National Toxicology Program
Board of Scientific Counselors
July 23-24, 2009
National Institute of Environmental Health Sciences
Research Triangle Park, NC

Summary Minutes
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I. Attendees

Tracie Bunton, Eicarte LLC
Edward Carney, The Dow Chemical Company
Russell Cattley, Amgen
Elaine Faustman, University of Washington
George Friedman-Jimenez, New York University School of Medicine
William Janzen, The University of North Carolina at Chapel Hill
Stephen Looney, Medical College of Georgia
Mitzi Nagarkatti, University of South Carolina School of Medicine
Raymond Novak, Wayne State University
Michael Pino, Sanofi-Aventis
Kenneth Portier, American Cancer Society (Chair)
Jim Riviere, North Carolina State University
Diane Robins, University of Michigan Medical School
Ruthann Rudel, Silent Spring Institute
James Sherley, Boston Biomedical Research Institute
Gina Solomon, Natural Resources Defense Council

Members not in attendance:
David Eastmond, University of California
Justin Teeguarden, Pacific Northwest National Laboratory

National Institute of Environmental Health Sciences Staff

Scott Auerbach
Linda Birnbaum
Jack Bishop
Susan Borghoff
Chad Blystone
John Bucher
Po-Chuen Chan
Rajendra Chhabra
Bradley Collins
Michael Cunningham
Helen Cunny
Donna Fisher
Kristen Fisher
Paul Foster
John French
Dori Germolec
Veronica Godfrey
Claudine Gregorio
Rachel Gross
Marc Hollander
Wanda Holliday
Michelle Hooth
JoAnn Lewis
Ruth Lunn
Robin Mackar
David Malarkey
Joyce Martin
Scott Masten
Deborah McCarley
Minerva Mercado-Feliciano
John Pritchard
William Schrader
Barbara Shane
Robert Sills
Cynthia Smith
Diane Spencer
William Stokes
Christina Teng
Kristina Thayer
Raymond Tice
Molly Vallant
Suramya Waidyanatha
Nigel Walker
Lori White

Gloria Jahnke
Patrick Kirby
Grace Kissling
Sandy Lange
Kristine Witt
Mary Wolfe
Michael Wyde

Other Federal Agency Staff
Paul Howard, Food and Drug Administration (FDA)
Michael Luster, National Institute for Occupational Safety and Health (NIOSH)
Mark Toraason, NIOSH

Public
Andrew Ballard, BNA, Inc.
Donna Browning, RTI International
Neepa Choksi, ILS, Inc.
Reshan Fernando, RTI International
Marc Jackson, ILS, Inc.
Joseph Manuppello, PeTA
William Thompson, Information Ventures

II. Introductions and Welcome

The National Toxicology Program (NTP) Board of Scientific Counselors (BSC) met on July 23-24, 2009 at the Rodbell Auditorium, National Institute of Environmental Sciences (NIEHS), Research Triangle Park, North Carolina. Dr. Kenneth Portier served as chair. He welcomed everyone to the meeting and asked the BSC members and attendees to introduce themselves. Dr. Barbara Shane made a few announcements and read the conflict of interest statement. She noted that no conflicts of interest were identified.

III. Report to the NTP Board of Scientific Counselors

Dr. Linda Birnbaum, Director of the NIEHS and NTP, welcomed the BSC members and expressed her gratitude to them for their advice to the NTP on its activities. She provided information on personnel and structure of NIEHS, key scientific issues and activities within the Division of Extramural Research and Training (DERT).

a. Personnel and Structure of NIEHS

Since joining NIEHS, Dr. Birnbaum has met the staff and evaluated the Institute's programs for the future. There are four major leadership positions that will be filled in the near future: the Deputy Director, Director for the Division of Extramural Research and Training (DERT), Clinical Director and Scientific Director. NIEHS recently closed the search for an ethics officer. An announcement for an educational director to include K12, undergraduate and graduate students has been publicized. There will be searches for three liaisons to staff the NIEHS Bethesda office. She has made a commitment to increase the

Institute's interaction with federal partners and work with advocacy groups and stakeholders and respond to their needs. She is also establishing an MOU with EPA to share facilities.

Dr. Birnbaum mentioned a number of presentations she has made since her appointment at the Toxicology Forum, the Institute of Medicine Roundtable, the National Conversation on Public Health and Chemical Exposures, Green Chemistry Panel, and the 11th Annual Workshop on Brominated Flame Retardants. She recognized Dr. David Rall, a former director of the NIEHS, and her mentor.

She said that NIEHS, one of the 27 institutes and centers of NIH, is unique among the NIH institutes in that (1) it is located in RTP, NC and not in Bethesda, MD, (2) its mission focuses on public health (3) it administers the NIH's Superfund program and (4) the Director heads up both the NIEHS and NTP. The NTP as well as NIEHS now have a wonderful opportunity for research, as health and the environment are high priorities for the Obama administration. NIEHS' mission is public health as opposed to clinical medicine, the focus of the other institutes within NIH. NIEHS defines translation not as translation from the laboratory to the bedside but translation is bench to public health and bench to policy. NIEHS consists of three main Divisions – the Division of Intramural Research (DIR), DERT, and the NTP. DIR employs hundreds of scientists in 12 laboratories and branches who are conducting research in environmental health sciences and undertaking cutting edge basic research and epidemiology. The division is a highly interactive and interdisciplinary group conducting high risk and long-term research. DERT oversees the extramural funding program and NTP whose charge is to coordinate testing across the federal agencies, develop new methodologies for efficient and thorough toxicological assessments and communicate the toxicological data to the public.

Dr. Birnbaum noted that a new clinical research unit would open on July 27 where an array of research studies would be conducted involving human sample collection, analysis, and functional assessment.

Key Scientific Issues

Two major issues are the sensitivity of animals during development and early life stages and subsequent susceptibility, and dosimetry. Since healthy young adults are relatively insensitive to chemical perturbation, NTP's testing paradigm for long-term studies has changed so that exposures begin during gestation. As an organism ages it may show effects of *in utero* exposure resulting in differences in susceptibility. The genetic make-up of an animal also contributes to susceptibility. For example, there has been a possible association of low birth weight with the development of diabetes and cardiovascular diseases later in life.

It is important to accurately measure the administered dose, although it is perhaps more important to determine the internal dose of a toxicant. The internal

dose must be known for comparisons across species. She highlighted "low dose," which she defined as a low internal dose relevant to human exposure.

A major focus of the administration is nanomaterials and nanosafety and the President has asked for $9 million (M) for nanomaterials testing. The NTP and extramural program are concentrating in this area of research. NIEHS will receive the largest proportional increase in appropriations of all the NIH institutes with an increase of $32M in the budget.

Dr. Birnbaum does not believe that the National Academy of Sciences (NAS) report on Toxicology in the 21st Century covers all the issues relating to *in vitro* toxicology testing. Hence, she is considering having the NAS convene a panel to evaluate the implications of the data from short-term computational studies and how to link this information to effects in animals and people. NTP has a major interest in moving forward with its *in vitro* and high throughput computational approaches that are embedded in the EPA's Tox 21 program, but needs to know how to apply these data to public health.

She mentioned computational approaches that are being used in the Tox 21 partnership between EPA's National Center for Computational Toxicology and the National Human Research Genome Institute and the NTP. NTP formed this consortium to analyze the huge amount of data from the HTS program in order to obtain insight into cellular pathways and to understand systems biology and develop more predictive approaches to toxicology.

The current NTP research and testing areas are familiar to the BSC and differ from those areas that were studied ten or twenty years ago. Some of NTP's present foci are dietary supplements, herbal medicines and therapeutics that arose due to NTP's partnering with FDA. A similar partnering with NIOSH and CDC has led to more of an effort on occupational exposures and nanoscale materials. Dr. Birnbaum briefly discussed the development of nominations and how a decision is made on which chemicals or mixtures to study.

Activities within the Division of Extramural Research and Training
DERT receives the major proportion of the budget for NIEHS. Many of the BSC members are beneficiaries or grantees of the program. Currently, NIEHS funds 1200 grants across the country. Dr. Birnbaum highlighted some of the newer initiatives.

- The RFA for the Children's Environmental Health and Disease Prevention Centers Program is still out on the street.
- In partnership with the National Science Foundation, NIEHS is funding Centers for Ocean Health. NIEHS also has an interest in obesity and the built environment and is presently supporting grants in the area.
- NIEHS recently joined with an autism advocacy group "Autism Speaks" to fund a project at Drexel University School of Public Health in Philadelphia, known as the "Early Autism Risk Longitudinal Investigation." Researchers

are seeking 1,200 women who are mothers of children diagnosed with autism who are pregnant or planning to become pregnant with another child. Studying families already affected by autism will provide the best chance of learning how genetics and environmental factors might interact to cause this condition.

- The NIEHS sister study has recruited 51,000 sisters of women with breast cancer for a longitudinal prospective study in which they will be followed for the next 10 years. Physical examinations, follow-up questionnaires, and many hair and blood samples will be taken from these women. As the data becomes available they will be placed on the NIEHS website for investigators to mine and analyze.
- For American Recovery and Reinvestment Act (ARRA) funds, NIH was the recipient of $10.4 billion of which NIEHS received $187 million (M) with $20M charted for the Superfund Program. NIEHS may receive additional funds from the Office of the Director who held back about $800M for Challenge Grants. The Division of Extramural Research and Training (DERT) received 1800 grant proposals for ARRA grants. The success rate will be lower than usual because of the vast response. DERT is encouraging unfunded NIEHS grantees who submitted grants to the ARRA program, to revise and resubmit their grants if they receive meritorious reviews as there will be opportunities to submit them under the regular granting process. The short time interval of making the awards by the end of September has placed an enormous burden on DERT to complete the reviews. There will be oversight from the White House before the awards are made. Some of the ARRA funds have been awarded and there are indications that jobs are being saved and created fulfilling the focus of the program.

She thanked the BSC for their attention and service.

b. BSC Discussion

Dr. James Sherley hoped there would be closer interaction between NIEHS and NIH. He wondered if there is a parallel between the human genome project and determining the internal dose of a toxicant. He explained that the motivation for the human genome project is to reduce the price of sequencing individual genomes to about $1,000 so that predictive studies of gene vs. disease would be possible. In parallel, environmental health scientists could develop a profile of exposure history from samples of hair and blood sent by the public. He wondered if the internal dose effort actually includes a specific plan on how to get that information on a large-scale. Dr. Birnbaum replied that NIEHS is involved in such a project through the extramural program known as the “Genes and Environment Program,” which emanates from the director's office at NIH where NIEHS is focused on exposure issues. Initially the emphasis has been on the development of small portable sensors and the measurement of internal exposure. The next four to five years will focus on linking exposure with genotype.

Dr. Elaine Faustman asked about the NAS tackling the issue of the application of toxicity testing. Dr. Birnbaum replied that she has not formulated her approach yet and would be interested in feedback from the BSC. Some of the NTP studies are excellent and well done, but the program needs to ask whether the modified 2-year bioassay is the best approach to obtaining information on protecting public health.

Dr. Mark Toraason asked how the NTP would link the high throughput screening (HTS) data to human health because he cannot see a link between the *in vitro* data and public health. Dr. Birnbaum replied that it is important to understand how the mechanistic data from the computational approaches can be linked with *in vivo* events. The *in vitro* computational approaches will be useless unless one can understand their relevance and predictive power. If one can understand the early steps of complex pathways that lead to adverse outcomes one can either intervene or prevent specific exposures. She has explained to her colleagues at NIH that it is better to prevent a disease from occurring than to develop a treatment or intervene in the development of a disease. With environmental health, there is the opportunity for prevention.

Dr. Kenneth Portier asked about NTP's role in communicating environmental risk to the public, as the public often reacts dramatically to a scientific finding even if there is a very low risk or little concern regarding exposure. Dr. Birnbaum replied that this is really an important issue and risk communication is a science. DERT has programs to encourage extramural scientists to improve science communication and to communicate risk. Part of the problem is that good news does not make news. One approach is to develop relationships with the press and build trust so they consult with NIEHS to explain a specific issue. She is trying to increase interaction between and across scientists at NIEHS whether they are in the intramural program, are funded grantees or in the NTP. Funded centers and large program projects are involved in public environmental health partnerships with local community groups to ascertain their needs and concerns and to consult with them about the studies NIEHS supports. Some of the studies within NTP have resulted from consultation with community groups.

Dr. Portier asked about NIEHS's participation in social networking and social media where communication and outcomes are rapid as a large percentage of the population participates in these interactive media. The logical approaches that governmental agencies have used in the past do not connect with these interactive environments. Dr. Birnbaum replied under the ARRA program, NIEHS is communicating with the public on the Twitter site. NIEHS is working hard to ensure that their Internet sites are helpful, easily searchable, and appropriately linked. It is important the government adopt these newer communication models in which the young generation participate.

Dr. Paul Howard applauded Dr. Birnbaum's efforts to reach out to the public and scientists at large and noted that regulatory agencies depend on the data generated by NTP and NIEHS. Regulatory science can only improve if federal agencies are involved intimately with NTP.

Dr. Gina Solomon said she appreciated that NTP is the "one-stop shop" for toxicity testing in the federal government. She was concerned that potential changes in TSCA might result in competition for NTP and whether NTP could take the lead in the implementation of changes in this law. Dr. Birnbaum replied that NTP is not the only governmental agency doing toxicity testing. Rather NTP's legislative mandate is to coordinate toxicity testing across the government. The best way to accomplish that goal is by cooperation, collaboration, and working to inform NTP's sister agencies.

Dr. Solomon asked about NIEHS' role in studying climate change. Dr. Birnbaum replied that NIEHS and the Fogarty International Center are taking the lead for NIH to study the health effects of climate change, which is a high priority agenda item for NIEHS. Dr. Christopher Portier, NIEHS, was asked to take the lead to develop a cross-federal-agency white paper by the end of summer on the factors related to the potential health effects of climate change. Most of the focus has been on whether climate change alters the biology of insects connected with vector-borne diseases such as malaria. NIEHS is also interested in increased flooding and hence mold contamination following further warming of the planet. A number of proposals under ARRA are likely to be funded on climate change.

Dr. Raymond Novak commended Dr. Birnbaum on her acknowledgement of team science and its importance in future research particularly as it pertains to public health.

Ms. Ruthann Rudel said there appears to be a number of initiatives on how to improve the utility of toxicity data for risk-management and decision-making. The recent NAS report on "Science and Decisions" and an initiative in California suggest that thought be given on the amount of information needed to make specific policy decisions before a study is initiated. Exposure is an area that has not been well funded and needs to be better understood. It is essential to understand the relationship between the source of a pollutant and its effect on an organism to plan an intervention strategy. She asked whether NIEHS plans to fund or participate in studies on exposure measurements. Dr. Birnbaum replied that a key issue is to understand how an organism is exposed. NIEHS is co-funding an NAS committee with EPA to evaluate exposure pathways. NIEHS is not involved in risk assessment but rather hazard identification. She believes that the U.S. spends too much time focusing on quantifying uncertainty and small risks instead of identifying hazards, determining alternatives, and arriving at a solution. Perhaps a new paradigm will begin to evolve over the next couple of years.

IV. NTP Update

a. Presentation

Dr. John Bucher welcomed the members of the committee and thanked them for their input at the meeting. He discussed staffing changes, recent meetings, and selected program initiatives.

(i) Personnel

Since the last meeting, there have been a number of staff changes reflecting Dr. Birnbaum's commitment of reinvigorating the NTP. He welcomed Dr. Michael Devito, a well-established scientist at EPA, and a number of post-doctoral candidates in various parts of the program. There are active searches for a number of other positions as well as additional post-doctoral candidates.

(ii) Meetings

An expert panel meeting for glass wool was held on May 9-10, 2009, one of the candidate substances being reviewed for the 12th Report on Carcinogens (RoC). A panel meeting for formaldehyde is scheduled on November 2-4, 2009. This will complete the panel meetings for the 12th RoC. The substance profiles for these two nominations and cobalt-tungsten carbide powders and hard metals will be brought to the BSC in June 2010.

A Technical Report Review Subcommittee meeting will be held on November 18-19, 2009, to review the reports on a number of herbals and other substances.

(iii) Selected Program Initiatives

Evaluation criteria for non-cancer studies

The NTP established a new level of evidence criteria for non-cancer endpoints including reproductive, developmental, and immunotoxicology studies. This was accomplished by holding two pathology workshops to discuss the approach to be used to analyze pathology and two working groups with selected members of the BSC and *ad hoc* experts to develop the criteria. The BSC accepted the criteria of the working groups at its meeting on November 20, 2008. The criteria were revised in January 2009 and unveiled at the Society of Toxicology Meeting held March 15-19, 2009. The first application of the criteria on immunotoxicity studies will be on resveratrol in Winter of 2010. Drs. Dori Germolec and Paul Foster were instrumental in compiling these criteria for non-cancer endpoints. Similar to the wording of the carcinogenicity studies the conclusions will begin with "Under the conditions of this study…" This has given the NTP more leeway in describing the findings, but the NTP is confident that by applying mechanistic toxicology tools, the program will generate the scientific information and understanding necessary for public health decision-makers to use to reduce the burden of environmental disease.

Now the NTP needs to go beyond this statement "under conditions of the study". The program has a responsibility to post all the preliminary and completed data

generated through the NTP HTS and genomics programs on its website in a format that can be used by the public, and the NTP needs to provide a context for the data and be able to explain the importance of the results. This activity will require considerable amount of internal discussions to formalize the context of the studies and will involve the BSC and the Executive Committee. This is going to be an exceptionally difficult challenge for the program in the coming years. It is conceivable that the organizational structure of the NTP and programmatic expectations will have to be changed to implement such an activity.

It is hoped that through sustained leadership, and creating and applying mechanistic toxicology tools, the NTP will generate the scientific information and understanding necessary for public health decision-makers to use to reduce the burden of environmental disease.

NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICETAM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

NICETAM and ICCVAM have their own science advisory panel known as the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM); hence, NTP does not discuss their activities often at BSC meetings. Dr. Bucher outlined the accomplishments of NICEATM and ICCVAM and said that federal agencies adopted and/or endorsed 9 alternative test methods in 2008-09.

He said an implementation plan was developed and published for establishing a NICEATM-ICCVAM Five-Year Plan (2008-2012) to help federal agencies identify and validate alternative toxicological methods. An interagency working group was established within ICCVAM to oversee the implementation of the plan, identify promising assays, and develop a mechanism to promote methodologies to validate these assays for regulatory use. An international validation study is underway for endocrine disruptor transcriptional activation assays.

An International Memorandum of Cooperation (MOC) was signed at NIH on April 27, 2009, by Dr. Birnbaum, representatives from the National Institute of Health Sciences in Japan and the Institute of Health and Consumer Protection Joint Research Centre in Europe, and the Director, Health and Safety Bureau of Health Canada. The objective of the MOC is to coordinate the validation of assays worldwide to prevent the duplication of efforts among the different agencies involved in *in vitro* testing to speed up the adoption of new methods.

Update on the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) Review On Bisphenol A

The BSC peer reviewed the draft NTP Brief on Bisphenol A (BPA) on June 11, 2008, and the final NTP-CERHR Monograph on BPA was issued on September 3, 2008. The monograph contains the NTP Brief, the CERHR Expert Panel Report, public comments and the peer review report on BPA. The conclusions were *some concern* for effects on brain, behavior, and prostate gland, and

minimal concern for effects on the mammary gland and an earlier age for puberty in female fetuses, infants, and children at current human exposures.

The NTP made a presentation to the FDA Science Board Subcommittee on September 16, 2008. In response to the Science Board's recommendations, FDA responded on February 24, 2009, and agreed to address the concerns of the Science Board. Several of the research recommendations made to the Science Board are being addressed by NTP in conjunction with NCTR.

The NTP and DERT issued a request for information (ROI) October 20, 2008, to the academic community asking for their input on data gaps and their needs to study the biological effects of exposure to BPA. The NTP believes that addressing data gaps for BPA is important; however, before proceeding with studies to address research needs, the NTP wanted to understand what studies are underway or being planned by the extramural community. NTP received a number of responses on data gaps including the development of PBPK models for BPA in rodents and non- human primates, and the evaluation of long-term outcomes from perinatal exposure. There is an effort with the extramural community to share tissues and information. Dr. Bucher published an editorial in the March 2009 EHP entitled "Bisphenol A: Where to Now?"

Dr. Bucher indicated how the ARRA funds presented an opportunity for the extramural community to study the impact of BPA on human health. This is a 2-year award, with $5 M available for grants; to date 41 applications have been received. The thrust of the grants is to provide data in strategic areas to address existing experimental limitations on developmental exposure to low doses of BPA in both animals and human.

A NIH/FDA BPA Task Force has been created to survey existing epidemiology studies relating to BPA and to determine if tissues or samples that have been collected in other studies might be useful in relating early life exposure to BPA to subsequent outcomes. Three NIEHS Children's Centers, at Mt. Sinai, Columbia University and the University of California, Berkeley, are measuring BPA in urine and blood along with neurodevelopment and growth outcomes in longitudinal birth cohort studies. The task force is also reviewing analytical methodologies to measure BPA.

BPA was evaluated as part of EPA's ToxCast Phase I, a program for assessing the data from a large number of chemicals tested in many HTS assays. In the first phase of ToxCast, 467 biochemical and cell-based assays using 9 different technologies were used. BPA was found to be one of the most active substances in ~100 endpoints. Data from this study will be published in a manuscript in preparation.

Dr. Bucher outlined four other activities related to BPA. Senator Schumer announced the BPA-Free Kids Act on March 30, 2009, which prohibits the sale of

BPA containing food and beverage containers for infants and children. The Act also directs NIEHS to begin a 5-year research initiative to understand health effects of BPA. Twenty-four states have pending legislation restricting the use of BPA in many products. FDA Commissioner Hamburg announced a re-assessment of BPA by FDA. California EPA held a hearing to determine whether to list BPA under Proposition 65 and decided that at present it not be listed.

b. BSC Discussion

Dr. Mark Toraason asked whether the banning of BPA would impact continuing research since public health decisions have been made about BPA. Dr. Bucher replied that the information collected from proposed epidemiology studies, PBPK models, and relative levels of BPA in humans and animal would aid in understanding its internal dosimetry. If NTP can obtain an understanding of the potential hazards of BPA in short term studies, it may be possible to truncate the longer research programs. However, the important question of the fetal basis of adult disease needs to be addressed even though it may not affect regulation. Dr. Birnbaum added that although BPA does not bioaccumulate, humans are continuously exposed through BPA - lined containers.

Dr. Portier was interested in how the NTP plans to manage and analyze the huge amount of data from the HTS program and what the implications are for its use by federal agencies. Dr. Bucher replied that it would be important for the NTP to understand and explain what the data mean. The program has had difficulties discerning a positive from a negative response even when a dose-response relationship has been determined. NTP will post all the data on a public website for anyone to analyze.

Dr. Edward Carney was pleased that the NTP is willing to consider other aspects of the data from the non-cancer and cancer studies and has not fixed boundaries around the results by only stating “under the conditions of.” He was referring to the discussion on the criteria for the non-cancer endpoints by the working group, who wanted to include dose in the conclusion statement. Dr. Bucher replied that the conclusions from technical reports and non-cancer studies would be as specific as they have always been. However, the NTP is considering the inclusion of an additional section in the reports that contains information on the specific chemical in relation to similar chemicals and their tumor outcomes, and how this may relate to humans. Dr. Birnbaum added that these are some of the issues that the NAS might be asked to address.

Dr. Sherley was in favor of the scientific data being reported accurately in the complicated technical reports. He understood the motivation of adding additional information to give the public some context, but it is important that the scientific findings are not diminished in the interest of providing the public an explanation of the data.

Dr. Birnbaum concluded the discussion by mentioning that the Korean Center for Alternatives would like to join the MOU signed by NIEHS with the other overseas organizations. She added that there is bill in the House for $15M on endocrine disruptors in which the NTP will play a major role. She hopes it will not be written in proscriptive language so that the NTP is not restricted in what can be studied.

V. Report from the Technical Reports Review Subcommittee

a. Presentation

Dr. Novak, Chair of the NTP BSC Technical Reports Review Subcommittee ("the Subcommittee"), summarized the actions on the Draft NTP Technical Reports from the peer review meeting held on February 25, 2009. Before he began his report, he acknowledged a letter the BSC received the day before the meeting from Mr. Michael McGuffin, the President of the American Herbal Products Association with a number of requests relating to the goldenseal report. These included straightforward editorial changes and a discussion on the dose used in relation to human consumption as well as comments on historical controls. Dr. Novak said that in his opinion it is very difficult to gauge the average consumption of a product by the population as a whole, as some people might consume more that that which is recommended. Regarding the incidence of the findings, he referred to Dr. Bucher's comments that the conclusions of the study were obtained "under the conditions of the study." The historical controls are within a five-year period to maintain stringency of the comparison; beyond that time period other factors such as diet and genetic drift can modify the historical control incidences.

The Subcommittee reviewed the findings and conclusions from studies of goldenseal root powder, androstenedione, 2,3',4,4',5-pentachlorobiphenyl (PCB 118), 3,3',4,4'- tetrachloroazobenzene, β-myrcene and tetralin that used conventional F344 rat and B6C3F$_1$ mouse models.

- The Subcommittee voted unanimously 8 yes, 0 no, and 0 abstentions in favor of the conclusions that there was *clear evidence* of carcinogenic activity of goldenseal root powder in male F344/N rats based upon hepatocellular adenoma and hepatocellular adenoma /or carcinoma (combined), *clear evidence* in female F344/N rats based on hepatocellular adenomas, *some evidence* in male B6C3F1 mice based upon hepatoblastoma and multiple hepatocellular adenoma and *no evidence* female B6C3F1 mice.
- The Subcommittee voted unanimously 8 yes, 0 no, and 0 abstentions in favor of the conclusions that there was *equivocal evidence* of carcinogenic activity of androstenedione in male F344/N rats based on alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined) and *equivocal evidence* in F344/N female rats based on mononuclear cell leukemia and *clear evidence* in male and female B6C3F1 mice based on liver neoplasms (multiple adenoma and carcinoma and hepatoblastoma) in male mice and hepatocellular adenoma and hepatocellular carcinoma in female mice.

- The Subcommittee voted 7 yes, 1 no, and 0 abstentions in favor of the conclusions that there was there was *clear evidence* of carcinogenic activity of 2,3',4,4',5-pentachlorobiphenyl (PCB 118) in female Harlan Sprague-Dawley rats based on liver neoplasms (cholangiocarcinoma, hepatocholangioma, hepatocellular adenoma) and cystic keratinizing epithelioma of the lung.
- The Subcommittee voted unanimously 8 yes, 0 no, and 0 abstention in favor of the conclusions that there was *clear evidence* of carcinogenic activity of 3,3',4,4'- tetrachloroazobenzene in male Harlan Sprague-Dawley rats due to cystic keratinizing epithelioma of the lung, cholangiocarcinoma of the liver, and gingival squamous cell carcinoma, *clear evidence* in female Harlan Sprague-Dawley rats based on cystic keratinizing epithelioma of the lung, and gingival squamous cell carcinoma, *clear evidence* of carcinogenic activity in male B6C3F1 mice based on transitional epithelial gland carcinoma of the urethra, and alveolar/bronchiolar neoplasms of the lung and *clear evidence* of carcinogenic activity in female B6C3F1 mice based on fibrosarcoma and fibrosarcoma or malignant schwannoma (combined) of the skin.
- The Subcommittee voted unanimously 8 yes, 0 no, 0 abstentions in favor of the conclusions that there was *clear evidence* of carcinogenic activity of β-myrcene in male F344/N rats based on renal tubule neoplasms, *equivocal evidence* in female F344/N rats based on renal tubule adenoma, *clear evidence* in male B6C3F1 mice based on hepatocellular adenoma, hepatocellular carcinoma and hepatoblastoma and *equivocal evidence* in female B6C3F1 mice based on hepatocellular adenoma and hepatocellular carcinoma.
- The Subcommittee voted 7 yes, 1 no, 0 abstentions in favor of the conclusions that there was *some evidence* of carcinogenic activity of tetralin in male F344/N rats based on renal tubule adenoma, *some evidence* in female F344/N rats based on hepatocellular neoplasms (adenoma and adenoma or carcinoma, combined) and uterine stromal polyps, no *evidence in* male B6C3F1 mice and *equivocal evidence* in female B6C3F1 mice based on splenic hemangiosarcoma.

b. Board Discussion

Dr. Sherley commented on the letter from Mr. McGuffin. He said he was not sure what the process is for addressing the comments after the TRRS meeting, but he thought the comments were not germane to the discussion. The public comments requested that the NTP change its standard language regarding the conclusions. Dr. Bucher reminded the BSC that the TRRS recommendations to the NTP are not official and cannot be received by the NTP until they are ratified by the BSC. Many of the changes that were requested in the public comments at the TRRS meeting are in the process of being added to the goldenseal report. He said the final technical reports are not being presented today for the BSC's consideration, but rather the BSC is asked to ratify the subcommittee's recommendations that describe the actions on which they voted. If a member of

the BSC wishes to address any issues raised in this new public comment from Mr. McGuffin that may impact the deliberations of the TRRS, then it can be discussed now. The BSC had no comments on this topic.

Dr. Howard commented on the daily in take of goldenseal, which, as discussed at the subcommittee meeting, is only an estimate of the daily intake. It would be very difficult to determine the actual intake of the public. Dr. Portier said the only comment on the recently obtained submission on goldenseal was the discussion of historical controls. The NTP provided a clear justification for using a five-year period for these controls and, he saw no reason for any change.

Questions were raised regarding the wording of the conclusions in the technical reports. Dr. Faustman asked why in some cases the conclusions specifically state that there is evidence of a specific tumor associated with a specific exposure whereas in others the conclusions state that the tumor is related to administration. She wondered about the reason for the subtle differences in wording. Dr. Nigel Walker replied that administration is related to the route, site, or mode while exposure is not. He agreed that there are inconsistencies in the reports regarding the description of how the animals were exposed. Dr. Bucher said the conclusions for all studies are qualified by the statement “under conditions of this study.” The results of each report are specific for the conditions of the particular exposure of the animals. Dr. Walker added that differences also result from different study scientists authoring the reports. In the case of PCB 118, the findings were related to exposure, not administration. Dr. Faustman suggested that the wording be more crisp and consistent across all the reports.

Both Dr. Faustman and Dr. Solomon then asked for clarification on the wording regarding the calls in the conclusions. Dr. Walker discussed how the calls are made. He said that a determination of the classification of tumor(s) with the highest level of evidence is made first. The wording used is “clear, some, equivocal or no” evidence. Thus, when there is “clear evidence”, it is based on the highest call, when the finding is based on lesser evidence, the program uses “ some evidence” etc. Then the other tumors in the same species and sex are classified. When there is evidence at the secondary level of another tumor in the same group of animals the terms used are “may have been related” as was the situation with PCB 118 or “considered to be related”. The report does not state “ clear evidence” for a specific tumor and “some evidence” for another tumor, and “equivocal evidence” of a third tumor in the same sex and species.

Both Dr. Faustman and Dr. Solomon then asked for further clarification on the wording of the PCB118 conclusions. They asked why there is “clear evidence of carcinogenic activity” due to neoplasms of the liver and lung but that carcinoma of the uterus is “considered to be related” to the administration of PCB118 rather than “clear evidence” since the uterine neoplasms were also related to the administration of PCB118. She noted that Dr. Pino asked the same question at the subcommittee meeting. Dr. Walker replied that when the NTP looked at all

the data combined, *the program felt that the strongest level of effect was seen with the hepatocellular and pulmonary neoplasms resulting in a call of "clear evidence." The strength of the evidence was less for the occurrences of carcinoma of the uterus so these were not included as part of the "clear evidence" call but nonetheless they were considered to be related to treatment with PCB118.* An "equivocal" call is used when there are marginal increases but the data is not clear. If the equivocal call is not the primary call, then language such as "may have been related" is used. Dr. Walker confirmed Dr. Faustman's query that if the overall call had only been "some evidence" based on the hepatic and lung tumors, then the finding in the uterus would have been included as part of the call.

Dr. Portier added that the TRRS discussed the same classifications at the meeting. NTP uses this codified language to describe the levels of the specific neoplasms.

Dr. Jim Riviere suggested that the NTP include the description of how the calls are made to the Subcommittee and the entire BSC when they are ratifying the minutes of the Subcommittee findings. Dr. Walker replied that the reports contain a preamble that discusses how the levels of evidence and the strength of evidence for individual technical reports are decided. Dr. Birnbaum replied that in future this information would be provided to the BSC.

Dr. Portier thought that the NTP could add those explanations as an appendix to the minutes of the subcommittee meeting and Dr. Faustman agreed that such an addition would be helpful.

Dr. Portier asked for a motion from the BSC to accept or reject the subcommittee minutes. Dr. Michael Pino made a motion to accept the report and Dr. Riviere seconded it. The motion passed unanimously with 15 yes votes, 0 no votes, and 0 abstentions..

VI. Nominations to the Testing Program

A. Introduction

a. Presentation

Dr. Scott Masten, NIEHS, provided background information on the nomination process. Substances are selected for study based on their known or anticipated human exposure, production level, suspicion of toxicity based on structure or existing health effects data, availability of adequate toxicological data, public concern, and the utility of additional studies for public health decision-making. There are multiple levels of review of nominations to determine merit and priority that can be found at http://ntp.niehs.nih.gov/go/156.

There are five draft research concepts for review: deoxynivalenol, alkylanilines, Dong quai, indium tin oxide, *p*-chlorobenzotrifluoride, tris(4-

chlorophenyl)methane and tris(4-chlorophenyl)-methanol. Each research concept outlines key issues, data gaps, and hypotheses, and/or specific aims that the program plans to address.

The charge to the BSC is to determine whether sufficient justification is provided for the use of the NTP's resources to carry out the proposed research projects as outlined in the draft research concepts. The BSC is asked to comment on the clarity and validity of the rationale for the proposed research program, the merit of the program relative to the goals of the NTP, the scope of the proposed program and its appropriateness relative to the public health importance of the issue under study, and the priority of the proposed research program.

b. BSC Discussion

Dr. Portier asked whether the interagency review committee considers public concern of a substance when considering nominations. Dr. Masten replied that public concern is as an important a criterion as is high production volume, or the known toxicity of a chemical, but it is the one factor that is the most difficult to quantify. Google News has a new feature "Google Trends" which tracks the number of news articles published on a particular topic and can serve as a proxy for public concern when there is wide media coverage on a chemical of public interest. Sometimes a nomination emanates from the public.

Dr. Faustman asked about NTP's integration with international programs and whether there is a contact person in the European Union who NTP can contact to find out what their needs are with respect to REACH. Also, since NIEHS has an international coordination effort on the development of new *in vitro* test methods, she asked if NTP reaches out to the international community for nominations. Dr. Masten replied that many of the international organizations have participation from U.S. organizations. Often staff from one of the NTP's federal agency partners has liaised with them or they are aware of overseas activities. Some NTP research programs such as the concept on DON was developed in response to uncertainties and data needs that were identified as a result of a WHO evaluation. NTP has an open line of communication with a former NIEHS employee who now serves as the executive secretary of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)**,** a food additive evaluation activity under the International Programme on Chemical Safety.

Dr. Faustman questioned the use of Google because she believes that the public frequently is unaware of a chemical's potential toxicity. She asked if NTP has specifically approached various public groups and their membership regarding nominations. Dr. Masten replied that it is difficult to determine the public's knowledge of chemical hazards and their concerns. He does monitor reports from advocacy groups, but the NTP might be able to do better to engage those stakeholders.

Dr. Faustman suggested that the NTP work to raise its visibility, as many people are unaware of the program's amazing resources and the unique opportunity they have to provide nominations. Even if they do not know what chemicals they want tested now, it is important they are aware of the resource.

Dr. Solomon said she was impressed with the research concepts, their summaries and the thought that was put into the scientific information that is needed. She wondered if these chemicals, planned for testing by NTP, might be tested by other entities, such as a commercial entity that is responsible for manufacturing the chemical. NTP could have a role in laying out the direction for the research. It would help to leverage the resources so that even more testing could be done. Dr. Masten replied that this is not an approach the NTP has considered or planned. He appreciated her comment that she thought highly enough of the concepts that she would suggest that other entities could use NTP guidance to test a chemical. Dr. Birnbaum said this suggestion is an interesting idea but beyond NTP's resource capability. Clearly the role of the NTP is to coordinate toxicity testing. Dr. Solomon's approach could be considered but it would depend on legislative mandates. By law, the requirements for testing are very specific for producers.

Dr. Toraason said that about a decade ago NIOSH thought about publicizing their agenda for study and asking other groups to join with them in conducting research on a particular substance. The public responded that it was an unfunded mandate that created many problems, because there was no money set aside for such studies.

Dr. Solomon clarified her remark by saying that the producers would still be doing the testing but following a protocol outlined by NTP rather than developing their own protocol.

Dr. Toraason commented on the international component. About a decade ago the belief was that if it is not a U.S. problem, it is not a problem. He wonders if U.S. taxpayer money should be used to address a problem in another country. However, NIOSH is concerned about exposure to indium tin oxide in Asia even though it is uncertain whether or not or there are exposures in the U.S.
Dr. Birnbaum said that the environment is global and problems in other countries become international problems. Under the current administration, there is a move to understand the global effect of chemicals in the environment. There is no reason that NTP should not test a chemical if there are concerns even if the known exposure is not local. Dr. Masten added that even if the primary production of ITO is in Asia and there is occupational exposure there, products incorporating ITO are in virtually every U.S. household. The concept of the whole life cycle must be considered, as someone has to ultimately dismantle the electronic equipment and ITO will end up in our waste streams.

Dr. Howard responded to the various issues raised. He said it is important to consider Dr. Birnbaum's remarks about a changing market place and exposures. It is necessary for the U.S. to gain an understanding of materials that are not manufactured in the U.S. but which might have considerable health importance. He responded to Dr. Solomon and said that the regulated industry is aware of NTP's specification for studies and how they are conducted. If industry plans to market a product, they are aware of the safety paradigm. U.S. federal agencies such as EPA and FDA are in contact with international agencies, hence, nominations from overseas do occur. The public awareness and their concern may launch the development of a nomination. At the ICCEC meeting, the scientific information presented in the background document provides the justification and the driving force for moving forward with a nomination although public concern is considered.

Dr. Mitzi Nagarkatti asked about the NTP mission and goals on which the BSC was asked to judge the merit of the concept and how important the coordination of the toxicology program across DHHS is. Dr. Masten replied that this is the least important of the NTP criteria for the BSC to consider in evaluating the concepts, the most important being to strengthen the science base and provide information on hazardous substances. Coordination across the federal government is accomplished through the NTP's various interagency committees.

B. Deoxynivalenol

a. Presentation

Dr. Dori Germolec, NIEHS, presented the draft research concept for deoxynivalenol (DON), a trichothecene mycotoxin produced by certain Fusarium species on corn, and other grains. Elevated levels of DON are found on grains during periods of increased rainfall or when the mold is stressed under high temperatures.

DON was nominated by the NIEHS for chronic toxicity, carcinogenicity and reproductive toxicity studies based on its potential to contaminate human foods, demonstrated toxicological activity, lack of definitive long-term studies and defined data gaps regarding its toxicity. Because DON is extremely stable during processing at high temperatures, it can contaminate edible grains. Acute toxicity is manifest as gastrointestinal distress.

NTP is collaborating with Dr. Jane Hoppin, NIEHS, and Dr. Donald Beezhold, NIOSH, to evaluate the allergic sensitization of a subset of farmers from the NIEHS Agricultural Health Study. Serum samples from 700 farmers are being screened for total IgE, mold-specific IgEs and DON levels to assess whether these parameters are associated with adverse respiratory outcomes, allergic disease and neurologic function.

A few published epidemiology studies from China indicated that elevated DON levels were found in the urine of people living in several areas with high

esophageal cancer rates although most studies correlated grain intake with urinary levels of DON rather than with specific disease risk.

DON is not mutagenic in bacteria or in *in vitro* assays in mammalian cells although it appears to induce DNA damage or chromosomal aberrations in *in vitro* assays in mammalian cells. Short-term and subchronic exposure of rats and mice to DON resulted in decreased body weight, decreased weight gain and decreased feed consumption and histological alteration in the gastrointestinal tract and spleen.

DON is a reproductive toxicant in male mice and rats causing decreased epididymal weight in mice, and epididymal and seminal vesicle weights in rats. DON reduced the pregnancy rate in Sprague Dawley rats and the numbers of live pups in a study with Swiss Webster mice. DON is a developmental toxicant in Sprague Dawley rats and Swiss Webster mice causing skeletal anomalies. Interpretation of these latter studies is difficult because the teratogenic effects in the fetus may be related to the gastrointestinal effects in the dams.

In a 2-year carcinogenicity study, both male and female B6C3F1 mice lost a significant amount of weight when animals were exposed via the feed. A dose-related decrease in preneoplastic and neoplastic hepatic lesions was found in males, which was attributed to reduced caloric intake.

Since humans may be exposed to a variety of tricothecene mycotoxins in the diet, the establishment of toxic equivalency factors (TEFs) may be important in assessing cumulative risk of mycotoxins.

The overall goal of these studies is to characterize the reproductive, chronic toxicity and carcinogenicity of DON following oral exposure using a tiered approach. The first tier is divided into three specific aims. The first aim will be to conduct toxicokinetic studies in Harlan Sprague Dawley (SD) rats and B6C3F1 mice. The second aim will be to conduct prechronic dosed feed toxicity studies in the SD rat to obtain information for dose setting for the reproductive and developmental toxicity study, chronic toxicity studies and to provide data needed to set TEF values for DON. The third aim will determine if exposure to DON induces chromosomal damage *in vivo*.

In Tier 2 a guideline reproductive toxicity study will be conducted to examine the effects of DON on fertility and fecundity in SD rats. Under this tier a 2-year dosed feed chronic toxicity and carcinogenicity study will be conducted in SD rats.

The proposed studies will address the data needs for DON including the need for a cancer study in a second species as suggested by the Joint FAO/WHO Expert Committee on Food Additives and the European Commission's Scientific Committee on Food, and studies of the effects on reproduction in males. These

definitive studies will provide necessary information for evaluating the cumulative risk from exposure to DON and other tricothecene mycotoxins. Additional toxicology studies may identify more sensitive endpoints relative to human exposures.

b. Public Comment

Mr. Joseph Manuppello, PETA, said DON is regulated in 37 countries at levels lower than exposure of the general population and suggested that one needs to control exposure rather than identifying more sensitive human endpoints.

c. BSC Discussion

Dr. Sherley asked whether there are follow-up data on humans with gastrointestinal symptoms, and Dr. Germolec replied there are no chronic studies except for those on esophageal cancer. Most epidemiological studies have specifically studied fumonosin and esophageal cancer rates rather than DON and disease.

Dr. Nagarkatti, the first BSC discussant, said the rationale for the proposed studies is well described and clear. The widespread contamination of foods such as grains with ***Fusarium*** species producing DON and the epidemiological findings necessitates an extensive and systematic evaluation of its toxicity. The studies will also address the recommendations of the Joint FAO/WHO Expert Committee on Food Additives and lead to development of Toxic Equivalency Factors (TEFs) for DON and other mycotoxins with varying toxic potencies. This project has significant merit in terms of the mission and goals of the NTP.

Dr. Nagakatti approved of the division of the testing protocol into two tiers. It was unclear why rodents would be dosed via the feed as earlier studies were inconclusive because mice consumed low amounts of feed and thus received lower doses of DON. She suggested that oral gavage would be more appropriate. She agreed with the testing of Harlan Sprague Dawley rats and not mice in the chronic and developmental and reproductive toxicity studies. Since inhalation or dermal exposure to DON may occur, inclusion of these routes of exposure might be useful to determine both systemic and local effects in the lungs and skin. She assigned DON a moderate to high priority for testing and said that overall, the studies are significant from the public health perspective and DON merits testing.

Dr. Russell Cattley, the second BSC discussant, said the rationale for the project was laid out clearly. In humans, known toxicity is limited to acute effects resulting in gastrointestinal symptoms while in animals, DON causes reproductive, developmental, and immune system toxicity, but not carcinogenicity in the mouse. A data gap is the lack of information on the effect of DON on functional reproductive capacity. Although guidelines allow for low-level contamination of grains by DON, it is unlikely that humans are exposed to toxic

levels from chronic exposure but further information on this possibility would be significant.

The overall significance and public health impact of this research program is low to moderate. Sufficient information exists to support FDA's advisory guidelines for DON concentrations in finished wheat for human consumption. Similar guidelines exist in several other countries.

He suggested that if studies are implemented that the high throughput screening (HTS) program be included in the first tier as it would aid in identifying key molecular targets, signaling pathways and the potency of DON and would allow for a comparison of DON with other trichothecene mycotoxins as they elicit different toxic effects. If the toxicokinetic assessment indicates a similar sensitivity of rats and mice to DON, a 2-year rat study could be abandoned or given a low priority.

Dr. Germolec replied that the plan is to administer DON by gavage to lessen the acute toxic effects and avoid food aversion, and because this route would be more relevant to human exposure. Intravenous administration is used for toxicokinetic studies and metabolite profiles. NTP is procuring mycotoxins and different mold extracts for testing in the HTS program.

Dr. Faustman said an important facet to the experimental design is the possible weight loss of exposed animals and thus control animals will have to be fed a restricted diet to mimic weight loss of exposed groups.

The BSC discussed the issue of low chronic exposure to DON, the approach for the TEF studies and whether reproductive studies are warranted. Dr. Solomon wondered whether a Dutch study where 80% of the diet of one-year old children contained levels of DON that exceeded the total daily intake was an anomaly. Dr. Germolec replied that although children consume more cereal than adults they do not have gastrointestinal effects. She added that there has not been a long-term follow up on this study and Holland probably uses EU wide regulations for allowable levels of DON in foods, which would likely be stricter than the U.S. Dr. Nagakatti thought that people who consume large amounts of cereal might ingest more than 1ppm of DON on a daily basis even if each cereal component contains less than 1ppm. Dr. Germolec said the levels of DON are not truly regulated in the U.S. but limits in grains are managed and there is no U.S. acceptable daily intake (ADI) for DON. Dr. Masten added that FDA regulates DON by providing an advisory level of 1ppm in grains but this is not enforceable. If new data shows an effect at less than 1ppm, the total daily intake (TDI) could be lowered. Dr. Howard added that any commodity that contains more than 1ppm of DON is considered an adulterated product and it is illegal to market it in the US.

Dr. Faustman wondered how the NTP plans to analyze data for the TEF because as mentioned by Drs. Cattley and Howard mycotoxins have diverse effects. Dr. Howard suggested that NTP coordinate its studies on TEF's for mycotoxins with regulatory agencies so they can determine how they might use the data in a regulatory context. Mr. William Janzen asked if purified or semi-purified mold extracts would be tested in selective screens in the HTS and Dr. Germolec replied general screens would be used, as a targeted panel of assays has not been developed for mycotoxins.

Dr. Carney gave the reproductive toxicity studies a low priority. Because DON is regulated at low levels he does not anticipate that a high dose 2-generational study to assess fertility, is warranted. Also, he was unsure how these additional studies could separate systemic effects from reproductive effects. Dr. Germolec said the selected doses of DON would be low enough to avoid anorexia. Dr. Birnbaum added that the levels to which children are exposed are not so low. She thought it important to address early life exposures and evaluate multiple endpoints to determine possible effects at a susceptible age. Dr. Riviere agreed with Dr. Birnbaum and said that although DON is regulated in grains, the chronic effects at low levels of exposure in infants is unknown.

Dr. Portier summed up the discussion by saying that the general consensus is to go forward with the studies but some members thought it should be given a low priority.

C. Alkylanilines

a. Presentation

Dr. Scott Auerbach, NIEHS, presented the proposed studies on 2-ethylaniline (2-EA), 3-ethylaniline (3-EA), and 3,5-dimethylaniline (3,5-DMA). They were nominated by NIEHS for toxicological characterization because of their ubiquitous potential for exposure, limited availability of published toxicological data for this subclass of alkyl-substituted anilines and their structural similarities to two known animal carcinogens, 2,6-xylidine (also referred to as 2,6-dimethylaniline (2,6-DMA), and *o*-toluidine (also referred to as 2-methylanline). He pointed out that 2-MA and 2,6-DMA are known rodent carcinogens while 2-MA, 3-ethylaniline (3-EA), 2,6-DMA and 3,5-DMA are associated with human bladder cancers.

Alkylanilines are used in the manufacture of numerous household and industrial products. The likely primary source of exposure to the general public is mainstream and side stream cigarette smoke. Biomonitoring studies have indicated widespread human exposure to a variety of alkylanilines based on the identification of hemoglobin (Hb) adducts.

Alkylanilines cause methemoglobinemia and are irritants. 2-MA is a mouse liver carcinogen and is classified by IARC as a Group 2A, while 2,6-DMA is a rat nasal cavity and liver carcinogen and is classified as a Group 2B by IARC. There is no

study with 2,6-DMA in the mouse. Alkylanilines are generally negative in Ames mutagenicity studies and positive for clastogenicity. Some alkylanilines form DNA adducts *in vivo.*

He described the metabolic pathways that alkylanilines undergo and stressed the polymorphism of N-acetyl transferase in humans, the enzymes involved in the formation of the unstable hydroxylamine that forms nitrenium ions, which in turn react with hemoglobin and DNA to form the respective adducts.

Dr. Auerbach proposed that the NTP undertake a class study of 14 alkylanilines because of the widespread exposure of humans at low doses, the reported carcinogenicity of some alkylanilines in animals and their likely metabolism by similar enzymes with relevant polymorphisms in animals and humans. The study would be centered around mode of action to determine a possible relationship between mechanism of carcinogenicity and the influence of genetic variation on genotoxicity. As evidence that the proposed approach has the potential to determine a relationship, Dr. Auerbach presented the results of a study with alkenylbenzenes that showed such a relationship between DNA adduction and tumorigenicity in the newborn mouse model.

The first specific aim will be to conduct *in vivo* DNA reactivity studies by measuring DNA and hemoglobin adducts in multiple tissues of C57BL/6 mice. *In vivo* mutagenesis (*Pig-A* assay) and clastogenesis (Comet assay and micronuclei in erythrocytes) studies will be performed in parallel with the DNA adduction studies to establish any relationship between genotoxic potency and carcinogenic activity.

The second specific aim will involve a short-term carcinogenicity bioassay in *Xpc*$^{-/-}$*/p53*$^{+/-}$ knockout mice constructed on a C57BL/6 background. This strain was selected because of its sensitivity to arylamine-induced carcinogenesis in the urinary bladder and liver. Four alkylanilines will be selected for study; one known carcinogen, one high potency genotoxin, one intermediate potency and one low potency genotoxin.

Since genetic variation in Phase I and Phase II enzymes confers differential carcinogenic risk to arylamine exposure, specific aim 3 will involve the measurement of DNA adducts in hepatocytes from multiple inbred mouse strains with genetic diversity at loci known to influence arylamine metabolism (N-acetyl transferase, CYP1A and glutathione -S-transferase). The cells will be evaluated for DNA adduction and DNA damage using the Comet assay.

The proposed research program will determine, relative to known rodent carcinogens, the genotoxic and mutagenic hazard of a collection of largely untested alkylanilines with widespread human exposure, the carcinogenic activity of a selected subset and the influence of genetic variation on alkylaniline

genotoxicity. The measurement of hemoglobin adducts will provide a metric of internal dosimetry for more accurate scaling of carcinogenic hazard to humans.

b. BSC discussion

The BSC asked specific questions about the presentation. Dr. Sherley asked if the structures of the alkylaniline DNA adducts are known, and Dr. Auerbach said 3,5-DMA binds randomly to DNA whereas the bicyclic alkylanilines bind to specific bases. Dr. Novak asked if the adducts were isolated from the same animals that developed tumors in the published study on alkylbenzenes, and Dr. Auerbach responded that the CD1 mice were injected at a young age and sacrificed a year later when tumors were found. Dr. Faustman asked if 32 P-postlabeling would be used to measure adducts, and Dr. Auerbach responded that this assay is not specific; thus, accelerated mass spectrometry is being considered.

Dr. Novak, the first BSC discussant, said the rationale for the proposed examination of 2-EA, 3-EA, and 3,5-DMA is clearly stated. The documented exposure of humans to a large number of alkyl-substituted anilines, as reflected by alkylaniline - or arylamine-Hb adducts, and the correlation of Hb adducts with bladder cancer further supports the need for an assessment of the toxicity and carcinogenicity of these chemicals.

The use of primary cultured hepatocytes and transgenic mouse models will advance the knowledge of the role of genetics in determining the genotoxic potential of the alkyl-substituted anilines and whether there is an association with target organ tumorigenesis. Such studies have the potential to result in improved screening and testing methods, structure-activity data, and identification of genetic susceptibility.

The importance of these data to human health is amplified by the knowledge that structurally similar agents are known animal carcinogens and that alkyl-substituted aniline- or arylamine-Hb adducts have been detected in non-smokers. Although the number of alkylanilines selected for testing is relatively large, the rationale for examination of this battery is reasonable as it reflects the wide-array of toxicants to which humans are exposed. These data should provide valuable information on the role of genetics in target organ tumorigenesis.

Dr. Sherley, the second BSC discussant, said cigarette smoking is a “well documented” route of non-occupational exposure to alkylaniline compounds. However, if cigarette smoking and environmental tobacco smoke (ETS) are the main sources of exposure, he does not believe that these studies will have an additional impact on human health due to existing efforts to reduce tobacco smoke exposure.

He did not believe that the studies proposed to evaluate 7 of the 9 additional aniline dyes beyond the three nominated are warranted because there is minimal

evidence of human exposure and 4 of them lack an ortho substitution. However, evaluation of the toxicological properties of 2,6-DEA and 2,6-MEA might be justifiable because of their high production volumes, and their ortho alkyl substitutions at the same positions as 2-MA and 2,6-DMA, established rodent carcinogens. He deems that the public health impact of the expanded proposed research program is low. However, information on the exact biochemical and chemical mechanisms responsible for alkylaniline dye DNA adduction would contribute to the understanding as to whether other aniline dyes have similar modes of action to 2-MA and 2,6-DMA.

Genotoxicity evaluations using human hepatocytes and bladder epithelial cells are greatly needed. Due to the absence of substantial source data and human exposure data, there is little justification of studying the additional 9 aniline dyes, although the initial 3 are justified. He would give the study a higher rating if the focus were on the 3 original nominations.

Dr. Auerbach agreed that a large proportion of exposure is to ETS but there is significant exposure in non-smoking individuals based on a German study that found a correlation between cotinine levels and alkylaniline-Hb adducts in nonsmokers suggesting other sources of exposure. He believes that the mode of action approach is reasonable for studying this class because hydroxylamine is the major intermediate of monocyclic, bicyclic or heterocyclic amines in humans and animals. Also, the identified human hemoglobin adducts are similar to those found in exposed rodents. Mouse hepatocytes were selected rather than human hepatocytes because the defined genetic background of the mouse strains from which the cells are derived, is known. The NTP will consider using human hepatocytes.

Dr. Pino asked why the initial genotoxicity studies would be *in vivo* while studies on genetic variation will be *in vitro*. Dr. Auerbach replied that the *in vivo* studies in mice will measure dosimetry by ascertaining whether hemoglobin adducts are detected for comparison with human data. Although isolated mouse and human hepatocytes have decreased cytochrome P450 activity, they are able to metabolize alkylanilines to reactive intermediates.

Dr. Faustman liked the approach as information about the mode of action and structural relationships will be obtained, thus, decreasing the number of animals required.

Dr. Nagarkatti thought that homozygous mice might be more useful but Dr. French, NIEHS, said that control homozygous P53 knockout mice develop spontaneous tumors early in life. Dr. Sherley enquired about the rationale for using cancer-prone animals rather than regular strains and Dr. Auerbach replied that use of such animals allows for more rapid assessments of carcinogenic activity and the knockout mice usually respond to genotoxic chemicals, such as the alkylanilines, but not to nongenotoxic chemicals.

Dr. Cattley questioned why genotoxicity and genetic differences were not being studied in the bladder, as it is the main target tissue. Dr. Auerbach replied that it is very difficult to culture bladder cells and to recapitulate its acidic environment *in vitro*. Also, he believes that the same genes are involved in the metabolism of the compounds in the liver and the bladder.

Dr. George Friedman-Jimenez agreed that the public health aspect is important and he thought that studying a broad range of chemicals in the different mouse strains with different genetic backgrounds is a strength of the concept.

Dr. Torasson asked about the number of knockout mice that would be used, and Dr. Auerbach replied that usually 3 dose levels and a control group of male and female mice are used with 25 animals/group. The study is for 9 months. XPC/P53 knockout mice will be used rather than XPA/P53 knockout mice because the former are less sensitive to higher doses of chemicals that are typically used in studies on non-transgenic rodents.

Dr. Portier summarized the discussion and said that the consensus seems to be that this is a high-priority study.

D. Dong Quai

a. Presentation

Dr. Michael Wyde, NIEHS, presented the concept on Dong quai. The root of the plant, *Angelica spp*., contains over 90 constituents including alkyl phthalides, furanocoumarin, terpenes, phytosterols, organic acids and immune-stimulating polysaccharides. It is the second most frequently ingredient in traditional Chinese medicines. Dong quai is commercially available in many formulations as a dried root powder or in a mixture with other herbs, vitamins, or minerals. The various methods for processing the root lead to differences in the constituents of Dong quai, which are not normalized to a specific component. Since the effects of these differences in the extracts are unknown, serious consideration needs to be given to the specific formulation selected for study.

Dong quai was nominated by a private individual for comprehensive toxicological characterization due to its widespread use in dietary supplements in the United States and potential adverse effects during pregnancy and lactation. Its purported effects include maintenance and balance of sex hormones, the treatment of menstrual irregularities and menopausal symptoms, and as a general tonic for the female reproductive system. The amount of Dong quai used in the U.S. is unknown, but it is regulated according to the FDA Dietary Supplement Health and Education Act of 1994.

There are no subchronic or chronic toxicology, carcinogenicity, or reproductive and teratology studies available in the literature on Dong quai or its primary constituents. Two constituents, bergapten and psoralen, may induce dermal

photosensitivity while a third, safrole, is a carcinogen. Dong quai induces hepatic cytochrome P450 enzymes and interacts with estrogenic drugs, anticoagulants, and nonsteroidal antiinflammatory drugs (NSAIDs) as well as dietary supplements.

The research plan is divided into two tiers. In Tier I, the differences in toxicological response between various preparations of Dong quai will be investigated in *in vivo* and *in vitro* receptor binding assays and through the NTP High Throughput Screening (HTS) program to select an appropriate test material. This will entail the testing of commercially available formulations and chemical analysis and characterization of the formulations and their biological activity. The same samples will be tested in short-term *in vivo* Hershberger and uterotrophic assays. A decision tree will be developed for selecting test material to evaluate in Tier II. A botanical expert will be consulted to achieve this objective.

In Tier II, oral 14- and 90-day toxicology studies and 2-year toxicology and carcinogenicity studies in rats and mice will be conducted. Consideration will be given to using an *in utero* and lactational exposure paradigm and evaluation of blood clotting parameters. Reproductive and developmental toxicity studies with emphasis on landmarks of sexual maturation will also be conducted as well as studies to evaluate the immunotoxicity and phototoxicity of Dong quai. For the latter study, individual components and the chosen mixture will be tested and photo-induced DNA adducts will be identified.

b. Public comments

Mr. Manuppello, PETA, was disappointed that the NTP is still using animals for testing and has not adopted the suggestions of the National Academy of Sciences that recommends *in vitro* screens of potential toxicity tailored to the endpoints of concern. Dong quai has been used for thousands of years in China with no adverse effects. FDA has designated Dong quai as GRAS-generally recognized as safe-as a spice and oil. A recent publication reported that exposure of rodents to an oral dose of 5 g/kg of an alcoholic extract of Dong quai for 14 days or 100 mg/day for three months had no adverse effects. PETA is concerned that a private, unidentified individual nominated this benign substance and questions how the NTP can justify the use of 18-36 rats per test for the uterotrophic and Hershberger assays. PETA suggests that the preliminary studies be limited to *in vitro* receptor binding and transcriptional activation assays and the *in vitro* micronucleus test. Studies have reported weak reproductive and developmental effects and showed questionable differences in fetal weight between control and exposed animals. PETA does not believe that the proposed immunotoxicology and photoxtoxicology studies are justified based on data from one study.

c. BSC Discussion

Dr. Faustman asked how one can determine that a nomination is not made for personal gain and why the name of the nominator is not provided. Dr. Masten

replied that the NTP does not disclose nominators' name to protect privacy, and he does not believe that the nomination was made for financial gain.

Dr. Faustman asked about substances listed as GRAS. Dr. Masten replied that the GRAS listing is confusing because the Code of Federal Regulations lists *Angelica spp.* as GRAS when used as a spice or seasoning in food. The levels used in traditional foods is low relative to the concentration in dietary supplements, which by definition are not GRAS.

Dr. Carney, the first discussant, said the project was very complicated. Dong quai is an important mixture for the NTP to study because of the high levels of exposure, particularly in women, that often exceed 15 g/day. This amount is orders of magnitude higher than the concentration of many chemicals the NTP typically studies. Although there are clinical data, only a few animal studies have been reported.

Dr. Carney thought the rationale for this project is less clear with respect to how the proposed studies would be used in risk assessment. Also, Dong quai is an ill-defined, naturally occurring product comprised of several different compounds that vary from batch to batch, and it can be sold in combination with several other herbal products. He was concerned that the formulation(s) selected for study might have limited applicability to all Dong quai preparations. However, the study would have a strong public health impact and the approach NTP uses to study this complex mixture would be important for future studies of similar mixtures. He suggested a more unique, hypothesis-driven research program that would take advantage of the available clinical data rather than traditional rodent studies. The ill-defined nature of Dong quai preparations limits the applicability and overall value of the traditional descriptive studies of a single preparation in rodents.

He liked the tiered approach and thought that it would be better to test individual components in *in vitro* and short term *in vivo* studies than to test a mixture until the composition and toxicity of the major components of Dong quai preparations are better known. The clinical history suggests that the focus should be on studies of blood coagulation and interactions with drug metabolism enzymes. He was concerned about how a decision would be made regarding which preparations to test in the higher tiered studies.

Dr. Solomon, the second BSC discussant, agreed with Dr. Carney's remarks and said Dong quai is used widely in over 6,500 products; therefore, from a public health perspective, it is a strong candidate for testing. Dong quai is not sold by a single commercial organization and is not likely to be evaluated adequately without federal government involvement.

She liked the approach of selecting relevant preparations for analytical chemistry and using *in vitro* tests to determine the biological activity of the preparations

being sold. These preliminary studies might allow the NTP to narrow the most representative preparations for further testing. ADME studies would be helpful in understanding oral absorption, distribution, metabolism, and excretion of the key, active ingredients.

She questioned why subacute and subchronic studies were included. She thought the reproductive studies are important because women are taking Dong quai.

Dr. Wyde thanked the BSC for their comments and support for the study. He said the NTP had grappled with the applicability of the proposed Dong quai studies to public health. At a recent meeting with the FDA, the NTP discussed the approach of testing several different preparations in short term tests as a means for developing a decision tree to determine whether to test the herb itself or a specific commercial product. The plan is to characterize the toxicity of commercial products of Dong quai and possibly individual components. The analytical chemistry and the biological characterization will provide insight as to whether or not certain constituents should be studied individually. The subchronic studies will be designed to examine blood coagulation and drug metabolism. Depending on the results from the first tier, 14-day studies might be eliminated and only 90-day studies conducted. Data from both subacute and subchronic studies would be used to select appropriate doses for the chronic studies.

Dr. Sherley was not as enthusiastic as the other reviewers because previous studies in humans did not report adverse effects of Dong quai. Dr. Wyde replied that many of the clinical studies were poorly designed and the participants consumed many preparations besides Dong quai. Also, most of these human studies only documented selected, acute effects despite the long list of possible side effects that have been reported for Dong quai.

Dr. Friedman-Jimenez added that typically observational studies or clinical trials of these herbs are small, short term, and without sufficient statistical power to identify toxicity. What is needed is post-market surveillance to identify long-term side effects as is done for drugs. He shared the enthusiasm of the other reviewers but was concerned about studying a mixture and the diversity of the formulations. He was unsure if enough was known about the Dong quai products to rationally select representative formulations for study. He suggested adding an additional aim to test the possible formulations analytically before a decision is made on which formulation to study.

Dr. Howard said the FDA and NTP are collaborating to select a representative herb from the market place. He clarified that the nomination is for the herb Dong quai and not for a specific marketed product. The objective of studying the herb is to fractionate the preparation in the hope of identifying an active toxic

component so that the FDA can ban the active ingredient and preparations containing it.

Dr. Nagarkatti reminded the NTP of the National Center for Complimentary and Alternative Medicine that has compiled a list of important considerations when studying botanicals, such as shelf life, where the plant was grown, contamination when harvested etc., and said this resource might be helpful. She suggested that Dong quai be tested in immunotoxicology and phototoxicology assays in both short and long-term studies and Dr. Wyde said the NTP would consider her suggestion.

Dr. Faustman said human studies should not be completely discounted and if serum samples from these studies are available they might be useful for toxicokinetic studies. She added that the first tier should concentrate specifically on analytical chemistry and *in vitro* studies and should be completed before the animal studies are initiated or a rationale is developed for selection of the formulation to test.

Dr. Tracie Bunton agreed with the comments and asked whether background information about their pharmacology had been obtained on the components of Dong quai. Dr. Wyde replied that certain toxic components have been identified but the whole does not equal the sum of the parts because of possible additive interactions.

Dr. Riviere added that the U.S. Pharmacopoeia might be a useful source of information as it lists the active principles of many natural products and dietary supplements. Dr. Solomon added that QSAR modeling on different active ingredients might also be informative. Dr. Wyde agreed with her suggestion.

E. Indium Tin Oxide (ITO)

a. Presentation

Dr. Patrick Kirby discussed the NTP research concept on indium tin oxide (ITO). In 2001, NTP demonstrated that indium phosphide (InP) was carcinogenic in both rats and mice. ITO was nominated by NIEHS due to its increased worldwide usage in the manufacture of flat panel TV screens, lack of adequate toxicity data, reports of clinical cases of “indium lung” in Japan, and the carcinogenicity of InP. ITO is also used for field emission displays, heat reflective coatings, solar panels, and photovoltaics.

The goals of the study are to understand if ITO and indium alone are toxic and to provide this information to regulators. ITO is formed when indium oxide (IO) and tin oxide (TO) in a ratio of 90% IO and 10% TO are sintered and the particles are bound to a solid material such as a TV screen. Human exposure occurs during deposition of the ITO film and during recycling of ITO containing electronics. NIOSH plans to monitor ITO in the workplace; however, the TLV, TWA and REL are based on unsintered IO data.

Interstitial pneumonia is the most prevalent condition in association with “indium lung”. Elevated serum indium and KL-6 levels were reported. Two cases of fatal pneumothorax are also documented in the literature.

Indium salts cause testicular and developmental effects in animals. Sintered ITO has been found to be more toxic in the lung than an equivalent mix of unsintered IO and TO possibly by increasing its solubility.

The NTP studies will address solubility of indium compounds, characterize ITO toxicity and potential carcinogenicity in both rats and mice following inhalation, provide data for use in setting occupational exposure limits, and compare the toxicity between ITO, $InCl_3$ and InP. It is hypothesized that following inhalation, particles of InP are solubilized by lung macrophages releasing free indium. Proposed studies are divided into three tiers. In the first tier the solubility of indium compounds at various pH’s will be measured using soluble indium chloride as a positive control. Then, the relative solubility of indium compounds following inhalation of sintered vs. non-sintered ITO particles in rats and mice will be determined. Tissue concentration of indium in the lungs will be measured using ICP-MS. The second tier will investigate the effects of inhalation of ITO for 14-days on developmental and reproductive toxicity. Tier 3, which will entail chronic inhalation of ITO and indium chloride in a 2-year carcinogenicity study will be undertaken, if necessary. The collected data may allow similar regulation of all indium compounds, whether sintered or nonsintered.

b. BSC Discussion

Dr. Pino, the first BSC discussant, agreed with the rationale for the proposed research program due to its increased use, the evidence of pulmonary toxicity in animals and humans, and the finding that other indium compounds are teratogenic and carcinogenic in animals. The proposed research should provide important data that can be used for risk assessment of all indium compounds.

Since most of the exposure to ITO is in the Far East, he asked if there was an estimate of the number of workers in the U.S. that work with ITO and are potentially exposed, or whether this will be determined by the NIOSH assessment.

Studies with both indium chloride and ITO are needed for the carcinogenicity and developmental toxicology studies. He suggested that it may be useful to pretreat dams with ITO for a period of time before mating so that the compound has time to be solubilized and maximum plasma levels are obtained before gestation begins. He gave the program a high priority.

Mr. Janzen, the second BSC discussant, said the research program and rationale is clear, and appropriate to the mission of the NTP. While the metabolic pathway from ITO to indium is not known, the hypothesis that the indium burden

results from ITO exposure is well reasoned. The key issues of solubility and particle size should be more closely linked. He said it would be important to study particles in the respirable range. His major concern is the poor solubility of particles for the proposed 2-week teratology studies.

The possible synergistic effects between indium and tin are not being addressed by using indium chloride and this issue needs to have more thought.

He gave this study a high priority because indium compounds have adverse toxicological effects and production will continue to rise for the foreseeable future. The impact of human exposure through handling of discarded electronic equipment in landfills in the U.S. warrants further study.

Dr. Kirby said that NIOSH would be addressing the exposure of the U.S. workforce in the sintering/deposition process and exposure in landfills. He said that a study with tin oxide could be included to address the possible synergistic effects.

Dr. Birnbaum addressed Dr. Pino's enquiry regarding exposure of workers and said most of the occupational exposure is in the making of ITO and the reclamation of discarded equipment in the Far East. The concentration of ITO and other heavy metals is extremely high in the recycling facilities.

Dr. Nagarkatti asked why immunotoxicology studies are not included since it appears that pulmonary macrophages play a roll in solubilizing ITO and Dr. Kirby said this aspect might be added. Dr. Toraason said NIOSH is aware of a couple of cases of pulmonary toxicity from indium exposure on the east coast of the U.S.

Dr. Solomon asked whether nanoparticles would be studied and Dr. Kirby replied that the particle size would be addressed in the preliminary analytical studies.

Dr. Portier summarized the discussion by saying that the BSC gives ITO a high priority for study.

F. 1-Chloro-4-(trifluoromethyl)benzene (PCBTF)

a. Presentation

Dr. Scott Masten outlined the rationale and proposed studies for PCBTF on behalf of Dr. Mathew Stout. PCBTF was nominated for toxicological testing by a representative from Kowa American Corporation due to lack of OSHA, NIOSH or ACGIH exposure limits. It is used to replace ozone-depleting solvents in paints and automobile body coatings. A public commenter had pointed out its expanding use as a volatile organic exempt compound and asked the NTP to evaluate its toxicity. Human exposure is likely via inhalation.

PCBTF is no longer manufactured in the U.S. but four different companies import a total of 1-10 million pounds per year. PCBTF causes liver and kidney toxicity in

subacute oral toxicity tests in rats and mice and in subchronic inhalation tests in rats. It is cytotoxic but not genotoxic to bacterial and mammalian cells. There is limited data on its reproductive toxicity and no data for immunotoxicity, developmental toxicity, chronic toxicity or carcinogenicity. A structural analog, benzotrichloride, is an established rodent carcinogen, but no benzotrifluorides have been evaluated in chronic toxicity studies. The NTP is recommending it for chronic toxicity, reproductive toxicity and carcinogenicity studies.

A tiered approach to testing will be used. In Tier 1, subchronic inhalation studies will be conducted in rats and mice in which standard reproductive tissue histopathology and analysis of sperm morphology and vaginal cytology will be included to determine the need for further reproductive toxicity studies. The potential for PCBTF to induce alpha2µ-globulin nephropathy in male rats will be evaluated.

In Tier 2, 2-year toxicity and carcinogenicity studies in rats and mice will be conducted as well as a teratology study in rats. Consideration will be given to conducting a current guideline functional reproductive toxicology study in rats.

In Tier 3, a perinatal toxicity study will be conducted to evaluate the effects of PCBTF on functional endpoints in the reproductive, nervous and immune systems. The tier 3 studies will be conducted based on the Tier 1 and Tier 2 study results.

b. Public comments

Mr. Manuppello, PETA, said that in 1985 EPA declared that no further testing of PCBTF was required under the Toxic Substances Control Act (TSCA) as it had been shown to be a weak toxicant and not a genotoxic agent. Also, it is not metabolized to toxic intermediates and is largely excreted unchanged as the glucuronide conjugate. Subchronic studies have reported no reproductive effects after 90 days following oral exposure. He questioned whether human exposure is increasing. Occidental Chemical, a previous manufacturer reported tremors following subchronic inhalation of 500ppm but no hematological changes, nervous symptoms or body weight loss was reported at levels of 250ppm. There was low subchronic toxicity when the oral route was used with no effects at concentrations as high as 10mg/kg/day.

An epidemiology study that linked stomach and lung cancer to PCBTF in an occupational setting failed to consider that the cohort was exposed to 25 other chemicals manufactured in the plant that were likely confounding factors. Since PCBTF is an orphan chemical, EPA may require testing. If this is the case NTP should coordinate their studies with EPA. PETA requests that NTP accurately determine the potential for human exposure before any animal studies are initiated.

c. BSC Discussion

Dr. Friedman-Jimenez, the BSC discussant, said that the NTP's rationale for further research of PCBTF was justified. However, minimal information was provided on use and environmental exposures to PCBTF in occupational and community settings and it is important for NTP to determine the extent of exposure. Since PCBTF is a solvent in commonly used metal cleaners and is a non-VOC universal diluent, he suggested that NTP collaborate with NIOSH, ATSDR, EPA, ACGIH, state and local health departments, and other federal and state agencies to determine exposure levels of workers and the general public.

He agreed with Mr. Manuppello that the human cohort mortality study mentioned in the NTP profile is very difficult to interpret for either PCBTF exposure or health effects because the predominant exposure in the plant was not to PCBTF, but rather magnesium perchlorate, as well as possibly 22 additional chemicals.

Although there is a reasonable amount of preliminary scientific information on the toxicity of PCBTF, a notable data gap is the lack of carcinogenicity studies in animals and the inadequate reproductive, immunotoxicity, neurotoxicity, and developmental toxicity studies in animals. Although the no-observed-effects-levels for oral and inhalation routes in animals seem to be relatively high from past studies, characterizing these parameters better as well as dermal absorption are important. He had no opinion as to whether PCBTF would be a suitable representative of the class of benzotrifluorides.

The scope seems reasonable, and if positive results are obtained, they would provide an adequate basis for future epidemiological studies of exposure in humans. All of the specific aims contribute necessary information, and the tiered sequence of studies is appropriate.

Dr. Masten replied that he tried to confirm the changes in the use patterns but there is little or no direct exposure information available.

Dr. Faustman asked whether NTP would continue with the chronic studies in rats if alpha2µ-globulin were found in male rats in tier 1. Dr. Masten replied that it was difficult to predict before the studies were conducted and all the data evaluated; for example, it would depend on whether renal toxicity is found in the females of both species. Dr. Faustman suggested that non-cancer endpoints including developmental and immunotoxicity be given a higher priority than carcinogenicity studies.

Ms. Rudel asked if PCBTF is used in low VOC paints, and Dr. Masten said he could not recall. She added that she thought PCBTF an important chemical to test because of the likely consumer exposure from many products.

Dr. Portier asked for clarification regarding the re-review of PCBTF by EPA. Dr. Masten explained that PCBTF is a substance that falls into the high production

volume (HPV) category but has been given orphan status because no company has offered to provide information or study the chemical. Until recently, EPA evaluated HPV chemicals under their Chemical Assessment and Management Program (ChAMP), which was recently discontinued until further revision. The requirements under the ChAMP program might be met with the current information except for data on developmental toxicity. Under the present situation, it is unlikely that additional toxicological data will be collected unless NTP does the proposed testing.

Drs. Faustman and Nagarkatti suggested that immunotoxicity testing be added to a higher tier, and Dr. Masten replied that this might be done. Dr. Friedman-Jimenez said he would be more supportive of the study if the data collected could be extrapolated to other benzotrifluorides. Dr. Masten said that the data collected on this compound might be useful to limit future studies on additional benzotrifluorides.

Dr. Portier summarized the discussion by saying that the BSC gives PCBTF a moderate to moderate-high priority for study.

G. Tris(4-chlorophenyl)methane (TCPMe) and Tris(4-chlorophenyl)methanol (TCPMOH)

a. Presentation

Dr. Scott Masten presented the research concept on behalf of Dr. Po. Chan. TCPMe and TCPMOH were nominated by NIEHS for toxicological characterization due to their apparent widespread occurrence and persistence in the environment, limited toxicity data, and their detection and possible bioaccumulation in human tissues. TCPMe is believed to be a by-product in the production of DDT and TCPMOH is a presumed metabolite of TCPMe.

There are some industrial uses but no production data is available. The primary human exposure is presumed to be from the consumption of food because both compounds have been identified in human adipose tissue, liver, bile, and breast milk in non-U.S. countries.

The toxicity of TCPMOH has been studied in a 28-day oral study in mice where changes in liver, spleen, and blood counts were observed. In sexually mature male rats an increase in serum FSH was recorded, but no effect was noted on serum LH and testosterone levels, or testicular morphology. TCPMOH is a potent competitive inhibitor of human and rodent androgen receptors *in vitro* and alters human sperm motility, vitality, and the acrosome. There is no information on ADME, genotoxicity, immunotoxicity, developmental, or reproductive toxicity.

A tiered approach will be used for testing. The first tier will entail ADME studies following single and multiple oral exposures when blood and tissue levels will be measured and metabolites identified. Confirmatory *in vitro* and *in vivo* endocrine

modulation studies such as binding to the androgen and estrogen receptors and genotoxicity studies will be included. In Tier 2, subchronic toxicity studies, with *in utero* and perinatal exposure will be conducted. The F1 will be evaluated for immunotoxicity, developmental and reproductive toxicity. In Tier 3, a multigenerational reproductive toxicity and carcinogenicity study will be conducted based on data from tiers 1 and 2.

b. Public Comment

Mr. Manuppello, PETA, said that although TCPMe and TCPMOH have apparently been used in the manufacture of polymers, the major source of TCPMe is believed to be an impurity in DDT produced during manufacture. TCPMOH, a metabolite of TCPMe, is produced in living organisms. Since DDT is the major source of TCPMe and hence TCPMOH, and it is banned from use in most parts of the world, PETA is unclear how the data that will be obtained will alter exposure or decrease the source of TCPMe. He suggested that DDT currently in commerce be analyzed to identify whether TCPMe is a contaminant before any studies begin.

c. BSC discussion

Ms. Rudel, the BSC discussant, said that the chemical is widely distributed in humans and biota in lipid-rich compartments, and has endocrine activity. The rationale for the study would be strengthened if the NTP clarified how the data generated in the proposed program would be useful for making risk management decisions.

If the major exposure to TCPMe is as a contaminant of technical grade DDT, and all DDT uses in the U.S. have been banned, there is little utility to the toxicology studies proposed by NTP. However, additional information on the toxicity of this chemical could inform 1) U.S. support of DDT for malaria control in other countries, 2) cleanup levels for areas contaminated by historical production and use of DDT, 3) programs for monitoring levels of these compounds in food products or animal feed, 4) interpretation of epidemiological studies of DDT exposure, and 5) understanding the toxicology of endocrine disrupting compounds. In addition, if there is ongoing occupational exposure the proposed program becomes even more important and useful. She was unable to provide a priority for testing since she had little information on known sources of exposure. She is concerned because TCPMOH apparently binds to the androgen receptor at low serum concentrations.

She approved of the tiered approach but thought that the depth of the tier 2 and 3 studies was greater than necessary for an initial assessment. She suggested that (1) the tier 2 and 3 studies be based on tier 1 results; (2) thyroid binding assays be added; (3) the NTP use similar methodologies to those being used in EPA's Endocrine Disruptor Screening Program; (4) binary mixtures of TCPMOH with DDT and DDE be included in one of the early screens, (5) mammary gland

morphology and precancerous lesions in the mammary gland be included in the tier 2 tests and (6) for CDC to monitor for TCPMOH in its present NHANES measurements.

Dr. Masten said he appreciated Ms. Rudel's statements as to how the data could be used. The idea of measuring contaminants such as TCPMOH in epidemiologic studies is an important scientific issue that may not be widely appreciated. NTP works with CDC and will consider requesting that TCPMOH be added to the NHANES monitoring program. NTP will consider the addition of thyroid receptor screens and measuring additional endocrine related endpoints.

Dr. Faustman thought that the NHANES data may not be very helpful and suggested that banked serum samples from epidemiological studies on children in other countries might be more useful to examine temporal and geographic trends in DDT and TCPMOH levels. She said that children emigrees from Mexico that have high levels of serum DDT, might be a good cohort to study. She added that data from the *in vitro* studies in tier 1 and tier 2 should be assessed before more testing is begun.

Dr. Bunton asked for clarification on the use of DDT in other countries, and Dr. Birnbaum replied that the usage is high in some countries such as India. Dr. Novak added that DDT is wide spread throughout the world and can be transported on dust micro particles and has been found in the maternal cord blood of Inuit populations Dr. Masten added that TCPMOH has been identified in seals and whales.

Dr. Riviere thought that testing of TCPMe and TCPMOH is of low to medium priority and Dr. Portier agreed that this seems to be the view of the BSC. However, Dr. Faustman added that if TCPMOH is pervasive in serum, its presence would be a concern from an endocrine point of view even if the source is unknown. Ms. Rudel added that TCPMOH levels correlated with DDT levels in people in Japan and Vietnam but it is unknown if TCPMOH is more or less potent than DDT or its metabolites.

VII. Contract Concept Reviews

Guidelines for Review

Ms. JoAnn Lewis, Office of Acquisitions at the NIEHS, briefly outlined the guidelines for the BSC regarding the discussion of research concepts. She asked the BSC to review the concept on mold materials for its overall value and for its scientific relevance to fulfill the program’s goal of protecting public health. They should consider the availability of technology to achieve the required goals, adequacy of the methodology to be used to perform the activity, the scientific or clinical uses of the anticipated data, and scientific, technical, and programmatic significance of the proposed activities. The discussion should be limited to a review of the general purpose, scope, goal, and optional approaches to pursue the overall objectives. The meeting will be closed to the public should discussions turn to the development or selection of the details of the project such

as specific technical approaches, protocol, statement of work, data format, or product specifications. A meeting is closed to protect free exchange of the advisory group members' opinions and avoid premature release of the details of the proposed contract or request for proposal.

The NTP presented three concept contract reviews to the BSC.

Contract Concept Reviews

A. Investigative absorption, distribution, metabolism and excretion (ADME) studies of toxicants in NTP animal model systems

a. Presentation

Dr. Michael Cunningham, NIEHS, presented a concept for an ADME contract to perform ADME studies for NTP designated chemicals in multiple genetically defined strains of rodents and in genetically-modified animal models in order to identify polymorphic genetic loci that influence ADME kinetic parameters and toxicity outcomes. It will replace the previous and related contract on "Chemical Disposition in Mammals" that has been in existence since 1978 and has been awarded to universities and contract organizations. Approximately 100 peer-reviewed articles have been published as a result of this contract and, although not part of the scope, it has provided training for doctoral candidates and postdoctoral fellows.

This contract will specifically address ADME needs of the Host Susceptibility Branch (HSB) that has been charged to develop model systems that mirror the genetic diversity of human populations. Under a separate contract, the NTP has sequenced 15 laboratory and wild-derived mouse strains and used this data to impute the sequence of other mouse inbred strains (96 total) derived from 3 mouse subspecies from different parts of the world that are more genetically diverse than the human population. These animals have more than 8×10^6 single nucleotide polymorphisms (SNPs) and copy-number variants, i.e. there is more or less than one copy of a gene at a specific locus. These mice strains will be used to generate ADME data through this contract. It is expected that genetic diversity can be modeled and correlated with ADME and human metabolism data.

He said the contractor must demonstrate expertise in studying: (1) absorption by different exposure routes, (2) tissue distribution of the test article, (3) the identification of unique metabolites in biological fluids, (4) excretion rates of parent compound and metabolites, and (5) providing a mass balance of the chemical, i.e. what is administered is totally accounted for by what is in the body plus that which leaves the body. Studies will use low doses of radiolabeled chemicals to avoid toxicity to the animal.

Dr. Cunningham described a proof of principle study with benzene, a known human carcinogen, in 18 inbred strains. Enormous differences were found in benzene kinetics as well as qualitative differences in the metabolites in the strains. Although the pathway of benzene metabolism is known, an unknown metabolite was found in one strain. He summarized the kinetic functions that were measured including the internal dose. This parameter varied from very low to very high and correlated with the differences in clearance between the 18 mouse strains. Those strains with the highest internal dose had the lowest clearance rate with a 10-fold or greater difference between the strains. Since differences in clearance depend on a number of parameters, it is likely that multiple genes in many pathways are involved. He believes that this type of data may be useful in understanding the mechanism of toxicity.
Dr. Cunningham invited the BSC to suggest chemicals that might be appropriate for this multiple strain analysis.

The contractor will use these 18 (15 sequenced by NTP and 3 strains with imputed sequences) mice strains and more with known genetic sequences for the ADME studies and will test NTP designated chemicals. This will allow HSB scientists and collaborators to obtain quantitative measures for each strain-specific ADME trait and identify quantitative trait loci (QTL), i.e. genes affecting continuously varying traits such as skin color. Bioinformatics and functional analysis of the mouse candidate gene allelic variants may then be used to identify human orthologs, which will aid in extrapolation of ADME and toxicity data across species and support functional validation studies in mouse and human cells and tissues.

He said the NTP sought the BSC's approval to conduct ADME studies for the HSB in NTP animal model systems via a contract mechanism.

b. BSC Discussion

Dr. Riviere, the assigned BSC reviewer, said this ADME support contract is absolutely essential for proper interpretation of bioassay data and design of studies and to assess strain differences. The scope is well defined. ADME is needed to obtain an internal dose to allow the extrapolation and interpretation of the data between strains and species of mice and for possible extrapolation to humans. It is important to understand the effect of genetic diversity on internal dose so that one can determine the effect of genetic diversity on toxicodynamic parameters. The planned approach is well justified and consistent with NTP toxicity studies and will provide a mechanism to interpret genomic differences and the effect of ADME on the mechanism of host susceptibility.

A general discussion then ensued regarding the scope and purpose of the contract, the variability in the data from the benzene study, the possible use of other mouse strains, and whether or not these studies are limited to adult animals. This contract would only provide ADME data and the offerers would not be responsible for data analysis.

Dr. John French, NIEHS, in response to a question from Dr. Sherley, said an analysis of covariance between and within a parameter in the benzene study was significantly different in the strains. He added that the NTP would consider the suggestion that the severe combined immunodeficient (SCID) mouse and mouse strains with humanized cytochrome P450 and peroxisome proliferator-activated receptor (PPAR) genes be studied in the future. However, the 18 selected strains represent laboratory and wild type species with millions of SNPs in highly conserved regions of thousands of genes. Dr. French said the primary objective of the contract is to obtain data on chemicals being tested in the NTP and secondarily to identify human orthologs that might modify the metabolism of the same chemical in humans. Dr. Cunningham said biomarkers of exposure would not be measured and nominations of chemicals for this initiative would be through the regular channels for the nomination of any chemical. In response to Dr. Carney's question whether ADME in fetuses and pups would be included in *in utero* studies, Dr. Cunningham said ADME studies could be designed to measure these parameters in different life stages. Dr. Bucher explained that NTP has an ADME contract that includes dosing during the perinatal period when serum, urine, and breast milk are collected and which could be analyzed for the chemical under test. Dr. Faustman enthusiastically supported the contract, as she believed the approach holds promise for interpreting studies. She suggested adding a human physiologically based pharmacokinetic (PBPK) component.

Dr. Portier called for a vote to approve the NTP going forward with the contract. Dr. Riviere made a motion and Dr. Novak seconded; the BSC voted unanimously with 15 yes votes, 0 no votes, and 0 abstentions.

B. *In Vivo* Toxicology and Carcinogenicity Studies

a. Presentation

Dr. Cynthia Smith, NIEHS, presented the second contract concept review. She said this is not a new contract but the NTP asks the BSC to periodically review contracts that exceed five years. The general toxicity and carcinogenicity studies are a substantial portion of the NTP's focus, budget, and product. In the past, these studies have centered on a central paradigm of 14-day, 90-day and 2-year studies. Each year approximately 6 chronic and 17 subchronic studies are started. These studies are conducted through contracts because of facility and personnel requirements and the need to use FDA GLP guidelines. Groups worldwide consider data from NTP toxicology and carcinogenicity studies as authoritative; therefore, the studies need to be conducted as well as possible.

Recently a number of additions or modifications have been made often to the standard 90-day study designs. These changes include perinatal exposure in rat studies and collection of tissues for mechanistic studies such as RNA for toxicogenomic evaluation. Non-standard designs are used to address specific

questions such as changes in the QT interval, studies of cardiotoxicity, and adenoviral vectors for gene therapy.

The NTP needs to continue to conduct toxicology and carcinogenicity studies in rodents to characterize the hazard potential of agents of public health concern and it seeks the BSC's approval for continuing to use contract mechanisms for this type of activity.

b. BSC Discussion

Dr. Bunton, the BSC discussant, said that this NTP contract is critically important for providing information on the short term and long term effects of exposure to physical and biological agents of public concern to human health. Data collected through this contract and others has resulted in the compilation of a database that can be applied by scientists, clinicians and the public to specific environmental situations. This testing is important, as the data is a public resource that might not otherwise be available. The methodology used is well established and accepted internationally. Ultimately, it is hoped that whole animal testing will become redundant and be replaced by alternative in vitro assays but at this time this is not practical. She approved of the tiered approach so that data from each previous tier can be used to design studies for the next tier. Careful decision-making depends on data that has been generated and/or is available in the literature to determine how studies should be designed to answer a specific question.

Dr. Stephen Looney asked whether the data collected by the testing laboratory is sent back to the NTP for analysis or whether the contractor performs any statistical analysis. Dr. Smith answered that the format for the studies are designed by the NTP and the design and characterized test article and directions for dose formulation is provided to the contractor. Chemistry is conducted through a separate contract. Data generated through the contract are entered into a data collection system that is based at NTP and evaluated by NTP statisticians. Pathology is conducted through this contract and evaluated though a pathology working group.

Dr. Faustman said she is excited about the optimal use of the tissues for non-cancer endpoints such as reproductive and vaginal smears in 90-day studies. She asked whether blood and tissues are collected for analysis from these studies and Dr. Smith replied that tissues are often collected depending on the study design. The tissues are analyzed through the chemical contractor except for inhalation studies.

Dr. Pino asked whether the smaller percentage of chronic studies being performed is due to the fact that the NTP is using a tiered approach so that chronic studies are often found to be unnecessary. Dr. Smith replied that the program relies on the data from the subchronic studies to determine if a chronic

study is appropriate. Sometimes non-cancer endpoints including reproductive or immunotoxicology studies are needed, rather than chronic bioassays.

Dr. Carney enquired whether the NTP has encountered any issues with these new study designs. He added that contract laboratories perform well if they are using study designs that have been used routinely in their facilities but changes in the protocol may result in issues with new designs. Dr. Smith replied that there have been some difficulties that the contract officer has to work diligently to get all the new parameters worked out before the definitive study begins.
Dr. Nagrakatti asked if every chemical is tested in immunotoxicology and reproductive toxicology assays through this contract and Dr. Smith replied that this contract only refers to the acute, subchronic and chronic toxicity studies and there are other contracts for reproductive and immunotoxicology studies.
Dr. Portier asked about GLP and Dr. Smith said that GLP testing requires that many types of records are kept documenting each aspect of the study. Compliance with GLP determines if a laboratory is certified. Smaller laboratories often do not work with this rigor. Not all the NTP studies are conducted under GLP.

Dr. Portier called for a vote to approve the NTP going forward with the contract. Dr. Bunton made a motion and Dr. Friedman-Jimenez seconded; the BSC voted unanimously with 15 yes votes, 0 no votes, and 0 abstentions.

C. Preparation of the Report on Carcinogens (RoC)

a. Presentation

Dr. Ruth Lunn presented the contract concept review by first providing background information on the RoC. The RoC is a congressionally mandated document that lists substances that are known to be human carcinogens or may reasonably be anticipated to be human carcinogens, and to which a significant percentage of the population residing in the United States are exposed. The RoC is a compilation of substance profiles that provides evidence for the listing of each substance in the above categories based on critical scientific justification from human, animal and mechanistic studies, exposure data and regulations regarding exposure. Substances are listed in the RoC by applying the RoC criteria to the body of knowledge on the substance. The current edition, the 11th RoC, has 246 entries.

The NTP prepares the RoC on behalf of the Secretary of the Department of Health and Human Services. The RoC, which identifies substances that cause cancer, falls into NTP's mission to provide scientific information for hazard identification targeted towards the prevention of diseases or adverse effects caused by environmental exposure to chemical, physical, or biological agents.

The preparation of the RoC follows a formal, multi-step process that includes scientific review and opportunity for public comment. As part of the review process, a document is prepared summarizing the relevant scientific information

on a substance. Scientists with expertise relating to the substance in question review the document in public forum and apply the RoC criteria to the body of knowledge and make a recommendation for listing.

The objective of the contract is to provide support to the NTP RoC staff to prepare draft scientific review documents for multiple substances by performing literature searches, drafting technical summaries or reviews of the literature, ensuring the technical accuracy of the information and managing and formatting the references. The contractor will also perform other activities including (1) scientific editing (2) formatting documents, and (3) providing administrative and technical support for convening the scientific review meetings. The contractor will also prepare a pre-publication draft of the RoC with updated information on the substance profiles of currently listed substances, by conducting literature searches on additional scientific information as well as for exposure and regulatory information.

The NTP has an ongoing responsibility to prepare the RoC and seeks the BSC's approval for continuing this activity using the contract mechanism.

b. BSC Discussion

Dr. Friedman-Jimenez appreciated receiving the citations of all the references in PDF format cited in the background document. He asked that this task be added to the contract. Dr. Lunn replied that this task is in the statement of work.

Dr. Faustman, the BSC discussant, said the RoC is an essential document provided by the NTP. Because the RoC provides background information for many subsequent actions, it is essential that the information be of the highest quality to withstand public and scientific review. Thus, support for the accurate preparation of the RoC is of utmost importance. She asked whether additional information on exposure and dose response could be included.

Dr. Lunn thanked the BSC for their comments.

Dr. Carney asked whether conflict of interest procedures had changed in response to a recent situation with the Center for the Evaluation of Risk to Human Reproduction (CERHR). Dr. Lunn replied that the statement of work for all the contracts has COI requirements.

Dr. Portier clarified that this is a support contract for the NTP who is responsible for the RoC's accuracy and information. There was a misunderstanding regarding the role of the contractor in the CERHR process.

Dr. Portier called for a vote to approve the NTP going forward with the contract. Dr. Novak made a motion and Dr. Nagarkatti seconded; the BSC voted unanimously with 15 yes votes, 0 no votes, and 0 abstentions.

VIII. Studies Supported Through the Interagency Agreement with NCTR

a. Presentation

Dr. Howard provided a disclaimer stating he is responsible for his presentation, and the opinions he expressed should not be interpreted as FDA policy. He summarized the NTP activities at the NCTR supported by an interagency agreement (IAG) with NIEHS. He said it is important to understand where the FDA and NTP programs interact. He described the mission of the FDA to (1) protect public health by ensuring safety, efficacy and security of human and animal drugs, biological products, medical devices, food supply, cosmetics, and products that emit radiation; (2) advance public health by speeding up innovations for more effective, safe and affordable medicines and food; and (3) provide the public with accurate, science-based information. He listed the classes of products regulated by the FDA.

The mission of the NCTR, which is one of the six centers of the FDA, is to conduct scientific research in support of the FDA, and to provide technical expertise for science-based regulatory decisions to improve the health of the US public by: (1) understanding the critical biological events in toxicology; (2) developing, characterizing, and incorporating new technologies to improve assessment of human exposure, susceptibility, and risk; and (3) increasing the understanding of the interaction between genetics, metabolism, and nutrition.

He discussed the partnership between FDA/NCTR, CDC/NIOSH, and NIH/NIEHS, the core NTP organizations. These agencies have representatives on NTP committees including the ICCEC, ICCVAM, BSC, BSC Technical Reports Review Subcommittee, and NTP Executive Committee. The IAG between FDA/NCTR and NIEHS/NTP was first established on December 10, 1992 to facilitate cooperation on compounds of mutual interest. The goals of the IAG are: (1) support the design and conduct of toxicological studies consistent with the needs and goals of FDA and NIEHS/NTP, (2) provide oversight and ensure studies are conducted in the most rigorous scientific manner, and (3) ensure data resulting from the studies are available to enable regulatory agencies (U.S. and worldwide) to make science-based, safety assessment and risk management decisions.

He described the nomination review process and the interaction between FDA, NTP, and the IAG Toxicology Study Selection and Review Committee (TSSRC) that oversees the studies conducted under the IAG and provides a forum for interaction among NCTR study scientists, FDA regulatory scientists, NIEHS/NTP scientists, and invited subject-matter experts. At the biannual TSSRC meeting testing protocols for studies are reviewed. Substances studied by NCTR under the IAG include endocrine active agents, dietary supplements, food contaminants, AIDS therapeutics, and nanoscale materials. In addition, phototoxicity and interactions between pediatric/translational drug/device are also studied.

Dr. Howard discussed the data that NCTR has obtained through the IAG. Genistein and ethinyl estradiol, two endocrine active agents tested in a multigenerational study, elicited endocrine effects in female and male Sprague Dawley rats without a generational amplification of effects. Three endocrine disruptor studies are ongoing with bisphenol A in rats. One study will develop a physiologically-based pharmacokinetic (PBPK) model in rats and non-human primates, a second will examine targeted endpoints in a subchronic toxicity study, and a third will study neuroanatomy and behavior.

A number of dietary supplements have been or are being tested at NCTR:

- A mechanistic study of riddelliine identified common DNA reactive intermediates for pyrrolizidine alkaloids.
- An ongoing study of the chronic, oral administration of the whole leaf of *Aloe vera* is ongoing to determine a dose-response relationship.
- A study on ephedra was cancelled when FDA banned its use in products.
- A developmental toxicity study is ongoing with Bitter orange (*Citrus aurantium)* to study its physiological effects in exercise-challenged rats in the presence and absence of caffeine.
- Toxicokinetic and mechanistic studies of usnic acid and *Usnea* lichen are being conducted in rats.
- *In vitro* and *in vivo* toxicokinetic and mechanistic studies of glucosamine/chondroitin sulfate in a diabetic rat model are ongoing.

NTP Technical Reports on two food contaminants namely fumonosin B_1 and malachite green have been published. Fumonosin B_1 caused hepatic and renal carcinogenicity through a non-genotoxic mechanism and a dose-response relationship was established that the FDA used for risk assessment. A dose response relationship to assess the risk assessment of malachite green, an aquatic fungicide, was established. Technical reports on chloral hydrate with and without dietary restriction were published. Both carcinogenicity in female mice and mutagenicity studies were equivocal following neonatal exposure. With dietary restriction, chloral hydrate caused liver carcinogenesis through peroxisome proliferation. A technical report on the administration of urethane in the presence and absence of ethanol showed that ethanol had a weak effect on urethane carcinogenicity.

There are ongoing studies for contaminants in foods, namely, acrylamide, furan, and melamine plus cyanuric acid. NCTR is conducting carcinogenesis, toxicokinetics, PBPK, neuroendocrine, and neurotoxicity studies for acrylamide; a carcinogenicity study in rats to examine the lower end of the dose-response curve for furan; and studies to establish a dose-response relationship in rodents and pigs to identify biomarkers and the mechanism of action of melamine plus cyanuric acid. There are ongoing studies on ketamine in which *in vivo* neurological apoptosis effects are being verified and quantified in the rat in association with behavioral studies. NCTR is developing mechanistic and

analytical methods for the effects of di(2-ethylhexyl)phthalate in rodents and pharmacokinetic studies in non-human primates to establish dosimetry in neonates. NCTR is providing support to the NTP on the cellular telephone radiation study by performing the brain histochemistry evaluation in rats and *in vitro* studies. The agency is studying the effect of combinations of AIDS therapeutics (zidovudine, nevirapine, lamivudine, nelfinavir, and efavirenz) on carcinogenesis following transplacental and neonatal exposure. Mechanistic studies are included to measure DNA adducts, mutagenicity, and clastogenicity. A related study is one in which the carcinogenicity of zidovudine and lamivudine is being evaluated *in* two genetically modified mouse models (C3B6F1$^{trp53(+/-)}$, FVBp16$^{Ink4a(+/-)}$/p19 $^{Arf(+/-)}$).

NCTR has a phototoxicology facility in which simulated solar light, UVB, UVA, or laser light can be used as exposure sources. Usually hairless or transgenic mouse models are exposed. NCTR scientists determined that the topical application of alpha hydroxy acid did not increase sunlight-induced carcinogenesis while beta hydroxy acid protected against sunlight-induced carcinogenesis. Application of aloe constituents to the skin had a marginal effect on the carcinogenesis of sunlight. Three studies are ongoing: the first is studying the photococarcinogenesis of topical application of retinyl palmitate, the second is investigating a potential immunogenic component in permanent makeup, and the third is identifying DNA adducts following exposure to lemon and lime oils.

NCTR is studying whether nanaoscale materials such as titanium dioxide and zinc oxides penetrate the skin and, if so, whether further studies are warranted. For titanium dioxide, the transgenic Tg.AC model is being used for phototumorigenicity; if warranted a photoactivation assay following nanoscale titanium dioxide application will be added to the study. Pharmacokinetic study and subchronic toxicity studies of nanoscale silver and gold are underway.

Dr. Howard indicated the public health impact of some of the NCTR studies. An average of 13 publications/year is derived from studies under the IAG.

- The carcinogenicity data from the fumonosin B_1 study was used to set U.S. and WHO acceptable levels in human and animal foods.
- The chloral hydrate studies concluded that pediatric risk was minimal and no labeling changes were required.
- The urethane studies resulted in a change in manufacturing methods to reduce levels in distilled spirits.
- The carcinogenicity of malachite green established its continued ban in aquaculture of edible fish in the United State and United Kingdom.
- The mechanistic studies of riddelliine identified a common active intermediate for pyrrolizidine alkaloids that allowed FDA to issue a warning and establish contaminant levels for pyrrolizidine alkaloids.
- The alpha- and beta-hydroxy acid studies showed that there is no added risk of their use in the presence of sunlight.

Dr. Howard concluded noting that the IAG has been a successful partnership in protecting public health.

b. BSC Discussion

Dr. Sherley asked about assessment of the "Greening of America," such as the issue of mercury in green light bulbs, and Dr. Howard said that is an EPA issue. The FDA actively nominates substances to the NTP as a means for obtaining information useful for public health decision-making. Dr. Nigel Walker added that the FDA nominated nanogold and nanosilver because they anticipated their use in the medical field. Dr. Howard said FDA is working with regulatory agencies overseas to develop guidelines for nanomaterials.

Dr. Nagrakatti asked about the studies with glucosamine and chondroitin sulfate and whether this mixture would be regulated only after testing is completed. Dr. Howard replied that the FDA regulates dietary supplements according to the Dietary Supplements Health and Education Act (DSHEA). FDA regulates some supplements under a GRAS rule if no known toxic substance is in the supplement. The burden of proof is on the FDA to prove that it is unsafe unless there is evidence in the published literature that a component is unsafe. This is the reason that FDA is studying glucosamine and chondroitin sulfate to determine its potential toxicity.

Dr. Riviere was supportive of the interaction of the FDA with NTP through the IAG.

Dr. Solomon said she receives questions about fragrances from the public and has found that there are many chemicals that can or cannot be used in fragrances. She asked about the approach FDA has taken to evaluate these chemicals. Dr. Howard replied that fragrances fall under the Office of Cosmetics and Colors and there is no requirement for premarket approval. If there is an ingredient they are unsure about, they contact him and he brings it to the NTP for possible study under the IAG. The FDA has nominated about 80 compounds to NTP; for example, cell phone radiation and AIDS therapeutics are being studied under the IAG.

Dr. Portier asked about the approach the FDA is taking for the study of compounds that have an estrogenic effect at low doses. Dr. Howard replied that the focus for endocrine disruptors (ER) is on low doses and their effects on the developing neonate. There is an emphasis on PBPK models in their studies with bisphenol A, because the agency wants to obtain data to inform the best regulatory decision for risk management.

Dr. Faustman asked about the relationship of NCTR staff with different FDA centers and whether a staff member at NCTR is associated with a specific center. Dr. Howard replied that the NCTR is a stand-alone center. All nominations to the NTP come from FDA not NCTR. Dr. Faustman then asked

about the interaction between NCTR and NIEHS/NTP on toxicogenomics. Dr. Walker said there is interaction between individual scientists at the two institutes, but the studies are not part of the IAG.
One of Dr. Walker's roles as the project officer for the IAG is to brief the TSSRC on the progress of specific FDA nominations. Dr. Howard added that his role as FDA liaison to the NTP BSC is to provide the FDA centers information on the discussions at these meeting.

Dr. Howard commented that the newly appointed Commissioner and Deputy Commissioner are focused on activities important for strengthening FDA's role in public health.

IX. Studies Supported through the Interagency Agreement with NIOSH

A. Characterizing Occupational Exposures for Hazard Assessment

a. Presentation

Dr. Mark Toraason, NIOSH, presented the studies that NIOSH has undertaken under the NTP/NIOSH Interagency Agreement. He said that the purpose of the Interagency Agreement (IAG) with NIOSH is to (1) capitalize on NIOSH's unique access to occupationally-exposed populations which provides real world context for toxicology studies, and to (2) utilize NIOSH's expertise to complement NTP and NIEHS' capabilities.

Nominations proceed through the standard NTP process with the study scientist, nominator, and NIOSH staff working together to identify studies that are most appropriately conducted at NIOSH.

Dr. Toraason discussed some of the NTP-related activities in which NIOSH has been involved.

(i) Asphalt Fume

NIOSH nominated asphalt fume after it had determined that fractions of asphalt fume contained components that were dermal carcinogens in rodents. The Heritage Foundation developed an acceptable device for generation of asphalt fume to simulate road-paving conditions and to assess the toxicity of the fume. The objective was for NIOSH to undertake preliminary studies to characterize the fume.. Characterization of the fume and its constituents would provide the bases for maintaining the integrity of a well-defined mixture during chronic animal testing. However, chronic studies were not initiated because of the expense, the previous finding that the fume was carcinogenic and because the Fraunhofer Institute planned to study the fume in a chronic study in rodents in Germany.

(ii) Cellulose Insulation

A private individual nominated cellulose insulation for study. Before NTP embarked on an animal study, they asked NIOSH to conduct a health hazard assessment of exposed workers and to characterize the material which involved

determining the particle size. They found low levels of respirable dust in the air and little evidence of lower respiratory tract effects; hence, chronic animal testing was not recommended.

(iii) 1- and 2-Bromopropane

OSHA nominated 1-Bromopropane, a potential replacement for ozone-depleting chemicals, due to a lack of definitive data of its uses and exposures in the workplace. Thus NIOSH, with NTP support, assessed occupational exposures to the bromopropanes, which have been summarized in two peer-reviewed publications. NTP has completed chronic testing of 1-bromopropane in animals and the information from workplace exposure assessments will be included in the technical report.

(iv) Welding Fumes

NIOSH nominated real-world exposures of welding fumes to the NTP for chronic testing. Although there was some evidence that welding fumes are neurotoxic, there was also some suggestion that these fumes might cause lung cancer, hence an animal study by NTP would provide needed data. To mimic real world exposures to the greatest extent possible, NIOSH designed and constructed a proto-type fume generator and inhalation exposure system to characterize the fume and evaluate exposure conditions. Animals were exposed under acute conditions to the fumes. However; based on the data obtained, NIOSH did not recommend scaling up of the exposure system because it would be costly to transfer the technology to another laboratory, and would provide hazard information limited to a single type of welding fume.

(v) Tungsten Oxide

Soluble tungsten was nominated to the NTP by NCI because of a leukemia cluster in New Mexico that was associated with elevated levels of tungsten in drinking water. The nomination brought to light exposure assessments in Sweden reporting the presence of tungsten oxide fibers in tungsten refining and manufacturing processes. NCI subsequently nominated tungsten fibers for testing by the NTP. NIOSH exposure assessments funded by NTP corroborated the presence of tungsten fibers with a 3:5 aspect ratio in the hard metal industry. Presently, NIOSH is determining the durability of the oxides in a variety of artificial and biological fluids. Depending on the data obtained, NIOSH will decide if a more extensive hazard evaluation is necessary.

(vi) Nanomaterials

NIOSH has begun a study to assess the feasibility of industry wide exposure assessments and epidemiological studies of nanomaterials. The agency will (1) determine workforce size, (2) identify data repositories for materials, processes, and handling, (3) develop specialized air sampling equipment and (4) conduct exposure assessments.

(vii) New NTP/NIOSH Activities for FY2009

These include the following studies:

- Cardiovascular toxicity of inhaled fullerene C60.
  NIOSH found that intratracheal instillation of carbon nanotubes caused cardiovascular effects in animals; i.e. a systemic effect. NIOSH will determine if rodents exposed to fullerenes in NTP inhalation studies exhibit comparable effects.
- Use of indium and indium compounds in the workplace.
  Indium phosphide was found to be a potent carcinogen in animals, which has raised concern regarding the toxicity of other indium containing materials. Although indium tin oxide is widely used in the United States, NIOSH has not been successful in adequately characterizing occupational exposures because of the difficulty in obtaining proprietary information from manufacturers. Absence of exposure information reduces the justification for toxicological testing. The absence of toxicological information reduces the need for exposure characterization. The absence of both types of information may result in chemical hazards going unrecognized for years. NIOSH and NTP are working together on potential hazards such as indium tin oxide to establish whether previously unrecognized hazards pose a risk for exposed workers.
- Exposure assessments of 2-methoxy-4-nitroaniline and 2'''-dithiobisbenzanilide.
  These chemicals were nominated to the NTP for testing based on the potential for exposure in the workplace. However, there is little information on occupational exposures for either of these chemicals. NIOSH will identify facilities and undertake pilot studies on exposure and determine if there is a need for additional studies.
- Diacetyl and other flavorings in food production facilities.
  Diacetyl is recognized as an occupational hazard in microwave popcorn production. NIOSH will assess comparable exposures to diacetyl in a variety of food industries.
- Neurological effects of welding fume.
  NIOSH will determine the extent of exposure in the workplace to different types of welding materials that may contribute specifically to manganese exposure. The nature of exposure regarding the valence of manganese and body burdens will be investigated.

Dr. Toraason concluded by saying that it has taken some to time to determine the best approach for interaction with NTP but it appears that this hurdle has been overcome.

b. BSC Discussion

Dr. Faustman applauded the interaction of NIOSH with NTP where the former provides human data and the latter supplies the animal data on a particular chemical. She added that frequently an adverse exposure is identified in an occupational setting before the magnitude of a problem is understood. She asked if NTP could help NIOSH in initiating a study by following up on an alert

they have identified. Dr. Toraason replied that often the limiting factor is gaining access to a facility. NIOSH has a right of entry capability but it is only rarely invoked. For the past two decades, NIOSH's approach has been to seek cooperation with industries. In the case of indium, NIOSH knew of its carcinogenicity in animals but has yet been able to gain access to facilities to determine the extent of exposure. Industries may be cooperative to an extent, but adequate exposure assessments fail to materialize for a variety of reasons. Dr. Germolec said working with NIOSH is an interactive process and exposure data feeds into animal studies and *vice versa* so the information from human data can be used to aid in the design of animal studies.

Dr. Solomon asked if a strategy has been developed to study solvent substitutions so that NIOSH can provide this information to NTP to prioritize chemicals for testing. Dr. Toraason said there is no strategy; in the past NIOSH has tried to develop strategies for systematically assessing workplace exposures for unrecognized hazards but none have been found to be particularly fruitful. An *ad hoc* approach of monitoring emerging issues via a variety of sources has been more effective and timely. Often, agents perceived to be hazards by the public become a priority for the agency and these rarely come to light via systemic assessments.

Dr. Portier asked about studying complex mixtures such as welding fumes and Dong quai. He suggested that a strategy be developed between NIOSH, FDA, and NTP as how to tackle the testing of these mixtures. Dr. Toraason replied that a problem with some mixtures such as welding fumes is their varying composition and their continuous change during use. An approach has been to study the most common formulations to determine which ones cause cancer, as a proof of concept. Dr. Bucher said that during use, welding materials undergo fractional distillation and volatilize when they come in contact with the side of the chamber housing animals. Since NTP has strict requirements for exposure to a specific concentration of the test article, testing of these materials has posed enormous technical difficulties. Dr. Howard added that NTP is aware of the difficulties agencies face in making regulatory decisions on complex mixtures as to what is best to regulate based on the end user, and what is best to study.

Dr. Toraason said that testing the individual constituents of metal working fluids in an *in vitro* assay to determine their toxicity or mutagenicity has been used to assess their hazard. The mixture is then tested *in vivo* in a short-term test to determine if the *in vitro* tests predicted the toxicity *in vivo*.

Dr. Walker said that different approaches are used for the testing of different mixtures to optimize the collection of data that will be useful for regulation.

B. NIEHS-NIOSH Interagency Agreement: Environmental/Occupational Immunotoxicology Studies in Humans

a. Presentation

Dr. Michael Luster, NIOSH (Health Effects Laboratory Division), provided a historical perspective of the NTP/NIOSH IAG established in the early 1990s to increase efforts to study non-cancer endpoints including immunotoxicology. Early efforts involved the establishment of an immunotoxicology human testing panel, a questionnaire, which could be incorporated into epidemiological studies, a health and immunology study following exposure to toxigenic fungi and to lead in exposed workers. He presented the highlights of studies relating to fungal allergens, latex and rubber contact allergens and genetic risk factors for irritant contact dermatitis (ICD).

(i) Fungal allergens

The primary focus of the NIOSH Fungal Allergen Project was to better understand human exposure and sensitization to fungal spores and fragments as sources of aeroallergens. In collaboration with the West Virginia University skin testing program, they found that sensitization to one fungus often resulted sensitivity to another mold species.

In studying the exposure of children in the Head-off Environmental Asthma in Louisiana (HEAL) study, NIOSH found that all the atopic asthmatic children tested positive in a mold mix skin test. Although *Aspergillus* and *Chaetonium* species usually grow in moist conditions, the children were not highly sensitive to these species. However, a very high prevalence of responders to *Alternaria* and *Epicoccum* was found; the latter genus is not usually found in the U.S. After home remediation in Louisiana, the children will be tested again.

Specific environmental and clinical immunoassays are being developed to better characterize personal fungal exposures. He described a confocal fluorescent halogen immunoassay (HIA) in which immunostaining of fungal spores and hyphal fragments collected from indoor environments in New York City demonstrated IgE binding to expressed antigens in human patient serum from a Puerto Rican cohort. Spores, because of their large size do not usually deposit in the lung but this study showed that hyphae and other fungal fragments are immunogenic.

(ii) Latex and rubber contact allergens

Latex and rubber products contain chemical polymerization accelerators that cause allergic contact dermatitis (Type IV hypersensitivity) as well as Type I pulmonary hypersensitivity. Human exposure is mainly via the use of medical and industrial gloves, condoms, bandages, surgical drains, and clothing.

Under the IAG, NIOSH developed a spectrophotometric assay that measures the primary accelerators in natural rubber latex (NRL) and nitrile gloves. There are three common classes of rubber accelerators, the mercaptobenzothiazoles (MBT), thiuram, and thiocarbamate type compounds. They assayed 38 brands

of latex and nitrile gloves and 14 brands of lubricated and non-lubricated latex condoms and provided the concentrations of accelerator content in glove brands to dermatologists to help manage patients with allergic contact dermatitis (ACD).

The guinea pig maximization test has been used for mechanistic studies of the two most prevalent latex contact allergen classes namely MBT and zinc dithiodiakylcarbamates. MBT must be oxidized to the disulfide for sensitization to occur. The sulfur center in thiocarbamates is important in vulcanization. When carbamates bind to metals and zinc centers in proteins, the latter become antigenic.

(iii) Genetic risk factors and Irritant contact dermatitis

Two approaches can be used to study genetic risk factors, namely, identifying candidate genes or performing genome-wide scans. NIOSH researchers prefer the candidate gene approach because it is hypothesis driven and gives fewer false positives than a genome-wide scan. They are evaluating polygenic responses rather than rare alleles as these are more likely to be associated with immune-related diseases that are influenced by the environment.

Skin diseases are the second most common occupational disease and 80% of all occupational and environmental skin diseases are irritant contact dermatitis (ICD). NIOSH is conducting a workplace-related ICD genetic study by determining if they can identify individuals who are sensitive to a panel of common irritants and determine whether their genetic make-up is correlated to their responsiveness. It is hoped that their sensitivity can then be used as a predictor for ICD. The study population consists of 700 health care workers with no existing skin disease, who wash their hands at least 8 times a day. The study duration is approximately 2.5 years.

Phase I consisted of a pilot study to determine an irritancy dose range using a patch test with three irritants; namely benzalkonium chloride (BKC), sodium hydroxide (NaOH) and sodium lauryl sulfate (SLS). They assessed inter- and intra-individual variability and determined an irritant threshold, i.e. the lowest concentration producing a visible inflammatory response. Some individuals responded highly to the irritants while others did not.

In the second phase, the final dose range for each irritant was selected. Two genetic analyses panels will be used; one includes a customized panel of ~5000 SNPs and a second a commercial panel that measures the major histocompatibility complex (MHC) genes. Workers were tested monthly over a 6-month period. The study will assess a possible association between the Patch Test versus transepidermal water loss as a measure of dermatitis and associations between variants.

Preliminary observations showed that 63% of volunteers developed hand dermatitis; of those with dermatitis, 72% hand washed their hands ≥10 times/day.

Of these subjects, 77% who reacted to 2.5% SLS and 74% of subjects who reacted to 5% SLS, developed dermatitis whereas non-reacting subjects only developed dermatitis at a rate of 33% and 26%, respectively. They found strong genetic associations with two groups of genes: those involved in anti-oxidant activity and those involved in pro-inflammatory responses.

It appears that frequent hand washing (≥10 times/day) may predispose to development of ICD and that Patch testing with SLS and genetic make-up may predict future development of dermatitis and identify sensitive individuals.

He identified the FY 09-10 studies.

- A mold study will characterize a hemolysin from *Aspergillus terreus* and develop an animal model for airway exposure to dry fungal aerosol as part of the HEAL study.
- The role of genetics in environmental/occupational diseases will be studied for chronic beryllium disease, allergic contact dermatitis to metals and occupational asthma to isothiocyanate and baker's asthma.
- Asthma prevalence from disinfectants in the service sector.
- Allergic sensitization from exposure to indoor air products such as diacetyl.
- Determination of total IgE, antinuclear antibodies and atopy in participants of the Upper Midwest Health Study.
- Production of toluene diisocyanate-conjugated protein monoclonal antibodies and epitope recognition mapping.
- Immune and inflammatory response to isocyanates and its effect on occupational rhinitis.

b. BSC Discussion

Dr. Luster agreed with Dr. Nagarkatti that the TH1 and TH2 cytokines would indicate the type of antibody being produced and that they have correlated IgE production with the type of cytokines produced. She wondered if functional studies with mast cells undergoing degranulation could be used as a biomarker for isocyanate exposure and Dr. Luster replied that this is not feasible. In Canada, researchers have collected nasal and lung gavages from patients challenged with isocyanate. If they see a variant of TNFβ, or IL1, which might be associated with asthma, they will measure IL2 levels in the gavaged samples.

Dr. Faustman was excited to learn that immune response to some molds is due to hyphae rather than spore proteins. Dr. Germolec said that EPA scientists have reported that hyphae fragments cause a significant allergic response. She added that in the HEAL study, which is a collaboration of NIEHS with Tulane University and NIOSH, biomarkers of exposure are being measured in children post Katrina and this data will be useful for the mold studies that NTP is beginning. Besides the structured projects through the IAG with NIOSH, there are research projects at the agencies that provide mechanistic information that provides information to the more traditional endpoints that NIEHS measures,

hence leveraging resources. NIOSH has performed elegant work in determining the substances responsible for sensitization in humans following exposure to latex gloves and in developing an animal model to ascertain what component in the gloves is causing the sensitization.

Dr. Friedman-Jimenez was impressed with the work on latex materials as determining the mechanism of these allergies and being able to help persons with them has allowed many to remain in their jobs. He sees many types of allergic reactions, the causes of which are unknown. He added there is a dearth of information on allergic diseases and immunotoxicologic effects. This type of definitive information is extremely useful to physicians and aids them in making important decisions regarding treatment.

Dr. Solomon asked if the biomarkers of exposure to isocyanate and molds will be available for clinical use, and Dr. Luster replied that most of the kits for molds studied by West Virginia University are available commercially. NIOSH has a large number of monoclonal antibodies that recognize 2,4 diisothiocyanates that need to be better characterized before they become commercially available.

X. Concluding Remarks

Dr. Birnbaum thanked the BSC for their active review of the NTP's activities. She also thanked NTP staff for their presentations. She added that the interaction of the NCTR and NIOSH with NTP through the IAG have been extremely important and she wanted to develop similar collaborations and IAGs with other federal agencies. Dr. Bucher thanked Dr. Birnbaum for her support, the NTP staff for their work, and the BSC reviewers for their very useful comments.

The meeting concluded at 12:45 p.m.