to moisture, mold, cockroaches, dust mites, etc., (2) what the impact might be to the disrupted health care system and (3) the effect of stress. Approximately 450 children ages 4-12, who have a prior diagnosis of moderate to severe asthma, will be enrolled in the study. The NTP will measure the level of several antibodies to common allergens and molds through an IAG with NIOSH. Colleagues at EPA will monitor environmental factors including baseline levels of mold, other allergens, and moisture in the homes.

b. BSC Discussion

Dr. Toraason asked how HEAL would show that the outcomes are Katrina-related since Katrina occurred two years ago. Dr. Germolec replied that the mold culture that will be used in the NTP study was obtained from a water-damaged house in New Orleans.

Dr. Pino asked whether the mixed mold cultures from the house in New Orleans contained the same organisms that have been found in a building designated as having "sick building syndrome". Dr. Germolec said the culture from New Orleans has been well characterized and the constituent organisms are known, but it will be further characterized before using in the NTP studies.

Dr. Mirsalis said this project is a very ambitious and important, but noted that the data obtained would be complicated and difficult to interpret. According to the NRC guidelines, animals are usually kept at 30-70% humidity, which is much lower than the conditions in New Orleans. He asked several questions: (1) how would a humidity level of 50% represent a "real world" exposure, (2) how would the NTP ensure that the culture being administered is the same as that growing on the walls of homes, (3) were any of the NTP's contractors experienced in this type of study and, if not, how the NTP would develop this capability, and (4) how would the aerosols of the mold, mixtures of spores, fragments and live organisms be made. Dr. Germolec replied that the fungi would be grown on wall boards under humidity conditions similar to those found in water-logged homes, harvested, dried to a dust, and then administered by inhalation similar to any particle study exposure regimen. Characterization of the fungal material would be different from a conventional NTP study and it might not be possible to definitively

identify exactly what the animals inhaled, which Dr. Germolec acknowledged would be a departure from the NTP's usual characterization of a test article. This initiative will require novel contracts and novel strategies for data interpretation and the NTP has begun to investigate the capabilities of potential contractors.

Dr. McCarver asked whether exposure to dry particles would have the same pathogenesis as exposure to the same particles in a damp environment. Dr. Germolec said there is no evidence that differences in humidity would affect the outcome, but exposure at 50% humidity is what is feasible. People are exposed to dry dusts of mold, which are circulated in air-conditioned homes; thus, the proposed studies will simulate real-life exposure by inhalation. It is not possible to simulate 90% humidity in the rodent quarters or for the animals to inhale mold from wallboards. Care will be taken that the wallboard is biocide and LPS-free because bacteria might be in the cultures.

Dr. Riviere was similarly concerned about the rodent's susceptibility to mold at low humidity and ideal temperatures compared to high temperatures and high humidity. He cautioned the NTP regarding the interpretation of the study following exposure to dry mold compared to exposure to mold in a damp environment. Dr. V. Walker disagreed with Dr. Riviere and cited "sick building syndrome" where people are exposed to point sources of mold at normal humidity and temperature and develop respiratory symptoms. He added that New Mexico is among the top 10 states where mold is found, despite the fact that the state has an annual rainfall of only 8 to 10 inches. Dr. Deininger added that when people move back into a water-damaged house, they control internal temperature and humidity with air conditioning.

Dr. McCarver said molds might be more pathogenic in different human environments; therefore, negative results in the animal studies may underestimate risk. She asked if the studies would include pulmonary function tests and encouraged the NTP to include a methacholine challenge. Dr. Germolec replied that NTP would pursue the tests if health effects were noted in the rodents and added that the design team for the study includes NIEHS intramural scientists.

Dr. Kerkvliet expressed concern regarding hazard identification and asked how the data from these studies would be used. Dr. Germolec said that information relating to other target organ systems besides the respiratory system and the pharmacokinetics of mold and its toxins would be obtained in the rodents. In addition the study would obtain quantitative measures of the amount of particles or spores that cause a health effect, which will contribute to the assessment of potentially safe levels.
Dr. Howard commented that pulmonary distress was found in compromised children in Cleveland, OH following co-exposure in homes to cigarette smoke and *Stachybotrys spp.* He asked whether rodents are sensitized to the same toxicants as humans are. Dr. Gerrmolec replied that animals can be sensitized to dust mite and cockroach allergens under specific conditions, but a synergistic study with environmental tobacco smoke would not be possible. She added that the NTP might consider an ancillary study where different strains of mice were exposed to mold since there are clearly genetic components to asthma.

c. Public Comments

(i) Dr. Kenneth Hudnell, a private citizen and neurotoxicologist retired from EPA, spoke on behalf of Ms. Sharon Kramer, who was unable to attend. He commended the NTP for assuming a leadership role in studying mold. He was pleased that the NTP would address exposure to mixtures of mold since many types of toxins as well as fragments of fungi are released into the air in water-damaged buildings. Biotoxin-associated illness is frequently characterized by a variety of symptoms, due largely to inflammatory responses that vary between and within people from day to day. A useful indicator of neurological effects is visual contrast sensitivity, a process through which visual patterns are detected. Patients with biotoxin-associated illness show a 60% loss of their ability to detect visual patterns, which is regained when mold-related symptoms abate after treatment with cholestyramine. He suggested that the NTP consider using an intervention group in its studies.

He was shocked when the American College of Occupational and Environmental Medicine published a position paper concluding that it is highly unlikely that humans become ill from exposure to a complex mixture of mold and other organisms in water-damaged buildings. Their conclusions were based on the exposure of one non-susceptible mouse strain for three weeks to one type of spore. The timeframe of three weeks was too short, as most humans become ill after months of exposure. Their conclusions did not account for susceptible populations, short-term rather than chronic exposure, or developmental effects. People who are sickened by mold and then recover after treatment are more susceptible to a relapse if exposed a second time. He supported the NTP in partnering with other agencies to undertake research with humans.

(ii) Dr. Richard Shoemaker, Medical Director, Center for Research on Biotoxin Associated Illnesses, said he first became interested in biotoxins in 1997 when blooms of *Pfesteria* sickened people, and he did not know how to evaluate them. He is pleased that the NTP recognized the complexity and the number of variables that must be considered in their studies. He has examined 4500 patients that have consulted him about molds and found a strong genetic basis for their illness. HLA haplotypes, innate immune elements, deficiency in hypothalamic regulatory neuropeptides and levels of melanocyte stimulating hormone seem to be contributory factors. Some patients harbor methycillin-resistant bacteria in their nasal cavities and have T-cell and hormonal abnormalities. He has treated patients with cholestyramine, which seems to alleviate many of the symptoms. He concluded by saying that the symptoms seen in humans are reproducible and each abnormal biomarker contributes to the illness and must be corrected to achieve wellness.

BSC Discussion (continued)

Dr. McCarver asked whether the NTP had considered studying the effect of mold during the perinatal phase of development. Dr. Germolec said initially studies in neonates would not be considered; however, subsequent reproductive and developmental studies with perinatal exposure would be included to assess effects on the nervous and immune systems and on reproduction.

Dr. Howard suggested that advice from the March 2007 information group might be useful once NTP initiates its studies. He added that NTP should consider evaluating exposure to mold via ingestion in addition to inhalation. Dr. Germolec said she continues to seek input from the group, and two members, Drs. Jim Pesca and Tina Raponen, have agreed to serve as *ad hoc* members of the NTP design study team. She also consults with mold experts within the NIEHS intramural program.

V. Nominations

Dr. Scott Masten outlined the study nomination review process. Briefly, the Interagency Committee for Chemical Evaluation and Coordination (ICCEC) makes preliminary study recommendations for the nominations. Public comments are then requested and NTP staff develops research concepts that are provided to the BSC for their review. The concepts and the BSC comments are taken to the NTP Executive Committee for approval. The designs of the studies may be altered according to suggestions from these two bodies. He said the BSC would discuss six of the ten nominations reviewed by the ICCEC in December. Concepts were prepared for 5 of the 6 nominations; one is not recommended for study, hence no concept would be presented. The concepts include the background and rationale for the nomination, its significance, study approach, and expected outcome. There are two research concepts on phthalates: one for diethyl phthalate (DEP) and one for a phthalate initiative to fill data gaps identified in previous NTP studies and by a CERHR expert panel.

1. Aminopyridines

a. Concept

Dr. Dunnick reviewed the concept for three aminopyridines, 2-, 3- and 4-aminopyridine (2-AP, 3A-P, and 4-AP). This class was nominated by National Cancer Institute (NCI) because of a lack of suitable information to predict chronic toxicity and an interest in structure activity relationships. NCI proposed that NTP conduct toxicological characterization and short-term mechanistic and neurotoxicity studies of the three APs and a two-year cancer study of 2-AP.

Aminopyridines are used in the synthesis of pharmaceuticals and dyes and 4-AP is also used as a pesticide. Recently, Acordia Therapeutics obtained approval from the FDA to undertake clinical trials with 4-AP for the treatment of multiple sclerosis. NTP has requested information from FDA, as there are no standard toxicological studies reported in the literature.

2- and 4-AP were negative in *Salmonella* genotoxicity tests but 3-AP was positive. APs block potassium channels by binding to a site in the channel, thus occluding the lumen and preventing the conduction of K+ into and out of the cells. This results in the maintenance of a presynaptic action potential and an increase in nerve signals. Both the pyridine ring and the amino substituent are necessary for binding and blocking activity, with 4-AP being the most active and 2-AP the least. The structure of the K+ channels is conserved across species. Blockage of K+ channels also inhibits lymphocyte proliferation. Based on this information, the NTP hypothesizes that APs would cause neurotoxicity, cardiotoxicity and immunotoxity at exposure levels shown to block K+

transport. Pyridine is hepatotoxic and causes liver tumors in rats and mice, and pyridine and APs are metabolized via similar mechanisms; therefore, APs may also be hepatoxic. The rationale for the study is to provide hazard identification information on this class of chemicals and comparative toxicity information for the three AP's. The proposed research project would conduct a series of short-term toxicity studies to identify cardiac, hepatic, neurologic and immunologic effects as well as genotoxicity studies. The APs will also be included in the NTP's HTS initiative in collaboration with NIH.

b. BSC Discussion

Dr. Mirsalis, a BSC reviewer, was supportive of the program because of the high production volume of the APs and fairly extensive human exposure to 4-AP. He was surprised at the paucity of toxicology data. He thought cardiovascular and neurological endpoints should be the focus of the NTP studies since clinical reports have documented cardiovascular and neurological effects following exposure to 4-AP. He relegated immunotoxicity studies to a lower priority and suggested those studies be undertaken after the 90-day studies when an indication of toxicity to the immune system might be gleaned. He thought that a comprehensive *in vivo* neurotoxicity study would be more useful than the proposed *in vitro* electrophysiology study. He asked Dr. Howard, FDA, if he could obtain the studies submitted to the FDA by Acordia Therapeutics despite freedom of information issues and proprietary information. Dr. Howard replied that this information is considered privileged. If the company has an approved investigational new drug application (IND), it would suggest they have conducted 14-day toxicity studies.

Dr. Russell Thomas, an *ad hoc* reviewer, was supportive of testing the aminopyridine series. He thought this nomination of high priority because the toxicity data would be able to leverage the potential for the pre-clinical studies being done with 4-AP. If the drug is approved, the NTP should be able to obtain the data from the chronic bioassay. To understand the mode of action of the AP series and their role in cardiotoxicity and neurotoxicity, it would be useful to include a dose response and a time series in the protocol. If the objective is to identify hazard, then cross-species assessments of gene expression changes *in vivo* would indicate whether the mode of action is relevant to humans.

Dr. Howard responded that the FDA cannot compel industry to provide information on an IND and it is up to the sponsor to provide it. Since the data are proprietary, the FDA cannot release the information without the company's approval. However, he has requested the data from the FDA product center, which he will forward to the NTP, if available.

Dr. Dunnick said NTP plans to obtain mechanistic data from the shorter-term studies to better predict cancer outcomes. The NTP has published a manuscript on gene changes at two, six and 13 weeks in the liver of mice exposed to hepatocarcinogens and found that similar genes are upregulated by peroxisome proliferators. The NTP plans to study the expression of cytochrome P450s in association with liver weight changes at different time points to predict cancer outcomes.

Mr. William Janzen suggested that it would be useful to include a panel of chemicals that affect potassium channels in the *in vitro* HTS studies and Dr. Dunnick replied that the NTP is collaborating with researchers at NIH, who will be able to provide advice on a series of ion channel blockers.

Dr. Mirsalis moved that the BSC accept the nomination and Dr. Kerkvliet seconded the motion. The vote was 12 yes and 0 no votes and 1 abstention. Dr. Soper abstained as he works for a pharmaceutical company that develops drugs for neurological diseases.

2. 2-Methoxy-4-nitroaniline

a. Concept

Dr. Richard Irwin, NIEHS provided background information on 2-methoxy-4-nitroaniline (2-MN), which was nominated by the NCI for complete toxicological characterization and evaluation of its carcinogenic potential due to an increase in production volume, a significant potential for occupational exposure, inadequate toxicological, carcinogenic and mutagenicity data, and the finding that three closely related chemicals namely, 2-methoxy-5-nitroaniline, *o*-anisidine and 2,4 diaminaoanisole, are rodent carcinogens.

2-MN is used in the synthesis of pigment yellow 74, which is present in numerous consumer products including printing and tattoo inks. Human exposure is primarily occupational by dermal and/or inhalation routes, and is associated with the handling of the pigment yellow 74 as a dry powder during dye manufacturing and in the textile industries; however, the information available is inadequate to determine its risk and neither OSHA nor NIOSH have set standards or guidelines. A second concern is that 2-MN may be a contaminant of pigment yellow 74. However, 2-MN is not a metabolite of microsomal metabolism of pigment yellow 74. The two major metabolites from 2-MN are 2-methoxy-*p*-phenylenediamine and 2-amino-5-nitrophenol, the latter of which has been shown to be a pancreatic carcinogen in rats. No epidemiological or case control studies of cancer risk from pigment yellow 74 in humans have been reported, but 2-MN is released when sunlight interacts with yellow tattoo pigments, and there are over 90 studies reporting an association between tattoos and skin cancer.

There is little information in the peer-reviewed literature on the toxicology of 2-MN except that it caused myocardial necrosis following ingestion in a 28-day toxicology study in rats, and it has been reported to be myotoxic in humans, causing rhabdomyolysis, the release of myoglobin, and the development of kidney failure. Although structurally related compounds cause skin tumors, there is no information that 2-MN is absorbed through the skin. Initially ADME studies and identification of metabolites will be done following exposure by the oral, dermal, and inhalation routes. If bioavailability is high following dermal and inhalation exposures, then oral exposure for subsequent studies will be used. The second specific aim is to conduct pre-chronic toxicity studies using *in utero* exposure. Biomarkers to monitor skeletal and cardiac muscle damage and studies of reproductive toxicity will be included. Since 2-MN is positive in some bacterial mutagenicity assays, but negative in others, and three structurally related compounds are carcinogens, it may not be necessary to conduct a 2-

year carcinogenicity study if 2-MN exhibits significant DNA reactivity. DNA reactivity will be tested in bacterial mutagenicity assays in nitroreductase proficient strains, DNA damage in the Comet assay, and DNA adduct formation in target tissues. The results of these studies will form the basis for predicting the carcinogenic potential of 2-MN without undertaking a 2-year bioassay.

b. BSC Discussion

Dr. Michael Pino, a BSC reviewer, said the reasons for initiating this research program are clear and valid given the high production volume of 2-MN, potential for human exposure, positive mutagenicity, and the suspicion of carcinogenic activity based on structure activity relationships. The appropriate routes of exposure have been considered based on occupational exposure. Special attention should be paid to skeletal muscle and the heart as cardiac toxicity has been reported in 28-day studies. He supported the decision to curtail the carcinogenicity studies until results are obtained for DNA adduct formation and reactivity. He agreed that it might not be necessary to perform a 2-year bioassay. He gave this program a moderate priority because occupational exposure to pigment yellow 74 is high, but public exposure to the dye in printing and tattoo inks is low and it is unclear whether 2-MN is produced in the skin from the inks.

Dr. James Popp, an *ad hoc* reviewer, agreed with Dr. Pino that the NTP should undertake the study and delay a 2-year bioassay. However, he questioned the relevance of the study of 2-MN based on it being a contaminant of pigment yellow 74. He suggested that the program consider comparing the metabolite profile of MN in *in vitro* studies in animal and human cells. The NTP should determine the distribution of 2-MN in skeletal and coronary muscles, and identify urinary metabolites in the *in vivo* rodent studies. This is an important series of molecules from the point of view of structure activity relationships. Although he ranked the program as moderate and said it merits utilization of NTP resources, he was not confident of human exposure to 2-MN as a result of exposure to pigment yellow 74.

Dr. Howard addressed the issue of human exposure to pigment yellow 74, by referring to a study at NCTR where ultraviolet light was found to cleave pigment yellow 74 releasing 2-MN, hence there is a possibility of human exposure. The NCTR is presently studying the pharmacokinetics and metabolism of radiolabeled pigment yellow 74 in rodents and has isolated unidentified metabolites.

Dr. Popp raised his level of support since photodegradation seems to occur.
Dr. Kerkvliet asked whether 2-methoxy-5-nitroaniline should be included as a positive control in some of the studies as the NTP has found it to be carcinogenic. Dr. Irwin replied that the metabolite profile is based on a preliminary report that lacked quantitative information and the NTP needs to identify the major metabolites.

Dr. Carney expressed concern about an *in utero* exposure for the 90-day study. Although the present dogma is that children are more sensitive, this is not entirely true, but rather they often respond differently from adults and sometimes are actually less sensitive. If the NTP is planning to alter its exposure regimen to begin 90-day studies while animals

are *in utero*, the program should first construct a comparative database from 90-day studies where exposure begins with young adults or *in utero* exposure, before perinatal exposures are accepted as the default approach. Dr. Irwin replied that the NTP's default position is to begin exposure *in utero* unless there is a reason not to do so. In this case, it seems relevant since a substantial number of females work in the dye and printing industries.

Dr. Toraason said the *in vivo* comet assay would be more useful than the *in vitro* assay. He asked about the proposal that robust DNA reactivity would be a rationale for not performing the 2-year bioassay and whether NTP had an algorithm of how this data might be used for hazard assessment. Dr. Irwin said 2-MN is structurally related to three known carcinogens, and if it forms DNA adducts and responds strongly in the comet assay, this would suggest that it too is a carcinogen. He was not sure how the data would be used for hazard assessment or how a decision would be made to omit a 2-year bioassay.

Dr. Bucher said previously some predictions have been made based on short-term data; in one case, the compound was a carcinogen, and in the second it was not. For example, diazoaminobenzene was predicted to be a carcinogen based on short-term tests and this information was used to list it as *reasonably anticipated to be a human carcinogen* in the Report on Carcinogens. Whether that level of information will be obtained with 2-MN is unknown. However, the NTP needs to learn how to use this type of information to develop approaches not used in the past for hazard assessment, if it plans to create a different type of toxicology for the 21st Century.

Dr. V. Walker asked about the approach that would be used to evaluate DNA adducts in target tissues, and whether the finding of adducts in tissues and positive genotoxicity data would indicate that a compound is a carcinogen. He warned that DNA adducts do not predict mutations but it is the cell that makes mutations. If the NTP is moving to a new paradigm, it must be cautious in interpreting what DNA adducts represent.

Dr. Howard said hazard identification is being proposed, but regulatory agencies need dose response information from carcinogenicity studies to determine hazard. He requested that the NTP contact the regulatory agencies to ensure that the information they will provide in the absence of a two-year bioassay would be sufficient to develop a reliable risk estimate for this class of compounds. He commented on the suggested relationship between tattoos and skin cancer and said this data were from case reports and not epidemiology studies as stated.

Dr. Pino proposed and Dr. Mirsalis seconded the proposal that this nomination be accepted for study and it was approved unanimously with 13 yes and 0 no votes and 0 absentions.

3. Nanoscale Gold

a. Concept

Dr. Nigel Walker, NIEHS, presented the concept on nanoscale gold, which is the second nomination of nanoscale materials received from the FDA. Nanoscale gold is being used

widely in the medical field for targeted chemotherapy and as a dietary supplement in its colloidal forms. There is a dearth of toxicology data on nanoscale gold. One study showed differential uptake across the gut of particles ranging in size from 4-60 nm. The FDA requested that ADME studies be conducted in rodents following oral and intravenous administration of a single dose and repeated doses for 28 days.

The primary focus is to understand how the physiochemical properties of the different-sized particles impact ADME and the toxicity of nanogold. The program on nanoscale gold will be integrated with the NTP research program on the physicochemical properties of nano materials to provide information on the effect of size and coating. A key question for nanoscale gold is to determine the ADME of the zero valence metal and how ionization and surface modifications alter ADME. The dose metric is a key issue in studying the toxicity of nanoscale materials and as surface-related metrics are important, a thorough characterization is necessary.

The first specific aim will evaluate the effect of particle size and particle coatings on the pharmacokinetic profile of nanoscale gold after oral and intravenous administration. Time course and tissue disposition studies will be conducted. Since the National Institute of Standards and Technology (NIST) has characterized nanogold particles of 10, 20, and 60 nm, it is likely the NTP will use these particles to evaluate the effect of particle size and particle coating. The second specific aim will evaluate the effect of particle size and particle coatings on the toxicological profile of nanoscale gold *in vivo* following subacute and subchronic exposure in rodents. Studies will address an evaluation of systemic and organ specific toxicity with specific emphasis on the immune and nervous systems.

b. BSC Discussion

Dr. Riviere, a BSC reviewer, said it is necessary to define basic ADME parameters as a function of size and coating. The biological studies need to be completed before NTP can determine the proper characterization metric. He is concerned that using only three particles will confound the comparison of size and surface coatings and this design may not be able to distinguish whether the difference in ADME is due to particle size or coating. Characterizing particle distribution is also crucial. He agrees with the use of the NIST particles and said that the standard particles used for this study should be the same as those available to other investigators. A problem in this field is the study of particles across different types. He gave this activity a high priority.

Dr. Stephen Roberts, an *ad hoc* reviewer, joined the meeting via videoconference from the University of Florida. He agreed with Dr. Riviere's points and thought that the rationale for the study was well articulated. He said the program is a worthy activity for the NTP due to an increasing use of nanoscale gold particularly as a platform, but also through other sources of exposure. The literature has anecdotal statements that gold is not toxic. It is important to design well-controlled ADME and toxicity studies and to understand the distribution of the particles in the body. There will be difficulties with dosimetry and particle size as the particles will not be uniform in size but will exist in a distribution. He suggested for a more rigorous study that the NTP use more than two sizes of uncoated particles, some of which could also be tested following coating with

polyethylene glycol (PEG). He said the priority for this program should be high.

Dr. Crump reinforced the importance of quantifying exposure and distribution of the particles. The program should not only consider mass or surface area because there may be other ways of quantifying exposure. He agreed with Dr. Roberts that the NTP should use standard particles so that its results can be compared with data from other investigators. He said scientists studying nanoscale materials must avoid making the same mistakes as were made with asbestos where it was believed that fibers longer than 5 microns and with greater than a three to one aspect ratio were the most toxic. Despite all of the studies on asbestos, there is still uncertainty regarding its risk because there was no emphasis on carefully characterizing exposure.

Dr. Soper suggested that the NTP consider studying four types of particles: "small uncoated," "small coated," "large uncoated," and "large coated," to determine if size and/or coating are important. Dr. N. Walker responded that NTP would likely use the standard particles of 10, 30, and 60 nm diameter that NIST has produced as its uncoated particles. Dealing with the coated particles would be more complicated because coating the standard reference materials with PEG can add 20-30 nanometers to the diameter. The actual hydrodynamic size of a 10 nm particle would then be more akin to an uncoated particle with a diameter of 30 nm, hence a direct comparison might not be possible. Classifying particles as "small coated" is also not accurate because the size will be closer to the larger particles. NIST has gone to great lengths to characterize a tight distribution of the particles within a size.

Dr. Popp said he endorsed the importance of the pharmacokinetic and tissue distribution studies. He wondered whether the NTP had considered using scanning energy dispersal X-ray analysis to determine the location within a tissue of the nanoparticles. Dr. N. Walker agreed that this technology should be appropriate, but cautioned that interpretation might be tricky. Thus, a combination of inductively coupled plasma mass spectrometry and electron microscopy would be a better approach. He added that ionization is a key metric and it might be possible to distinguish the different forms within the tissues.
Dr. Riviere made a motion that the nanoscale gold be studied; it was seconded by Dr. Mirsalis and unanimously approved 13 yes and 0 no votes, and 0 abstentions.

4. 2',2'''-Dithiobisbenzanilide

a. Concept

Dr. Masten presented a brief outline of the concept for 2',2"'-dithiobisbenzanilide (DTBBA). The NCI nominated this chemical based on its high production volume, lack of adequate toxicity data, and a suspicion of toxicity based on its chemical structure. DTBBA is used in the manufacture of natural and synthetic rubber for tires and other solid rubber goods. Rubber industry workers are potentially exposed to DTBBA dermally and by inhalation during the compounding and mixing process. The general population may be exposed to small amounts of DTBBA that leaches from rubber and latex gloves. It has low acute toxicity, but is a skin sensitizing agent, suggesting that the

compound is reactive. There are no genetic toxicity or ADME data. Potentially, DTBBA could be metabolized to biologically active intermediates either in the body or in the environment. One aspect of the study would be to identify potential metabolites and degradation products. The proposed research program has four parts for study: *in vitro* mutagenic activity, *in vitro* and *in vivo* absorption through human and rodent skin, and *in vitro* metabolism using liver preparations. Based on the results of these studies, a bioavailability study with more extensive characterization requiring a radiolabeled compound may be undertaken in rodents.

b. BSC Discussion

Dr. Soper, the BSC reviewer, said there is a clear and valid rationale for the proposed research program and the scope is appropriate. Although toxicity is potentially low, there is substantial exposure to rubber industry workers but unknown exposure to the public. The latter depends on how much of the compound leaches from rubber or latex gloves or from rubber tubing used in food processing. Little is known of any reactive metabolites, thus, there is a rationale for studying the absorption and metabolic activation potential of the compound. He rated the importance of studying DBBA as moderate.

Dr. Popp, an *ad hoc* reviewer, agreed with Dr. Soper that the study is straightforward. The concept document was clearly written and indicates how the results would be used to make further decisions. There should be an emphasis to study bioavailability and metabolism. He gave the nomination a high priority.

Dr. Sikka suggested that a radiolabeled compound be used in the *in vitro* and *in vivo* metabolism studies, as it would be more efficient for detecting metabolites present in low concentrations. Dr Masten agreed that it might be more efficient, but the cleavage of the disulfide bond is a critical step. The first *in vitro* studies would be designed primarily to determine if cleavage occurs, which may be possible via this approach without the time

Dr. Kervliet asked whether the NTP is concerned about the sensitization potential of the parent compound and why no sensitization studies are proposed. Dr. Masten replied that it is not clear from the literature whether the parent compound or one of the metabolites

might be the sensitizing agent. The NTP will consider her comments when designing the experiments.

Dr. Soper proposed that the NTP study DTBBA; it was seconded by Dr. Kerkvliet and the vote was unanimously approved with 13 yes and 0 no votes, and 0 abstentions.

5. Pentaethylenehexamine

a. Concept

Dr. Masten said NTP did not develop a research concept for pentaethylenehexamine (PEHA) because the NTP's recommendation is not to study PEHA. The NTP is asking the BSC if they concur with this decision.

The NCI nominated PEHA based on its high production volume, lack of adequate

toxicological data, and its positive mutagenic response in *Salmonella*. It is used almost entirely as an intermediate in the production of a number of different products, but little is known of its potential exposure. There are a few reports in the literature indicating it is a dermal and ocular corrosive and moderately toxic following acute exposure. Although PEHA is a hazard, there are practical difficulties with carrying out studies due to its reactivity and corrosivity, such that only low concentrations could be used. Most of the studies of PEHA and other polyamines performed at low concentration levels were negative. NTP believes it would be inhumane to expose animals to PEHA. There are no occupational exposure guidelines or hazardous waste regulations for PEHA. It may be useful to test PEHA in *in vitro* toxicity studies.

b. BSC Discussion

Dr. Kerkvliet, the BSC reviewer, said she agreed with the decision not to study the compound and there was no further discussion.

Dr. Kerkvliet moved that the compound not be studied and it was seconded by Dr. Bunton. The motion carried unanimously with 14 yes and 0 no votes and 0 abstentions.

6. Diethyl phthalate (DEP)

a. Concept

Dr. Paul Foster, NIEHS, discussed the research concept for diethyl phthalate (DEP), which was nominated to the NTP for testing by NIEHS. DEP is used primarily as a solvent in personal care products and fragrances. It is metabolized to a more water-soluble compound, conjugated with glucuronic acid, and excreted. Phthalates cause reproductive toxicity with the fetus being the most susceptible life stage in rodents. Two phthalates, dibutyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP), have antiandrogenic effects in the developing fetus resulting in a syndrome that is similar to a testicular dysgenesis syndrome in man. The rat and guinea pig are more sensitive to the reproductive and antiandrogenic effect of DEHP than the mouse, while the hamster is the least sensitive. A number of multigenerational and transgenerational studies have been reported in the literature. An NTP study in the mouse found that up to 2.5% of DEP in the diet had no significant effects on the offspring except for small changes in the F1 generation in the adult. A study in the rat did not report any effects, although it did not monitor some phthalate-sensitive endpoints. Questions have been raised about the statistical power of standard multigenerational experiments to detect developmental changes in the adult, as only one male and one female pup from each litter is raised to adulthood.

The CDC, while monitoring the concentration of various phthalates in the US population, found that the concentration of DEP is the highest of all the phthalates in samples of human urine and amniotic fluid. Two small epidemiological studies found a positive correlation of urinary DEP metabolites in pregnant women with a decrease in the anogenital index in male offspring. The concentration of DEP metabolites in human breast milk correlated with the lutenizing hormone (LH): free testosterone ratio in 6-month old male infants. These findings are consistent with the effects of other phthalates on androgen signaling in rats. However, the findings in humans may not be related to an

individual phthalate but to the total body burden of phthalates.

NTP will test the null hypothesis that DEP does not affect reproduction in rats and is unlikely to present a risk to humans. A robust multi-dose, dietary, multigenerational study in rats will be performed where phenotypic end points known to be sensitive to antiandrogenic effects of other phthalates would be evaluated. The study will retain more than one pup from each litter and take measurements into adulthood. DEP concentration in the amniotic fluid will be measured and toxicokinetic measurements will be included to assess the dose delivered to the fetus. A similar study by NTP with DEHP showed an increased ability to detect reproductive tract effects in the F1 and F2 generations at lower dose levels compared to the conventional multigenerational study design. These proposed studies with DEP will fill critical data gaps for which public health concerns have been raised, particularly the apparently high exposure of humans to DEP and the possible link between maternal exposure to DEP and alterations in androgen levels in male offspring. It will be important to determine whether DEP itself contributes to any cumulative risk to humans exposed to multiple phthalates.

b. BSC Discussion

Dr. Crump, a BSC reviewer, said exposure to DEP is wide spread, and DEP exposure might potentially be a health issue. However, he gave this concept a low priority because of the extensive literature on DEP in rodents including two multigenerational studies in mice conducted by NTP. Extremely high doses (2.5% in the mouse and 1.5% in the rat) of DEP were used in the diet, the latter of which is 20,000 times higher than the average human blood level. Both these studies were negative and only minimal defects were observed at the high doses. The emphasis for any further studies should be the evaluation of DEP in humans. A definitive epidemiological study would not be difficult, as it would require the collection of blood and urine from the mother and a non-invasive technique to sample the amniotic fluid. If a change in anogenital length were found, the children could be monitored for possible developmental effects later in life. Such a study would help to resolve the issue in humans. Dr. Crump thought that repeating a multigenerational animal in rodents did not seem productive. Although DEP appears to be the phthalate of highest concentration in humans among the phthalates evaluated by CDC, it may not be the isomer of greatest concern.

Dr. Mylchreest, an *ad hoc* reviewer, provided comments through videoconferencing and said this proposal has some merit at addressing the questions regarding possible adverse effects in male children. She gave this proposal a priority of moderate to high, based on the human exposure data. Although the animal studies suggest a low concern for the antiandrogenic effects of DEP, there are deficiencies in the data set including the limited number of offspring that were evaluated, which reduced the power to detect a low incidence. She suggested that a dose range finding study where exposure is limited to the *in utero* phase would reveal whether high concentrations elicit adverse reproductive outcomes. Subsequently, a decision could be made whether further studies are warranted. The LH:free testosterone ratio could be examined as a biomarker to determine whether a correlation exists between the ratio, toxic end points, and metabolite formation. Since the mouse is less sensitive than the rat, it would be worthwhile

exploring the responsiveness between rats and mice. It would be useful to develop a database on phthalates to determine which animal model is more predictive of human risk. Perhaps the rat is not the most appropriate model due to differences in pharmacokinetics and the development of the reproductive tract.

Dr. McCarver agreed with Dr. Crump that a human epidemiological study would be extremely useful, but she supported the rodent studies. She thought the NTP's proposal of a multigenerational study using a wide dose range is appropriate, and the scope of the study would be sufficient to address the data gaps. There are several problems associated with the present studies on DEP, namely, inadequate statistical power due to limited sample size, apparent bias, and the suggestion that there was not a clear outcome. She had serious concerns whether the animal data would be sufficient to prove that DEP is truly negative in humans. The issues raised about the appropriate model, doses and dosing time, whether both *in utero* and perinatal dosing are necessary, and whether hormonal changes as well as structural changes should be monitored are all relevant. The highest concentrations of phthalates (2-3 orders of magnitude greater than in the general population) are found in pregnant women and newborns, possibly from exposure to plastics. She suggested that the gene expression studies be curtailed for subsequent analysis. She expressed concern about zinc deficiency and suggested that abnormal anogenital distance in humans might be due to zinc deficiency, while negative findings in the rodent may be related to zinc sufficiency. If DEP will be a component of the phthalate initiative (see later) information on DEP alone would be important.

Dr. Sikka said studies indicate that peroxisome proliferator receptors in rats are not relevant for humans. If this were the case, he asked why the study is being proposed in rodents.

Dr. Mirsalis was ambivalent about this concept and agreed that the rat is more sensitive than the mouse, but noted that human sensitivity in relation to these two rodent species is unknown. He also agreed that more information would be gleaned from an epidemiology study.

At this stage, it was agreed that the BSC would vote on the DEP concept after the concept on the phthalate initiative had been discussed.

Dr. Carney agreed with several of the comments and thought that little additional information would be obtained with the proposed study in rats. The difference in exposure between humans and rodents is dramatic, and even if the "low effect level" in rodents were tenfold less, this concentration is much higher than human exposure levels. He suggested that more effort be made toward developing a relevant biomarker that could be measured earlier in development. He had animal welfare concerns, because the study would require 3-4 thousand animals if the proposed dose levels were used.

Dr. Kerkvliet suggested that the studies focus on the developmental period, finding additional biomarkers, and comparing responses in mice and rats.

Dr. Foster noted the difficulty in following development of the prostate, a target organ, in

a weanling. At high dose levels, only the animals from the F1 and F2 generations that survive are available for selection. Thus, the most sensitive animals may be selected against, since they cannot breed. The NTP is planning a robust study that meets regulatory norms to definitively find the potential for adverse effects by DEP.

Dr. Foster added that the concentration of DEP in maternal human urine and rodent urine differs and cannot be extrapolated to the concentration to which the fetus is exposed. However, the concentration of DEP in the amniotic fluid in rodents and humans are more similar. A Danish study focused on the surge of testosterone levels in the first six months of life in young boys; such a surge does not occur in rodents and cannot be modeled. It will take 20 years to obtain data on adult reproduction in an epidemiology study and although certain parameters could be measured after birth, other endpoints such as fertility and reproductive tract development would take a generation. He justified the use of the rat by stating that the mouse is less sensitive probably due to pharmacodynamic and pharmacokinetic parameters. Also, the mouse is quite different from the human in development of the reproductive tract and is much more sensitive to estrogens than either the rat or human.

Dr. Mylchreest asked Dr. Foster to address the need for a multi generational study rather than a study where exposure is during the critical *in utero* window. Dr. Foster said the treatment regimen suggested would not monitor whether the animals could reproduce. In the mouse study, there was an effect on sperm count and litter size in the F1 generation. If one were only evaluating an antiandrogenic effect, an *in utero* exposure would be sufficient.

Dr. McCarver mentioned an ongoing National Children's Study in which phthalates will be measured before conception through childhood to adulthood, but data from this study will not be available for many years.
Dr. Howard asked the Board whether they were suggesting that the NTP fund an epidemiological study. Dr Crump replied that an epidemiological study would be the most suitable and that it seems reasonable for NIEHS to fund such a study. Dr. Crump reiterated that the studies in rodents are totally irrelevant because of the very high doses used and the lack of any positive response. He suggested that the NTP design a study in humans that would not take 20 years to complete. If the study replicates CDC's findings, the children could be monitored to determine if there are other adverse effects.

Dr. Kerkvliet encouraged the NTP to obtain human data to compare with data from animal studies. She asked Dr. Bucher if the NTP could sponsor human epidemiology studies. Dr. Bucher replied that traditionally NTP has not sponsored epidemiology studies, but the NTP could work with the epidemiology branch at NIEHS or with an epidemiologist in the program. There are other avenues that could be pursued to address this issue.
Dr. Crump moved that the DEP concept not be approved for study and Dr. Mirsalis seconded the motion. All voted in favor of disapproval with 11 yes and 1 no vote, and 0 abstentions except for Mr. Janzen who thought it should be taken forward.

7. Di-(2-ethylhexyl) Phthalate (DEHP) and Phthalate Mixtures

a. Concept

Dr. Paul Foster introduced the research concept to investigate the potential for adverse effects of di-(2-ethylhexyl) phthalate (DEHP) and phthalate mixtures. The NTP has received many nominations to study phthalates including a nomination from FDA for DEHP and a request related to critical needs derived from a CERHR monograph on DEHP. Liver and testicular Leydig tumors were seen in a life time study in Sprague Dawley rats exposed to DEHP. The International Agency for the Research on Cancer (IARC) and EPA designated DEHP as a *probable human carcinogen* (Category 2b) based on the NTP bioassay in Fisher 344 rats. IARC subsequently changed its classification to *not classifiable as a human carcinogen (Group 3)* based on its mode of action, namely that the liver tumors were initiated through a PPARα receptor, which has limited relevance to humans. A 22-month study with DEHP in the PPARα null mouse showed more liver tumors in the knockout mouse than in wild type mice suggesting that the induction of liver tumors may be through mechanisms other than PPARα. Phthalates induce developmental effects after *in utero* exposure through an antiandrogenic mode of action. Postnatal developmental effects were not seen in the PPARα null mouse treated with perfluorooctanoic acid, while prenatal effects were observed. This poses the question of whether PPARα is developmentally regulated.
The hypotheses to be tested are:

- lifetime (perinatal + 2-year) exposure to DEHP affects the dose response, incidence, and/or severity for cancer of the liver, testis, and pancreas compared with adult-only exposure;
- PPARα is developmentally regulated in the rat and unlikely to contribute to toxicity initiated *in utero* after exposure to DEHP;
- exposure to mixtures of phthalates, based on their individual potencies, would result in dose-addition for cancer (and other) outcomes. For this hypothesis, the NTP will need to obtain information on each of the individual chemicals.

Dr. Foster said the proposed study design would consider the route of exposure, pharmacokinetics, and estimates of internal dose in the Wistar Han rat during pregnancy and lactation following exposure by both dietary and gavage routes. He noted that the NTP studies would use gavage as the route for perinatal exposure because it would be easier to control the external dose of DEHP during pregnancy and lactation.

The NTP would conduct short-term assays on a number of phthalates [e.g., dibutyl phthalate (DBP), di-isobutyl phthalate (DiBP), butylbenzyl phthalate (BBP), di-isononyl phthalate (DINP), DEHP and DEP] to develop potency estimates in the Wistar Han rat for the mixture studies. The potency estimates would be based on fetal testicular testosterone levels for *in utero* exposures and on hepatic peroxisome proliferator activity (e.g. CYP 4A1, Acyl CoA oxidase etc.) for weanlings. Individual toxicokinetic data also would be needed for each phthalate. Once the potency estimates were determined, the long-term mixture study would be designed for three phthalates. .

Dr. Foster noted that it would be appropriate to consider cumulative risk for the

phthalates class since human subjects (including fetuses) are typically exposed to multiple phthalates. Due to similar modes of action *in utero*, phthalates show dose-addition when administered in combination. For example, when DEHP and DBP were administered separately to rodents there were no malformations, but when administered together during a critical window of development more than 50% of males had hypospadias.

b. BSC Discussion

Dr. Cattley, the BSC primary reviewer, said the focus for the initiative is cancer risk assessment. The program has a clear rationale and it is largely based on a 2007 publication in which uncertainty was expressed over the mechanism of PPARα-mediated carcinogenesis in the liver of mice. Since there are critical exposure windows for development, there is a rationale for comparing the outcome following *in utero* versus adult onset of exposure. He asked why the Wistar-Han rat would be used instead of the Sprague Dawley rat, since the earlier definitive study used the latter strain. He suggested that interim endpoints and time points be included in the study design, especially during the developmental window. He questioned the strategy of investigating gene expression to determine if PPARα is developmentally regulated, as it is likely that a very small subpopulation of cells would be responsible for mediating the effect. PPARγ, δ and τ should be included if a gene expression study is implemented.

When studying mixtures, understanding exposure, tissue concentrations, and kinetics are tantamount. He did not consider acyl-CoA oxidase activity as a relevant end point to calculate toxic equivalency factors. He questioned whether the chosen phthalates are representative of the class because two of the phthalates (DiNP and DEHP) are associated with liver tumors and two (DEHP and DBP) are associated with the risk of Leydig cell tumors. He asked why toxicokinetics and toxic equivalency factors would be determined for all the phthalates when not all of them would be studied in the mixtures. He suggested that the NTP include two additional strains: 1) the PPARα knockout mouse because non-phthalate peroxisome proliferators mediate their carcinogenic effects though PPARα, and 2) the mouse model with the human PPARα receptor. He considered this study of high priority due to the relevance of human exposure to phthalates. It should provide mechanistic data that may have a significant impact on the assessment of human health risk to this class.

Dr. Mylchreest, the *ad hoc* reviewer, said the proposal was excellent and addressed an important issue, namely the effect of perinatal exposure of a mixture of phthalates to cancer risk. She liked the idea of a tiered hypothesis approach. The results of the first hypothesis would influence the design of the mixtures study. If there were no increase in tumor incidence from perinatal exposure to DEHP, then such a finding would reduce the concern for exposure to other phthalates and mixtures. She agreed with Dr. Cattley regarding the study design, but thought that an adult-only exposure regime would be useful to address the first hypothesis. She questioned how NTP would interpret an increased tumor incidence from perinatal exposure. She also asked how NTP would distinguish the role of prepubertal exposure in tumor incidence if animals were exposed throughout their lifetime including during the perinatal window.

Dr. Deininger was enthusiastic about the study but was troubled whether the rat is the right model. It would be important to characterize the experimental animals regarding PPARα. He asked whether co-modulators to the other PPARs were known. He had low enthusiasm for the third hypothesis because it is fairly broad and was presented as the null hypothesis. However, he felt that studying mixtures of phthalates was important because of possible synergistic effects.

Dr. Carney said he liked the initiative as it is based on human exposure, and noted the importance of evaluating cumulative exposure. He said it would be important to use concentrations that are below the threshold to determine if effects are observed at low dose levels.

Dr. Crump said he had little enthusiasm for the study in terms of its utility for regulatory decision-making. He did not favor a 2-year bioassay with perinatal exposure because it would require a large amount of resources, need a comparative control, and two conventional bioassays have been completed on DEHP. He was more excited about the third hypothesis, to develop a scheme to assess the toxicity of mixtures of different phthalates. One downside is the limited number of combinations that can be evaluated *in vivo*. He asked if there were an *in vitro* test or group of *in vitro* tests that could provide an answer in a shorter time period and where many more combinations of phthalates could be tested. *In vivo* studies could then be performed to validate the findings of the *in vitro* studies, which would allow a comprehensive assessment of the risk of exposure to mixtures.

Dr. Foster said if the NTP studies find no differences between adult and perinatal exposure that would not affect the mixture studies. He noted that there are no simple *in vitro* studies that could be used; however, some relatively short *in vivo* studies would provide reasonably good potency estimates. He added that the EPA has asked the National Academy of Sciences to evaluate the cumulative and additive risks of phthalates.

Dr. Deininger moved that the phthalate initiative be approved, which was seconded by Dr. V. Walker. The vote for approval was unanimous with 12 yes and 0 no votes, and 0 abstentions.

VI. NTP BSC Technical Reports Review Subcommittee

Dr. Nancy Kerkvliet, Chair of the Subcommittee, summarized the actions on the Draft NTP Technical Reports from the peer review meeting on May 16-17, 2007. The Subcommittee reviewed the findings and conclusions from studies of sodium dichromate dihydrate, formamide, cumene, cresols, and propargyl alcohol that used conventional F344 rat and $B6C3F_1$ mouse models.

- The subcommittee voted 6 yes, 0 no, and 0 abstentions in favor of the conclusions that there was *clear evidence* of carcinogenic activity of sodium dichromate

dihydrate in male and female F344/N rats based on oral squamous cell carcinoma and in B6C3F$_1$ mice based on to intestinal neoplasms.

- The subcommittee voted unanimously 6 yes, 0 no, and 0 abstentions in favor of the conclusions that there was *no evidence* of carcinogenic activity of formamide in male and female F344/N rats, *clear evidence* of carcinogenic activity in male B6C3F1 mice based on hemangiosarcoma in the liver and *equivocal evidence* of carcinogenic activity in female B6C3F1 mice based on hepatocellular adenoma and carcinoma combined.
- The subcommittee voted unanimously 6 yes, 0 no and 0 abstentions in favor of the conclusions that there was *clear evidence* of carcinogenic activity in male F344/N rats based on nasal epithelial adenoma and renal tubule adenoma and carcinoma combined, *some evidence* of carcinogenic activity in female F344/N rats based on nasal epithelial adenoma, and *clear evidence* of carcinogenic activity in male and female B6C3F1 mice based on alveolar bronchial neoplasms.
- The subcommittee voted unanimously 6 yes, 0 no and 0 abstentions in favor of the conclusions that there was *equivocal evidence* of carcinogenic activity of 60:40 m/p-cresol in male F344/N rats due to renal tubular adenoma and *some evidence* of carcinogenic activity in female B6C3F1 mice based on forestomach sqaumous cell papillomas.
- The subcommittee voted 4 yes, 2 no, 0 abstentions in favor of the conclusions that there was *some evidence of carcinogenic activity* of propargyl alcohol in male F344/N rats based on nasal epithelial adenoma and mononuclear cell leukemia and *no evidence* of carcinogenic activity in female F344/N rats. The Subcommittee recommended *some evidence of carcinogenic activity* in male and female B6C3F1mice based on nasal epithelial adenomas.
- The subcommittee reviewed a multigenerational study and a 2-year bioassay following dietary administration of 2, 10 or 50 ppb of ethinyl estradiol. The Subcommittee voted 7 yes, 0 no, and 0 abstentions in favor of the conclusions. For the 2-year bioassay, there was *no evidence* of carcinogenicity in the F1 generation exposed from conception, through lactation until 2 years of age. There was *equivocal evidence* of carcinogenicity based on uterine stromal polyps in F1 females fed ethinyl estradiol from conception to 20 weeks of age, and in F3 males exposed from conception to weaning based on preputial gland epithelial neoplasms and mammary gland adenomas, and uterine stromal polyps in the females.

Dr. Walker proposed to accept the Subcommittee report as presented. Dr. Soper seconded the motion. The vote was unanimous in favor of the motion with 12 yes and 0 no votes and 0 abstentions.

VII. Review of Lead and Cadmium for the Center for the Evaluation of Risk to Human Reproduction (CERHR)

Dr. Michael Shelby, CERHR Director, outlined the process for compiling NTP-CERHR monographs. There are three phases: (1) chemical nomination and selection for

evaluation, (2) preparation of the expert panel report and its review, and (3) preparation of the monograph and NTP Brief. The NTP Brief presents the NTP's official position on the reproductive and/or developmental hazards of the substance. The CERHR relies on advice for its activities from a Core Committee (CC), consisting of representatives with expertise in reproduction and development from the NTP, EPA, FDA, CPSC, NIOSH, and CDC. The CC meets quarterly to discuss action items, such as nominations. CERHR prepares a preliminary dossier on every nomination received to ascertain the availability of relevant literature, production volume, information on human exposure, and the extent of literature on the reproductive and developmental toxicity of the nomination. If the CC suggests that sufficient information is available, a final dossier is prepared. In selecting a nomination for evaluation, CERHR considers several factors including the extent of the relevant literature, public concern, production volume, and extent of human exposure. The nominations of lead and cadmium were being brought to the BSC to obtain their input about whether CERHR should undertake an evaluation on either chemical.

a. Cadmium

Cadmium was nominated anonymously in 2000. At that time it was deferred because of insufficient relevant literature, but in 2007, with the availability of new literature, the CC recommended cadmium for evaluation. The rationale for evaluation is its high production volume and widespread human exposure through numerous routes including increased use and disposal of cadmium/nickel batteries from electronic devices. In Europe, fairly strict controls have been imposed regarding the importation of products containing cadmium.

BSC Discussion

Dr. Carney, the primary BSC reviewer, said human exposure is high and cadmium has been detected in human blood and urine samples. The latest, comprehensive review of cadmium was prepared in 1999 by ATSDR. The present dossier mainly has information on studies since 1999 with the area of focus being the testis. Many animal studies were conducted using quite high doses of cadmium administered by the intraperitoneal and subcutaneous routes. Toys from China are contaminated with both lead and cadmium. Overall, he was supportive of CERHR conducting an evaluation of cadmium and gave it a moderate priority.

Dr. Michael Collins, an *ad hoc* reviewer, addressed the meeting via videoconferencing. He has studied the importance of the developmental toxicity of cadmium for the past 15 years. He said it would be very difficult to determine if cadmium causes adverse human developmental and reproductive toxicity because exposures to humans are relatively small. However, cadmium is an extremely potent developmental toxin in all the animal species in which it has been tested; hence, it is likely to be a reproductive and developmental toxicant in humans. He said the dossier was somewhat incomplete with substantial omissions of historical literature. None of Dr. Bill Webster's publications from Australia are cited and he has spent over 10 years working on the developmental toxicity of cadmium. In addition, some review articles on cadmium published in journals

that are not widely disseminated were omitted. He considered the evaluation of cadmium a high priority.

Dr. Shelby clarified that the emphasis of the dossier is on publications post 1999, hence the exclusion of some of the earlier historical publications.

Dr. McCarver said there was no discussion on exposure of potentially susceptible subpopulations or other end points besides the testis, some of which may be worth mentioning.

Dr. Collins added that it is likely that susceptible populations exist based on Dr. Daniel Nebert's work on the ion transporter CDM gene, which determines susceptibility or resistance to testicular toxicity in mice. Certain mouse strains are susceptible and others resistant, predicated on the specific gene they carry. He said his group has published on another gene, the calcineurin gene subgroup a isoform, which may also be involved in the susceptibility to cadmium toxicity. The processes involved in the transport of other metals, including iron and calcium, could also be very important in the overall susceptibility to cadmium.

Dr. Deininger was skeptical about predicting susceptibility to cadmium toxicity based on one or two genes and said there is a debate about which of the two genes are the most important mechanistically. He thought that a study to elucidate the mechanism of cadmium-induced testicular toxicity would be important for determining susceptibility.

Dr. Mirsalis moved that CERHR undertake an evaluation of cadmium. Dr. Carney seconded the motion and it was approved unanimously by 12 yes votes, 0 no votes, and 0 abstentions.

b. Lead

Dr. Shelby provided background information on lead. Over 22 billion kg of lead are released annually and exposure is widespread. There is an extensive literature on lead with 315 studies listed in the EPA Air Quality Criteria Documents. Lead was nominated for CERHR evaluation by Dr. Elizabeth Whelan from NIOSH. The rationale is that occupational exposure limits allow blood lead levels of 40 µg/dl in workers including women of child-bearing age. The Association of Environmental and Occupational Clinics has been working with NIOSH and reported that levels of 10 to 19 µg/dl of lead are associated with possible spontaneous abortion and reduced birth weight in humans. Epidemiology studies have indicated that blood lead levels below 10 µg/dl are associated with adverse effects. The CERHR CC recommended an evaluation of the potential for reproductive and developmental effects when blood lead levels are below 40 µg/dl. Besides contributing to the overall public health, a CERHR evaluation could help confirm the need to revise the recommended exposure limit. It would be the first time that CERHR has evaluated a known human developmental toxicant.

BSC Discussion

Dr. V. Walker, the primary BSC reviewer, said the CERHR evaluation of lead is a very important public health concern and should be given a high priority. In the EPA Criteria Document on lead only a few pages were devoted to discussing the reproductive and developmental effects and there is no discussion of the weight of evidence. There is an effort to minimize exposure of children to lead so that blood lead levels do not exceed 10 µg/dl, but no consideration has been given to the exposure of pregnant women in the workplace to lower levels than other workers. He suggested that developmental neurotoxicology, which was omitted from the present document, be included in the dossier.

Dr. Guilarte, an *ad hoc* reviewer, who addressed the BSC via videoconferencing, said Dr. Shelby provided a comprehensive background to lead. He said he has studied developmental neurotoxicity, an extremely important topic for human health, for over 15 years. He said there is a rich literature on the neural effects of lead that is missing from the dossier and there needs to be a concerted effort to add this and other missing literature.

Dr. Sikka asked about the criteria for choosing nominations, why lead and cadmium were chosen, and whether other chemicals were nominated for evaluation. Dr. Shelby replied that CERHR relies on the scientific community and the public at large to nominate chemicals that are of concern. CERHR has about 400 nominations on file, and expert panel evaluations have been completed on more than 20. Only those that are the most important to public health are evaluated.

Dr. Kerkvliet was pleased with both the nomination of lead and cadmium and said that older studies are often forgotten even though they can contribute to the understanding of toxicity. It is important to have expert scientific panels to evaluate the literature and prepare expert panel reports. Dr. Shelby said CERHR does periodic literature searches during development of the expert panel report and makes this literature available to the panel for its consideration in developing the expert panel report. Members of the expert panel also identify pertinent literature. The public is invited through Federal Register notices to comment on the draft expert panel report and supply additional literature.

Dr. Deininger proposed a motion in favor of the CERHR evaluation of lead. Dr. Kerkvliet seconded the motion and the BSC voted unanimously in favor with 12 yes votes, 0 no votes and 0 abstentions.

VIII. Report on Carcinogens

a. Presentation

Dr. C. W. Jameson provided an update on the process and time line for the twelfth Report on Carcinogens (RoC). The RoC is mandated by law for publication every two years, and it is submitted to Congress by the Secretary of Health and Human Services. The RoC contains a listing of all the chemicals that are either *known* to be or are *reasonably anticipated* to be a human carcinogen. The 11th RoC was published in January of 2005. The reason for the tardiness in completing the 12th RoC relates to the issuance of the Information Quality Act and guidelines from the Office of Management and Budget for a

more in-depth peer review of draft documents prepared by the federal government. The NTP made a number of changes to modify the process for review of nominations for the 12 th RoC to increase transparency. The revised process was completed and released in April 2007.

Dr. Jameson briefly summarized the revised review process, which has 4 major parts:

- Nomination and selection of candidate substances
- Scientific review of candidate substances
- Peer review of draft substance profiles
- Preparation of the RoC and transmittal to Congress and release to the public

The process includes steps to peer review the draft background documents by an expert panel at a public meeting, obtain recommendations on listing status of candidate substances from the expert panels and two government groups, and peer review of the draft substance profiles by the NTP Board of Scientific Counselors at a public meeting. Public input is invited multiple times throughout the process.

The first expert panel meeting was held in October 2007 and the second meeting is scheduled for January 2008. The NTP anticipates submitting the 12th RoC to the Secretary in January 2010.

BSC Discussion

Dr. Toraason asked (1) how the NTP handles a situation where one group recommends that a candidate substance not be listed, and (2) how the NTP records that a chemical was reviewed, but subsequently not listed. Dr. Jameson responded that the recommendations from the three review groups are considered in making the final determination of listing of a candidate substance. For the more recent candidate substances that were reviewed but not listed, there is a short profile at the back of the RoC.

A member of the public asked about the time period between the 12 th and 13th reports and Dr. Jameson responded that historically the RoC takes three years to complete, but there is an overlap between the compilation of two reports so it is a staggered process.

IX. Implementation of the Workshops and Retreat Recommendations.

The NTP Roadmap proposed that the NTP evaluate its current testing strategies to determine if changes/improvements are needed. Three workshops were held. The first examined the current stocks and strains of rats and mice being used in the testing program, and the possibility of using multiple strains of mice with different genetic backgrounds. The second workshop discussed the relevance of the bioassay for hormonally induced tumors and the third workshop was held to identify biomarkers for the lung, heart, and lipid and carbohydrate metabolism.

a. Animal Models for NTP Rodent Cancer Bioassays: Strains and Stocks Should We Switch?

A recommendation at this workshop, held in June 2006, was to discontinue the use of the NTP Fisher 344 rat in the testing program, but to continue the use of the B6C3F1 strain of mouse despite its high incidence of spontaneous liver tumors. No specific recommendation was made to implement the multiple strains approach. The recommendation to use a different rat strain was based on breeding problems and periodic seizures that occurred during the second half of some rat studies. The experts suggested using an F1 hybrid of another F344 strain that had been crossed with the Brown Norway rat. The experts, who debated the multiple strain approach, considered the advantages of genetic variation and pointed out that statistical power probably would not be lost if the total number of animals used were spread across the different strains. However, they noted numerous disadvantages namely the added cost for range-finding studies, increased animal resource costs, the need to develop historical data for multiple strains, and the possibility that regulatory acceptance might be questionable.

b. Hormonally Induced Reproductive Tumors: Relevance of Rodent Bioassays

This workshop held in May 2007 addressed the problems associated with identifying hormonally induced cancers that are common human cancers in the two-year bioassay. Four breakout groups examined the breast, prostate, ovary, and testis. The experts were not overly impressed with the rat or mouse bioassays that are currently used to identify chemicals that might cause hazards to these tissues. They suggested that endocrine end points such as early patterns in hormonal changes be built into the prechronic studies because they might provide information to predict cancer in humans. They recommended that the NTP discontinue using the Fisher rat as they recognized its limitations for detecting tumors in these tissues. They pointed out the importance of developmental programming, which has led the NTP to adopt perinatal exposure as the default mode for its two-year bioassays.

c. Biomarkers for Toxicology Studies

The expert panel at the third workshop held in September 2006 suggested that the NTP monitor for troponin T as a biomarker for cardiovascular metabolism in pre-chronic studies in rats. They also recommended measuring insulin levels rather than fructosamine as an indicator of long-term glucose overload.

d. Implementation

The NTP has implemented a number of changes in response to these workshops and an NTP retreat held in October 2007. The NTP we will continue to use the B6C3F1 hybrid mouse, but discontinue use of the Fischer 344 rat and replace it with the Wistar-Han. The Wistar Han rat has a low incidence of spontaneous tumors, a long lifespan, is of moderate size with robust reproductive capacity, and is plentiful commercially. If there is evidence that a particular strain of rat should be used instead of the Wistar-Han, it will be considered. The NTP has decided to dose rats during the perinatal stage because of developmental programming considerations unless there is evidence that this information will not be relevant. Perinatal dosing will not be considered for the hybrid mouse

because it will be too difficult. The program has formulated study designs for *in utero* and lactational exposures. The NTP is now monitoring for troponin T in pre-chronic studies in rats at 3, 21 and 90 days.

BSC Discussion

Dr. Crump recalled that NTP had reported that studies with male rats and female mice capture most of the carcinogens in the animals and asked whether the NTP had considered only using these two groups in the 2-year bioassay. Dr. Bucher replied that this approach is considered routinely, but the design teams have been reluctant to adopt this strategy as the default, because sometimes only one gender responds and, thus, a probable or possible carcinogen might be missed. For IARC and the RoC, a response in two species of both genders is often needed.

Dr. Bucher thanked the BSC members for attending the meeting and for their useful advice, which will be considered in the NTP's future activities.