National Toxicology Program
Board of Scientific Counselors

June 22, 2007

National Institute of Environmental Health Sciences
Research Triangle Park, NC

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I. Attendees

Members in attendance:
Kenny Crump, Louisiana Tech University
Prescott Deininger, Tulane University
Katharine Hammond, University of California at Berkeley
Nancy Kerkvliet, Oregon State University (on the phone for part of the meeting)
Gail McCarver, Medical College of Wisconsin (Chair)
Jon Mirsalis, SRI International
Harish Sikka, State University of New York at Buffalo
Keith Soper, Merck Research Laboratories
Vernon Walker, Lovelace Respiratory Institute

Members not in attendance:
Christopher Bradfield, University of Wisconsin
Germaine Buck Louis, National Institute of Child Health and Human Development (NICHD/NIH)

***Ad Hoc* Members**
Agnes Kane, Brown University
Raymond Novak, Wayne State University
Martin Philbert, University of Michigan
Jean Regal, University of Minnesota
Jim Riviere, North Carolina State University

NIEHS Staff
Alma Britton
John Bucher
Rajendra Chhabra
Helen Cunny
Greg Danko
Allen Dearry
Gordon Flake
Christine Bruske-Flowers
Paul Foster
John (Jef) French
Melissa Gentry
Donald Gula
Angela King-Herbert
Michelle Hooth
William Jameson
Grace Kissling
Ruth Lunn
Scott Masten
Robin Mackar
Daniel Morgan
Joseph Roycroft
William Schrader
Barbara Shane
Robert Sills
Cynthia Smith
William Stokes
Matthew Stout
Gregory Travlos
Molly Vallant
Nigel Walker
Samuel Wilson
Kristine Witt
Mary Wolfe

Other Federal Agency Staff
Diane Frasier, NIH

Paul Howard, FDA/NCTR
Dennis Lynch, NIOSH
Linda Pellicore, FDA

Public

George Burdock, Burdock Group
William Calore, Constella Group
Reshan Fernando, RTI International
Samuel Garner, ILS
Susan Kinney, ILS
Sandy Lange, Public Citizen
Sue Leary, Alternatives Research & Development Foundation
Allen Levesque, ILS
Joseph Manuppello, PETA
Bobbie Peterson, RTI
Catherine Price, RTI
Ivan Rusyn, UNC
Timothy Schur, Constella Group
Catherine Willett, PETA

II. Introductions and Welcome

The National Toxicology Program (NTP) Board of Scientific Counselors (BSC) met on June 22, 2007, at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. Dr. Gail McCarver, Chair, welcomed everyone to the meeting and asked the BSC members and attendees to introduce themselves. Dr. John Bucher, NTP Associate Director, NIEHS, welcomed and thanked the BSC members for their attendance and service to the NTP. Dr. Gail McCarver introduced Dr. Samuel Wilson, Deputy Director of the NIEHS and the NTP.

III. Accomplishments at NIEHS and NTP

Dr. Samuel Wilson, acknowledged attendance by Ms. Diane Frasier, Head of the contracting activity at NIH, who he said would address the BSC later, and thanked the BSC for their involvement in the NTP. He congratulated Dr. Bucher on his appointment as Associate Director of the NTP.

A. Presentation

Dr. Wilson reviewed the budget at NIEHS and NTP. He said the budget for NIEHS is acceptable for next year with an appropriation of $721M including Superfund. The budget for FY 2008 appears to be stable. A key emphasis of the institute is to maintain the funding rate of RO1s to at least 20% of submissions. The NIEHS makes 400-450 awards per year, which is in the middle of the funding range of all the NIH institutes. Since 2001, the number of applications has increased but, despite this increase, the funding rate has remained at about 20% annually.

At the last BSC meeting, Dr. David Schwartz, Director of NIEHS and NTP, said the NIEHS would begin implementing certain aspects of the NTP Roadmap and integrate those activities into NTP's ongoing research and testing program. Dr. Wilson said in the future, the NIEHS would try to link basic research programs to public health outcomes. The challenge will be to integrate fundamentally new approaches, such as high throughput screening and the use of animal models, into the research and testing program. A team approach is needed to implement these new initiatives, which cannot

happen in isolation but needs to involve the entire environmental health community. In conclusion, he challenged the BSC to help the NTP link these new technologies with human health, disease, and public health policy. Dr. Wilson said he was looking forward to working with the BSC, Dr. John Bucher, the NTP, and the entire community to implement these new programs.

B. BSC Discussion

Dr. Paul Howard asked whether the average grant award has remained constant over the last five years or whether it is decreasing. Dr. Wilson responded that he thinks the awards are increasing slightly. During the past few years the requested budget for some proposals has increased. In the future with a relatively fixed budget, the size of the awards may be reduced to maintain the 20% funding goal.

Dr. McCarver said the perception among the extramural community is that very few grants are being funded on the first-round and they have to be re-submitted. She asked whether NIEHS examined whether the approval rate for funding of first submissions has changed over the past few years. Dr. Wilson replied that he has heard this same concern and criticism, but he is not sure it is true. Budgets are complex and are probably study section specific. Understanding and differentiating the way in which different study sections decide on funding has become more difficult and more challenging for the extramural community.

Dr. Wilson then presented certificates to BSC members whose term of duty would end in December 2007. He thanked Drs. Deininger, Louis, Sikka, and V. Walker for their service to the NTP.

IV. NTP Update

Dr. John Bucher, NIEHS, thanked Dr. Wilson and Dr. Schwartz for their confidence in his ability to lead the NTP as Associate Director. He praised Dr. Allen Dearry, the interim associate director of NTP, for his leadership over the past 18 months and said many of the new initiatives became realities because of his persistent efforts. Dr. Bucher said there are plans underway to realign NTP within the Division of Intramural Research (DIR) resulting in the creation of a distinct National Toxicology Program that is separate from the Environmental Toxicology Program. He believes this new structure will help the extramural community understand where the NTP lies in the NIEHS organizational structure.

NTP will report to Dr. Perry Blackshear, Acting Scientific Director, NIEHS, and Drs. Wilson and Schwartz. This realignment of NTP will more clearly define its roles and functions, provide an identifiable home for the NTP within NIEHS, bring analysis activities into the program office, and create a structure for program development. The Laboratory of Experimental Pathology will become the Cellular and Molecular Pathology Branch. Two new branches will be created, a bimolecular screening branch, which will house the high throughput screening initiative, and a host susceptibility branch. These new branches will aid in accomplishing some of the goals Dr. Wilson articulated earlier

regarding collaboration with the DIR. This new structure makes the NTP budget distinct within DIR as opposed to being incorporated within the Environmental Toxicology Program.

1. Technical Reports Review Subcommittee (TRRS)

The TRRS held a meeting on May 16-17, 2007 to peer review seven draft NTP Technical reports, two of which received considerable press coverage. The first report, which detailed studies of hexavalent chromium in drinking water, found a dose-response relationship between the intake of hexavalent chromium in drinking water and squamous cell papillomas and carcinomas of the oral cavity in rats and adenomas and carcinomas of the small intestine in mice. The second study investigated whether feeding ethinyl estradiol to the F_0 and F_1 generations causes changes that would be reflected in offspring in multiple generations, and whether this effect would be enhanced in subsequent generations. Body weight suppression, accelerated puberty and estrous cycles in females and mammary gland hyperplasia in continuously exposed males were reported, but there was no consistent carryover into unexposed generations. There was equivocal evidence of carcinogenicity in male and female rats exposed to ethinyl estradiol in various F_1 and F_3 arms of the study.

2. Center for the Evaluation of Risks to Human Reproduction

Prior to an expert panel meeting on bisphenol A (BPA) held on March 5-7, 2007, allegations were raised about potential conflict of interest (COI) by the support contractor. The contractor provided administrative support for preparation of the first draft of the expert panel report, which consists of factual synopses of papers considered relevant for review. The expert panel used the first draft as a template for preparing its draft report, which included requesting changes and additions to the text. The draft report was released for public comment in December 2006. The draft report was edited and prepared as a track-changed version for discussion at the March meeting. Because of potential COI, the contractor was removed from providing support at the March meeting. The expert panel made extensive revisions to the draft report; however, due to the volume of information for consideration, the panel did not reach conclusions about the potential reproductive and developmental hazards of BPA. An interim draft report based upon discussions at the March meeting was released for public comment in April and a second expert panel meeting is planned for August 6 -7, 2007.

In response to the allegations of potential COI, the NTP formed a working group of the NTP BSC to review all NTP contracts for potential COI. NTP also worked with NIH and the Office of General Counsel to develop COI language for incorporation into all current and future contracts.

The NTP has begun an audit of the August “template” report and the December and March draft reports that included the publications identified by the contractor for the panel’s consideration for the draft BPA reports. The NTP is also auditing the fidelity of changes requested by the panel that the contractor made in the various drafts of the expert

panel report. Dr. Bucher said the review of the NTP contracts would be discussed later in the meeting.

The NTP Briefs on genistein and soy formula are in revision and the NTP Brief on hydroxyurea is in preparation. The NTP Brief gives the government's opinion on whether a chemical is a hazard for human reproduction or development. It is based upon information in the expert panel report, public comments, and any new information available following the expert panel meeting.

3. Update on Nominations

The NTP has changed the nomination review process to include presentations and discussion by the BSC of research concepts that outline initial plans for the research program for a nominated substance.

4. Host Susceptibility Initiative (HSI)

The NTP is creating a Host Susceptibility Branch under Dr. John French as the acting director. This branch will study the genetic basis underlying biological responses utilizing multiple strains of genetically modified inbred mice to distinguish genetic from kinetic differences in response to various toxic substances. Rather than using the contract mechanism, the NTP will involve research partners in this activity. The NTP is planning to implement this activity using a program modeled after the NCI Rapid Access to Intervention Development (RAID) Program in which projects are proposed by outside parties. These projects would be reviewed for technical merit and scientific feasibility by a study section arranged by NTP. The selected studies would be carried out utilizing NTP contracts and resources, and scientists proposing the study would analyze the data. NTP would not provide funding to the research partners.

Currently NTP is involved in the environmental airway disease project, which is part of the NIH Genes Environment and Health Initiative. The environmental airway project involves the exposure of 15 strains of inbred mice to four different agents, either acutely or for 5-weeks, with the measurement of a number of immediate or delayed endpoints.

5. NTP High Throughput Screening (HTS)

The NTP has established an interagency agreement with the NIH Chemical Genomics Center (NCGC), which is part of the NIH Molecular Libraries Initiative (MLI). This collaboration will enable NTP to generate information that can link data on the biological activity of environmental substances with the toxicity endpoints identified in the NTP studies. The NCGC tested 1408 compounds supplied by the NTP in cytotoxicity and caspase assays and will test these same compounds in 60 assays with varying endpoints including cell signaling and DNA repair. It is hoped that profiles of biological activity can be used to categorize different chemicals and different chemical classes as a means of prioritizing substances for further evaluation and more sophisticated testing by the NTP. A second set of 1408 chemicals will be sent to the NCGC shortly for testing in assays relevant to carcinogenesis and immunotoxicity. In the latter case, the data will be compared with that from human lymphocytes and hopefully provide a bridge between

human and animal data. The NTP presented three posters at the 2007 SOT meeting on the HTS initiative and organized a symposium at the Society of Bimolecular Sciences. Collaboration with the EPA continues in their development of a recently announced ToxCast program aimed at generating information on biological outcomes in a wide variety of *in vitro* and lower animal *in vivo* assays.

6. Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)/ NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) Five-year Plan

This plan is being compiled by ICCVAM with support from NICEATM in response to a request from the U.S. Senate and House Appropriations Committees to project their activities for the next five years. Presently, NICEATM is evaluating a number of *in vitro* assays for their validation status including assays in EPA's endocrine disruptor screening program, refinements to the *in vitro* ocular toxicity test, additional *in vitro* pyrogenicity assays, and *in vitro* alternatives for testing vaccine efficacy and botulinum potency.

NICEATM and ICCVAM are expanding their role in developing guidance documents for the Organization of Economic Cooperation and Development (OECD), which requires acceptance of data from regulatory agencies. Changes to guidelines in Europe relating to the acceptance of *in vitro* assays for various endpoints often affect the regulatory processes in the United States. NICEATM is becoming involved in the development of these guidelines from the outset and will continue its efforts in global harmonization through interactions with the European Center for the Validation of Alternative Methods (ECVAM) and the new Japanese Center for the Validation of Alternative Methods (JaCVAM).

7. Notes on Dr. Bucher's Appointment

Dr. Bucher said he has been affiliated with the NTP for 24 years and pledges to uphold the excellent goals of the program. He believes the location of the NTP program within NIEHS has been beneficial both physically and scientifically, as it has allowed the program a broader scope than if it were located within a regulatory agency. The NTP is recognized internationally as a leader in toxicology. Strengths of the program include its assessment activities, such as development of the Report on Carcinogens, identification of potential reproductive and developmental hazards by CERHR, and the work toward the validation and regulatory acceptance of alternative methods by NICEATM and ICCVAM. The budget is improving and the program needs to use its funds as efficiently as possible to tackle issues important for the protection of public health, which the NTP can generally address more ably than other government groups. While maintaining its present activities, the program will also focus on development of new methodologies such as high throughput screening and through the host susceptibility initiative how the genetic underpinnings of a particular species or strain might alter its toxicological responses. The NTP needs to provide guidance to regulatory agencies for the proper utilization of these new types of information for hazard identification, hazard characterization, and use in regulatory decision-making.

V. Review of Contracts

A. Presentation

Ms. Diane Frasier, Acting Director of the Office of Acquisitions and Logistics Management, NIH, presented the draft report from the NTP BSC Working Group for the Review of NTP Contracts (WG) summarizing the process, findings, and conclusions. The members of the WG included three contract analysts, a past and a future member of the BSC, and one NIH intramural scientist. The WG began its review on April 26, 2007, with a goal to be completed by July 1, 2007. The objectives of the review of NTP contracts were to (1) assess potential for conflicts of interest (COI), (2) consider what recommendations might be appropriate to reduce the potential for COI to occur, and (3) address how to mitigate any current or future COI.

Ms. Frasier provided information on current COI regulations, which the WG deemed insufficient. She noted that the Federal Acquisition and Regulation (FAR), a series of regulations that concern the requirements of contractors that sell goods or services to the government, addresses organizational conflict of interest (OCI) of contractors, but not personal COI. Personal COI can result from an individual's activity or personal relationship with an agency's contracting staff, their spouse, and their families. OCI addresses unequal access to information, unfair advantage in future competitions that might provide a competitive edge for a contractor, and impaired objectivity. At the present time, contractors are not required to provide their COI policies to the contracting officer.

NTP staff and NIEHS acquisition staff reviewed the 42 active NTP contracts and triaged them into one of three bins: high, moderate, and low, based upon a perceived level of risk for COI. The WG reviewed the contract statement of work, the contract, and a statement of objectives prepared by the contract's Project Officer that summarized the contract's purpose, management oversight by the government, and output or product of the contract. Using this information, the WG revised some of the initial COI risk determinations. The contracts were assessed as 9 high risk, 11 moderate risk, and 22 low risk.

The WG sought additional information from contractors determined to be at either high or moderate risk for COI. The WG was limited by the Paperwork Reduction Act of 1980 regarding the number of contractors that could respond to the questionnaire. Nine contractors received the full questionnaire that sought information about both OCI and personal COI, as well as information on their corporate affiliations and firewalls to separate work for the government from that of other corporate clients. A tenth contractor responded to a different set of COI compliance questions under the authority of 45 Code of Federal Regulation, Part 94, which addresses objectivity in research for government contractors.

None of the contractors reported an actual or potential COI. Each contractor's COI policy was evaluated to determine its compliance with the requirements of 45 CFR 94. Most of the contractors had written COI policies. Only a few contractors applied COI policies to subcontractors and collaborators. Most contractors complied with an official

review of financial disclosure statements, few complied with the requirement to update financial disclosures, and very few maintained those disclosures for three years. The majority complied with the guidelines for an institutional official who is responsible for COI issues, but few contractors said they provided for sanctions when COI was identified. The majority of the contractors had firewalls and a few met the requirement for nondisclosure agreements. None said they had contract staff with affiliations or relationships that could lead to potential COI and none were aware of any actual or potential organizational COI in any of their contracts.

The WG identified some best practices by NTP that encourage objectivity and minimize the risk for COI, which were either already in place or under development. They noted that NTP's pathology review process promotes objectivity in research by using multiple contractors to carry out different steps in the process.

The WG also reviewed a new COI clause that had been developed by the NIEHS in concert with the Office of General Counsel (OGC) and the NIH Division of Acquisition Policy and Evaluation in the Office of the Director. To ensure best practices, this clause is now being inserted into NTP contracts.

Summary and Recommendations

The WG did not find evidence of actual or apparent COI. The WG recommended a number of best practices and areas where NTP as well as NIH could make improvements regarding COI and contracts. Ms. Frasier noted those recommendations, which are detailed in the WG's report. The WG recommended that guidance on COI include OCI and personal COI and apply to both research and development (R&D) and nonR&D contracts, and that education and training about COI be available for both staff and contractors.

B. BSC Review and Discussion

Dr. Prescott Deininger, the first BSC discussant, thanked the WG for its tremendous effort in completing the review in such a short time period. He noted the importance of COI being adequately addressed for government contracts. He is pleased the government will provide guidelines on COI policies and suggested that the policies have standard wording so that completion of the paperwork is not onerous. He asked whether COI policies are in effect for grants and Ms. Frasier responded that the NIH grant community would be developing requirements for COI. She added that the government must address COI, but also be realistic about COI requirements otherwise contractors will not want to do business with the government.

Dr. Jon Mirsalis, the second BSC discussant, said he works for an institution that is one of the largest holders of contracts from NIH and he is the principal investigator on four contracts, but none from NIEHS. He congratulated the WG on its tremendous effort. He commented on what he called "scientific COI" or the notion that a person's financial holdings or relationships might impact their scientific decisions and judgments. He did not see scientific COI happening within NTP because it segments its contractors and has

review boards to review the science at different levels. In his opinion, the data generated by the NTP are absolutely impeccable. He said contractual COI, or the idea that a contractor through its activities could gain knowledge and insight that makes it more competitive, is much harder to control. A contractor who is awarded a contract gains experience, knowledge, and insight into the needs of the program that are far beyond what is specified in the statement of work and this gives them an advantage. He suggested that the turnover rate of contractors is probably very low and it is probably difficult to unseat an incumbent. He said there should be ways of separating a cozy relationship between existing contractors and program staff to truly encourage more competition. Finally, he said COI paranoia should not exclude the most qualified individuals in an organization from participating in reviews. He recently abstained from reviewing a draft NTP technical report because of the perception of a COI that could be misconstrued by the public. It related to funds his institution received about 12 years ago from an organization that was a defendant in a litigation case involving the compound being reviewed. He said the NTP should draw a rational line between what is appearance, but not really a COI, from what is really a COI. This distinction is important but difficult, and he encouraged the NTP to consider it in the future.

Ms. Frasier commented on the incumbency issue and said it is not just an NIH issue, but a government-wide issue. Congress is very interested in increasing competition, but when an incumbent does an excellent job in the performance of a contract, and thus has a better understanding of the work involved, the chances are that the incumbent will have an advantage in the next solicitation.

Dr. McCarver said she particularly liked the WG's recommendation to focus on education of contractors. She noted that although the contractors stated that they had no employees with COI, they did not request financial disclosure statements from their employees. Ms. Frasier said one way of educating contractors might be to work with trade associations to which contractors are affiliated, so that COI could be an item on future meeting agendas.

Dr. Katharine Hammond asked how the WG could determine that there was no COI if the relevant information was not collected on all 42 contracts, but only on 10 contractors, with some of contractors having inadequate procedures for determining COI. Ms. Frasier responded that by reviewing the statement of work and the contract document and questioning the Project Officers regarding the tasks, oversight, and products of the contract, the WG members understood the role of the government and the role of the contractor. The WG determined that even when a contract was binned as "high risk" for potential COI, there was no likelihood of it occurring because the government had strong oversight of the contract's activities.

Dr. Hammond asked whether the WG reviewed all the contracts for each contractor to ensure that a COI situation did not exist. Ms. Frasier replied that only NTP contracts were included in the review and that the WG relied on the contractor's responses to the questionnaire. Dr. Hammond wondered whether in the future a contractor would have to provide the government the names of all the organizations with whom they have a financial relationship. Ms. Frasier replied that the contractor and its employees would

have to provide that information to the institutional official in the company who would have to certify that no conflicts exist. However, if the government had concerns, they could audit the contract.

Dr. Hammond expressed concern regarding the difference in the binning of high-risk contracts by the NTP and the WG. Ms. Frasier said the NTP and NIEHS began binning the contracts at the same time that the WG was proposing its standards to categorize the contracts, and thus the WG had more information to determine the bin. Dr. Bucher added that he was involved in the initial binning by NTP and another reason for the differences in binning is that the NTP had a much greater knowledge of the management oversight for the contracts than the WG. As the review process proceeded, the NTP and WG reached the same conclusions on the categorization of each contract.

Dr. McCarver called for acceptance of the report. Dr. Mirsalis moved to accept the report and Dr. Deininger seconded the motion. The chair reminded the BSC that Dr. Vernon Walker would abstain from voting because of a COI. The motion passed unanimously with 6 yes and 0 no votes and one abstention.

VI. Nominations

Dr. McCarver said the discussion of the nominations would be handled differently for this meeting to allow the public to participate in the discussion and hear about the research proposals the NTP has developed for the nominations. The BSC will vote on whether the nomination warrants study by the NTP and not on the types of studies that the NTP might perform. She briefly outlined the process for this agenda topic. The study scientist will make a presentation on the nomination, followed by comments on the concept by a BSC member and an *ad hoc* reviewer. Public comments would then be entertained followed by further BSC discussion and voting. Following the meeting the Board and *ad hoc* primary reviewer would compile their comments and those that ensued at the meeting into a final document. These reviews are available at http://ntp.niehs.nih.gov/go/32562

Dr. Scott Masten contrasted the current process with how nominations were reviewed previously. In the past, the study design came later in the process, but this new approach allows the BSC and public to have more input on the future study of the nomination.

1. Nanoscale Silver

A. Presentation

Dr. Nigel Walker briefly outlined the nomination of nanoscale silver and its research concept by describing the molecular forms of silver, their antibacterial properties, its use as a dietary supplement and the rationale for the study. He outlined the known toxicity of silver salts to humans and indicated the paucity of knowledge regarding nanosilver. This study is part of a larger NTP initiative on nanomaterials to understand the key physical properties of nanoparticles and their absorption, distribution, metabolism and excretion (ADME). One key question is whether nanoscale silver will have the same properties

and biological effects as ionic silver and silver salts. The surface area of a particle increases as the particle size decreases; therefore, it will be important to evaluate multiple materials of different sizes to understand how a change in size changes the potency or the biological properties of the material. For all studies, a silver salt will be included as a standard.

Three aims are planned to:

- Characterize the relationship between the size of a nanoscale silver particle and its degree of ionization to the silver cation.
- Evaluate the effect of particle size and ionization state on the pharmacokinetic profile of nanosilver.
- Evaluate the effect of particle size and ionization state on the toxicological profile of nanosilver *in vivo*.

B. BSC Review and Discussion

Dr. Jim Riviere, the first *ad hoc* reviewer, said that silver is an interesting compound because it is ubiquitous and therapeutically useful. The basic question is whether the nanoscale nature of silver affects its toxicity and ADME properties. He is pleased the NTP will first characterize the different forms of silver, keeping track of both the ionized and metallic forms in *in vitro* studies, and then pursue ADME studies. He is concerned about the choice of a soluble silver salt as a control and suggested that another nanoparticle, such as a fullerene, be used instead. The toxicity of the nanosilver may be due to the amount of silver being ionized and released, which may depend on where it occurs and the structure of the nanoparticle. He said that the wide use of silver as a supplement and in other therapeutic mixtures justifies its study by the NTP.

Dr. Martin Philbert, also an *ad hoc* reviewer, agreed that the concept should move forward quickly so that high quality, reproducible data could be obtained that would benefit federal agencies. He said the fundamental difference between soluble silver and particulate silver is that the latter can occur at much higher concentrations within the local vicinity of the particle. While ADME might be important, it will be necessary to understand how much silver is released locally and how it affects a specific target cell in a tissue. It is known that these materials tend to congregate in the epithelial system and muscle capillary beds, thus NTP should consider micro as well as macro ADME. Since silver is not used in isolation but is mixed with palladium and platinum and often marketed in a mixture, he advises the NTP to examine silver in the presence and absence of these metals. In addition, he suggested that since silver is applied to the skin, its properties and toxicity should be considered in the presence of ultraviolet, infrared, and near infrared radiation.

Dr. N. Walker responded that integration of the study of nanoscale silver with other nanoparticles that the NTP plans to study is a key consideration, but he wants to keep this study focused on the comparison of ionic silver with its nanoscale form and is unsure whether the inclusion of another nanoscale material as a control will be useful.

Dr. Bucher asked whether the nanotoxicology community believes that there is a particular material that could be used as a control based on its particulate characteristics rather than its chemical characteristics. Such a particle could be used as a benchmark against which all the particles could be compared.

Dr. Howard agreed with Dr. Bucher and said there are no positive standards for nanotechnology or a particle that is precisely manufactured for which we know its toxicological effects. All the federal agencies are grappling with this question. The OECD, FDA, and NIST have discussed development of a panel of nanomaterials that could be considered as standard materials. Dr. N. Walker said NIST is planning a conference in September to develop a 5-year plan regarding the manufacture of reference particles and their characterization.

Dr. Philbert said there are a number of neutral polymers, such as methacrylates, with tight size characteristics and are nondestructible in biological systems that could be used as control materials. There are also more reactive organically modified silicates of ±1 nm in diameter that might be appropriate controls.

Dr. Paul Howard said the FDA commends the NTP for these enhanced reviews on the nominations, as the additional advice will result in better studies, which will be beneficial to regulatory agencies. He said FDA has no jurisdiction on the use of silver as a dietary supplement, but if manufacturers make health claims, it falls into the category of a drug for which FDA has jurisdiction. Nano-coated underwear and socks involve the Consumer Product Safety Commission and other federal agencies that are interested in the potential toxicity of nanosilver but who do not have the resources to study their potential adverse effects.

Dr. Hammond made a motion for approval of the concept and it was seconded by Dr. Keith Soper and approved unanimously by the BSC with 8 yes votes, 0 no votes, and no abstentions.

2. *o*-Phthalaldehyde

A. Presentation

Dr. Michael Wyde presented the background to the nomination of *o*-phthalaldehyde (OPA). He said more than 300,000 health care workers in the United States are exposed to the technical solution that contains 0.56 % OPA (w/v) but there are no vapor pressure measurements for this solution. There are no toxicology data for OPA only a number of non-peer-reviewed summaries that appeared after the 1999 approval of OPA use by FDA. The lack of toxicology data has been exacerbated by the unwillingness of the manufacturer to release unpublished toxicology studies to NIOSH and the legal inability of FDA to provide the data that were received in support of the submission. Although OPA is marketed as a safer disinfectant to glutaraldehyde, there are reports that health care workers exposed to OPA experience similar adverse effects to those experienced by workers exposed to glutaraldehyde. FDA approved OPA based on its use on medical devices.

The goal of these studies is to better understand OPA's toxic effects and better protect health care workers and patients. NIOSH plans a study of exposed workers and the NTP program will study its toxicity. The Material Safety Data Sheet (MSDS) for the 0.5% formulation of OPA states that it has a high LD50; however, based on the toxicity of glutaraldehyde and OPA's similar chemical structure, it is likely that OPA may elicit dermal and respiratory sensitization and irritant effects.

The aims of the study are to:

- Determine the vapor pressure of OPA in solution at workplace-relevant concentrations.
- Investigate the potential for OPA to cause dermal and respiratory irritation and sensitization in rodent models.
- Conduct ADME studies in rodents to determine systemic exposure.
- Assess toxicity and potential systemic effects following inhalation exposure.

Glutaraldehyde may be included for comparative purposes in some of these studies.

B. BSC Review and Discussion

Dr. Nancy Kerkvliet, a BSC reviewer, questioned whether dermal toxicity and hypersensitivity need to be studied in animals as glutaraldehyde, which is promoted as being safe, has been tested for dermal toxicity and hypersensitivity. She said chronic toxicity data are not needed because risk assessment is based on immunotoxicology, but she agreed with a study of health care workers to monitor exposure and determine if sensitization in the workplace had occurred. She thought that workplace regulations for OPA are probably based on glutaraldehyde exposure and therefore are probably quite strict. She questioned whether the proposed animal tests would be informative and said data from these studies would not change workplace practices. Rather, she encouraged the NTP to pursue the possibility of obtaining the requested toxicity data from FDA. In summary, chronic toxicity testing has a low priority, but the study of dermal and respiratory toxicities has a higher priority. In her opinion, the research proposal has a low priority.

Dr. Jean Regal, the *ad hoc* reviewer, agreed with Dr. Kerkvliet about the frustration of not being able to obtain the data from the manufacturer. However, she said because OPA is positive in the LLNA assay and elicited sensitivity, there is a high probability of it being a sensitizer and a hazard to humans. She stated that workplace exposures need to be monitored and workplace practices managed. Marketing practices might indicate that the substance is not hazardous; hence, health care workers are not minimizing exposure. She was more enthusiastic about the sensitization studies than the ADME studies in animals and said data from the former studies might be useful. In her opinion, the priority for testing is low to moderate and she would prefer that NTP be able to review the FDA data.

Dr. Wyde said that exposure of patients is very different from that of health care workers and that OPA was approved for use on medical devices. Multiple efforts have been made to obtain the information from the manufacturer and FDA, but NTP has been unsuccessful. The manufacturer claims that the 0.56% solution used on medical devices does not cause irritation or sensitization yet the health care workers exposed to this same concentration are complaining of these effects.

Dr. Dennis Lynch said NIOSH has received approval to undertake a study on health care workers, and to evaluate current work place practices. However, NIOSH would benefit from obtaining the toxicology data from NTP. In a future study, health care workers and workers in OPA manufacturing facilities will be tested for IgE antibodies and IgE-human serum albumin (IgE-HAS) conjugates through an interagency agreement with NTP.

Dr. Hammond said there have been a number of case reports to NIOSH about OPA sensitization, but no epidemiology study has been undertaken on workers exposed to OPA. It was approved as a substitute for glutaraldehyde because glutaraldehyde has not been well controlled in the workplace.

Dr. Howard stated that approval for use of a compound is based on its intent and purpose of use, which for OPA is as a residue on medical devices, independent of consideration of workplace exposures or toxicity to health care workers. He said legally FDA cannot release information from the manufacturer to other agencies, but the manufacturer could provide the data if it wished.

Drs. Mirsalis and Soper agreed that human studies would be the most useful and relevant; however, it would take two years until the data are available. Dr. Soper said since the data submitted to the FDA are confidential and cannot be obtained, it is unknown what information FDA has. He speculated that perhaps FDA would not release the data because there is high risk of sensitivity to OPA.

Dr. Bucher said each agency would study different aspects of the nomination; the worker and exposure studies would be undertaken by NIOSH and the toxicity studies by NTP. Dr. Wyde reiterated Dr. Lynch's statement that the toxicity data are important to NIOSH and they would complement NIOSH's program.

Dr. Howard said FDA does not have the data. He added that since it is used to sterilize medical equipment, it cannot be inert.

Dr. Hammond proposed and Dr. Soper seconded that NTP pursue this nomination. The vote was 6 yes, 1 no, and 0 abstentions with Dr. Mirsalis dissenting based on his earlier comments that the compound was not worthy of study in animals and it was of low priority.

3. Asbestos, Naturally Occurring and Atypical Forms

A. Presentation

Dr. Masten outlined the nomination for naturally occurring and atypical forms of asbestos (NOA), which is frequently found in mountainous regions of the country. He described the different types of asbestiform and non-asbestiform fibers. Asbestos in its natural form is not homogeneous; it consists of elongated particles and fibers of different lengths, chemical composition, and crystal structure. Each of these attributes is known to influence the type and severity of an adverse effect. The hallmarks of adverse health effects following exposure to asbestos include lung cancer, mesothelioma, and non-malignant pulmonary disease. The rationale for NTP to study these mineral fibers is the large knowledge gaps for the determinants of fiber toxicity, continual exposure in certain areas particularly of children, and uncertainties relating to the risk assessment of non-asbestiform fibers. One area of concern is Libby, Montana where vermiculite ore containing amphibole asbestos was mined, but the ore was not pure and contained NOA. Research is requested by the nominators and by regulatory and public health agencies because of increased mining and building activities and the ongoing public health implications for this geographic area and others.

The aims of the program are to:

- Identify and select natural mineral fibers including a Libby amphibole composite sample, a commercial source of amosite asbestos, and 2 to 3 other site-specific natural mineral fiber samples such as tremolite, actinolite, winchite, and erionite.
- Characterize the physical and chemical attributes of the of test materials.
- Assess fiber durability using in vitro dissolution and in vivo biopersistence studies.
- Undertake multiple-dose subchronic and chronic inhalation toxicology studies.

Several different materials with similarly sized particles will be tested to obtain a better understanding of the dose-response relationship and the optimal dose metric for evaluating risk. This value may not be the administered dose in terms of concentration, but perhaps the cumulative lung burden. The easiest way to assess relative potency of the different NOA asbestos would be to have pure materials or particles with a defined dimension of different crystalline structure and different mineralogy. This will be difficult to obtain but may be overcome by testing multiple samples with a range of sizes, compositions, and crystalline structure to infer how these different characteristics affect toxicity. The NTP is currently considering minimal processing of the test materials to avoid potential loss of bioactive structures and alteration of surface chemistry.

B. BSC Review

Dr. Mirsalis, the BSC reviewer, said this is a very important and ambitious undertaking. In spite of all the human data, we still do not know the mechanism of action of asbestos because human data have superceded animal data. Three important aspects should be factored into the studies, namely: (1) the use of juvenile animals; (2) measurement of cell proliferation, inflammatory endpoints, and body burdens following exposure; and (3) whether NOA acts via a genotoxic or nongenotoxic mechanism. His major concern with the research program is its expense and whether other programs will be relegated to a

lower priority due to the cost of these studies. He asked for a better clarification of what species and strains would be used, particularly whether mice, which respond reasonably well to fibers, are appropriate animal models for the *in vivo* studies.

Dr. Agnes Kane, the *ad hoc* reviewer, agreed that the study of NOA is highly relevant and important due to the circumstances at Libby MT. She said there is concern with exposure of children at an early age, as they could develop disease in their early 40s or 50s, while occupationally exposed men usually develop disease in their late 60s. She suggested that the NTP obtain a 2005 International Life Sciences Institute (ILSI) publication on a proposed strategy for testing fibers that was developed by a group of international scientists. This report states that no definitive single physical or chemical property of fibers will predict the carcinogenicity of all fiber types and it is unlikely that all fibers act through one mechanism to cause fibrotic diseases, lung cancer, and other non-malignant pleural diseases.

Dr. Kane agreed that the collection and characterization of representative samples of NOA from Libby, El Dorado, and taconite mines are appropriate. She emphasized that a standardized reference material, such as commercial amphibole that is known to produce lung cancer, be included as a positive control, and that wollastonite, which consists of calcium silicate fibers, or a soluble synthetic vitreous fiber be used as a negative control.

Dr. Kane said there is considerable controversy whether the *in vitro* acellular assays are suitable to screen for potentially hazardous fibers. One of the reasons scientists do not understand the carcinogenicity of asbestos fibers is because these acellular tests, which have durations of a few hours to a few days, do not mimic the conditions that might be occurring in the lungs and pleura of humans over a period of 10 to 20 years. She encouraged the NTP to develop new mechanistic assays and different testing strategies such as the use of *in vitro* co-cultures. Any mechanistic study that could determine whether these fibers are genotoxic, damage chromosomes, cause oxidative damage, or amplify persistent inflammatory responses in the lung would contribute to the understanding of their toxicity. Usually chronic assays are conducted with exposure over a lifetime rather than the 90-day exposure proposed for some arms of these NTP studies. As before, reference compounds, including a commercial amphibole sample as well as a negative fibrous sample, need to be included in the study. In some animals, it has been found that if they are exposed for some time and then exposure is halted, fibrosis may regress; however, disease progresses in humans throughout life even if exposure is halted.

A very important study would be to obtain lung and pleural specimens at autopsy to assess lung fiber burden, location of fibers and whether location is associated with tissue pathology. Such a study would require the cooperation and permission of the residents in Libby Montana.

Dr. Kane was skeptical that the NTP's study would be able to definitively rank the potency of different types of NOA asbestos unless a wide range of doses is used. An association has been found between the development of fibrosis and lung cancer with a cumulative dose of fibers in the lung. However, very few rats develop malignant mesothelioma when exposed over a lifetime to chrysotile asbestos fibers at 10 mg/m^3,

while mesothelioma develops in humans at lower exposures. Despite the difficulty of working with hamsters, she suggested that the NTP consider using hamsters for the *in vivo* studies because they are more sensitive than rats to developing malignant mesothelioma.

Dr. Kane summarized her review and said this is a very important endeavor, and the physical and chemical properties of the fibers as well as the surface properties need to be well characterized by multiple techniques. Assessment of the toxicity of these fibers and comparison to known commercial amphiboles are very complex, and whatever toxicological approaches the NTP can develop to accomplish this aim would be extremely useful.

Dr. Masten thanked the reviewers for their comments and said NTP would carefully consider them. He said NTP had discussed including a negative control but decided that resources would be better spent on testing other natural mineral fibers of specific interest. Since the BSC has now raised this issue, it would be reconsidered and more emphasis will be placed on characterizing the fibers' surface chemistry. He agreed with the reviewers' comments about the utility of the *in vitro* toxicity studies and said the NTP would consider developing new assays. He added that Wistar Han rats likely would be used for the *in vivo* studies.

C. Public Comments

Mr. Joseph Manuppello, People for the Ethical Treatment of Animals (PETA), thanked the NTP for the opportunity to comment and said his comments would focus on Libby amphiboles. Human data on commercial asbestos are abundant and the National Research Council has called for a change in the paradigm from animal testing to using non-animal methods such as epidemiology studies of human populations, computational toxicology, and *in vitro* studies. Since all the cancer data on asbestos are derived from human studies and there is no information that Libby vermiculite differs toxicologically from other forms of asbestos, PETA proposes that no further animal testing be undertaken with NOA. He stated that the ATSDR does not advocate that animal data from inhalation studies be extrapolated to humans due to anatomical and physiological differences between the species. Also, some individuals have postulated that rats are much less sensitive to these fibers than humans. The NTP could use MRI imaging technologies and 3D models of the architecture and tissue mechanics of the respiratory systems of rats, mice, monkeys, and humans to study fiber deposition and transport in the lungs.

D. BSC Discussion

Dr. Howard suggested that NTP fractionate the fibers at Libby as differently sized particles might have different toxicological effects.

Dr. Philbert said there are very few reliable physical methods to measure the zeta potential and other surface parameters of small particles including nanoparticles. He agreed this is an important characteristic to understand, but commented that these surface

measures can also be altered in biological fluids and this point must be taken into consideration.

Dr. V. Walker noted that the Wistar-Han rat is smaller than the other strains used by NTP in the past. He suggested that if the NTP uses the Wistar-Han rat in the future for chronic studies that the program expose the animals for 2 years and wait six months before the animals are sacrificed. He said most of the tumors in these chronic studies are evident from 18-24 months after exposure begins.

Dr. Mirsalis proposed that the BSC accept the nomination for study; it was seconded by Dr. V. Walker and the motion passed with 6 yes votes, 0 no votes, and 1 abstention. Dr. Hammond abstained as she was uncertain which fibers would be tested.

4. Artificial Butter Flavoring and Components (Diacetyl and Acetoin)

A. Presentation

Dr. Daniel Morgan outlined the background to this nomination. In 2006, artificial butter flavoring (ABF) and two of its components, diacetyl and acetoin, were nominated by the United Food and Commercial Workers International Union for long-term testing based primarily upon outbreaks of obliterative bronchiolitis (OB) in eight workers in a popcorn packaging plant and because potentially thousands more workers are exposed to these compounds in the food industry.

Butter flavoring is a complex mixture containing about 150 volatile organic chemicals. All proprietary formulations contain diacetyl and acetoin, which are water-soluble direct acting ketones and contact irritants. The vapors of diacetyl and acetoin when inhaled attack the epithelial layer of the bronchioles, resulting in the formation of scar tissue, reduced air-flow, and loss of lung function. Both diacetyl and acetoin have been implicated as the toxic components of ABF based on their inclusion in all ABF formulations. OB is a severe, debilitating, irreversible, progressive, and fatal disease that develops insidiously and has no known treatment. NIOSH found a strong correlation between exposure to butter flavoring and the extent of airway obstruction in mixers who add the components to ABF; however, workers do not usually wear protective clothing because there are no safety standards for exposure to ABF. Regulatory agencies need data to set exposure limits in the workplace, but there is a lack of inhalation toxicity data in animals exposed to ABF, diacetyl, or acetoin for use to determine what the exposure limits should be.

Four aims have been proposed to:

- Evaluate the contribution of diacetyl and acetoin to the respiratory toxicity of ABF by quantifying the toxicity of ABF with known amounts of diacetyl or acetoin and comparing this response with the toxicity of equal concentrations of either diacetyl or acetoin or a combination of the two.
- Evaluate the systemic distribution and toxicity of ABF and the two components following inhalation by measuring clinical chemistry parameters, histopathologic

- Investigate whether OB is immunologically mediated using a battery of immunotoxicity tests and dermal sensitization studies.
- Estimate a NOEL by determining the dose-response relationship of ABF.

The NTP has preliminary data in C57Bl6 mice. In a sub-chronic inhalation study, mice were exposed to diacetyl for six hours a day, five days a week, for 12 weeks. The nose and upper respiratory tract were the primary target organs with an accumulation of lymphocytes in the distal airways. In a second study, exposure of mice by oropharyngeal aspiration to one dose of 200 or 400 mg diacetyl resulted in fibrocystic lesions in terminal bronchioles four days later, indicating that sufficient doses to the airways can cause typical OB lesions in rodents.

B. BSC Review

Dr. Hammond, the BSC reviewer, said Dr. Morgan presented a clear case for the study, many workers have been affected, and OB is a very serious illness. She added that it is important to recognize that ABF is manufactured and added to foods at multiple plants and facilities, and not just the one facility where the problem was reported. In addition to the extremely serious lung disease, less serious adverse health outcomes have been reported such as shortness of breath, abnormal spirometry measurements, unusual fatigue, and skin irritation; however, no epidemiological studies have been performed. She expressed concern regarding inhalation experiments with rats because rodents do not show the deep lung effects observed in humans and, hence, may be a poor model for this outcome. She is not sure how one would extrapolate findings from animal studies to humans and how the results would be interpreted. Due to the seriousness of the problem, there needs to be an effort to find a more suitable model or to address the route by which the animals will be dosed. She said she has concerns regarding the exposure regimen; however, these concerns do not lessen her enthusiasm for the study.

Dr. Regal, the *ad hoc* reviewer, agreed with the high priority for the study, the aims, the large knowledge gaps, the severity of OB, other bronchiolar effects, and the lack of treatment. Mechanistic knowledge derived from these studies may be applicable to understanding pulmonary toxicity in general. She added that studying the two major components alone and with or without ABF is warranted because it would indicate which components are toxic. Initially, she had concerns about inhalation exposure because of the differences in distribution of the inhalant in rodents and humans, but she realizes that there is no perfect inhalation model besides using humans, which is not possible. She suggested that it might be informative to identify the other major components of ABF and to assess commonality. The rationale for immunotoxicity testing is weak except that the pathological changes are similar to what has been observed in the rejection of a lung transplant. The research concept does not address the nominator's request for the evaluation of carcinogenicity, which has a lower priority.

Dr. Morgan thanked the reviewers for their comments and suggestions. He said the most

troubling issue for the BSC, namely, the use of rodents in these studies, is not a new problem. A number of related ketones and gases affect the upper respiratory tract in rodents while in humans the lower tract is affected. He said the in-house studies indicate bronchiolar damage in rodents following longer periods of exposure. Dr. Hammond's comment about susceptible populations agrees with findings in humans because not all workers exposed to ABF succumb to the disease. To address this question, the NTP plans to study whether the genetic susceptibility of different strains of mice with different sensitivities respond differently to ABF. In the earlier NTP studies, the program studied hypersensitivity following dermal or systemic application of diacetyl to rodents and found immunosuppression. Lymphocytic bronchitis was evident by accumulation of fluid around the basement membrane of the bronchi. In humans, this accumulation is thought to be due to antibody production to human leukocyte antigen (HLA).

C. Public Comments

Mr. Manuppello, PETA, said legislation endorsed by PETA was introduced into the House of Representatives asking for a standard to regulate worker exposure to diacetyl. If this regulation is promulgated, animal tests would be irrelevant. Instead, OSHA and/or NIOSH need to implement practice controls to eliminate this health risk. As cumulative exposure increases, the incidence of airway obstruction is increased indicating a clear dose-response relationship with exposure. Both reviewers expressed their concern regarding extrapolation of inhalation results from rodents to humans. NIOSH scientists reported that the lowest average concentration of diacetyl in the air of six popcorn plants was 0.2 ppm. Since this concentration was measured in an area where a worker was affected, NIOSH concluded that the air concentration should be maintained below this level. In July 2006, the union petitioned OSHA for an emergency temporary permissible exposure limit of 0.05 ppm to provide a sufficient margin of safety and requested that exposure assessments and medical surveillance be required and that workers be trained to wear protective equipment. In follow-up studies of workers, similar practice controls have already proven effective in limiting exposure. He concluded by saying that more animal tests would be completely unacceptable considering that legislation has already been introduced that directs the establishment of an interim standard limiting exposure to diacetyl in the workplace.

D. BSC Discussion

Dr. Hammond said OSHA should issue an exposure standard and propose engineering controls to protect workers. Meanwhile, further information is needed to understand the potential hazard of diacetyl and its components, and the study should go forward. The NTP needs to determine whether diacetyl is the major toxic component because this is not clear from the present data. Although not reported, there is a concern that susceptible people who microwave popcorn at home could be adversely affected and succumb to an asthma attack.

Dr. Harish Sikka asked Dr. Morgan if metabolism studies were done in the earlier NTP experiments, which could explain the lack of toxicity of diacetyl if it were metabolized to

carbon dioxide and water. Dr. Morgan replied that metabolism studies were not done because it is known to be metabolized to carbon dioxide.

Dr. Mirsalis said there has been extensive discussion about the relevance of obligate nose breathers, as a model for humans who breathe through both their nose and mouth. There are many compounds such as formaldehyde that cause upper respiratory toxicity. He is less concerned about the interpretation of the results from rodents because upper respiratory models are informative and should not be discarded. He said well-designed studies of ABF alone and of ABF with and without diacetyl and acetoin are important for evaluating if these two components are the toxic agents or whether other components in the ABF are involved.

Dr. Kenny Crump agreed that it is important to ascertain the causative agent. Since there are known exposures in the workplace, the animal studies will add more information. He did have concern whether the results in animals could be extrapolated quantitatively to humans.

Dr. Hammond proposed and Dr. Mirsalis seconded the motion that NTP study this nomination and it was approved unanimously by the BSC with 7 yes, 0 no votes, and 0 abstentions.

VII. ICCVAM/ NICEATM Five-year Plan

A. Presentation

Dr. William (Bill) Stokes said the five-year plan is being undertaken in response to a congressional request from the House and Senate Appropriations Committees. They asked NICEATM and ICCVAM to partner with relevant federal agency program offices to build on the NTP roadmap and create a five-year plan identifying areas of high priority for research, development, translation, and validation activities for new, revised, and non-animal alternative assays that could be integrated into the federal agency testing programs. ICCVAM was advised to limit the report to 20 pages and to consider the broad audience including policymakers, members of Congress, and their staff.

Dr. Stokes described the structure of NICEATM, ICCVAM, and the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM). NICEATM provides scientific and operational support for ICCVAM by convening workshops, expert panels, and peer review meetings and by providing a mechanism for communicating and partnering with stakeholders. ICCVAM is a permanent interagency committee composed of the heads or their designates of 15 federal regulatory and research agencies whose mission is to facilitate the development, validation, and regulatory acceptance of new and revised regulatory test methods that reduce, refine, or replace the use of animals in testing while maintaining and promoting scientific quality and the protection of human health, animal health, and the environment. ICCVAM does not carry out the research, development, and validation activities, but depends on other stakeholders to perform those specific tasks. ICCVAM considers nominations of test methods from the public for review, evaluates them, and submits recommendations to U.S. federal agencies for

adoption of scientifically valid tests. During the past 10 years, NICEATM/ICCVAM has evaluated 185 test methods and recommended several test methods that have been adopted both nationally and internationally. SACATM provides advice to the NIEHS Director, NICEATM, and ICCVAM on their activities and priorities.

Dr. Stokes described the process for developing and completing the draft plan and provided an overview and highlighted some of the public comments received. An ICCVAM subcommittee gathered and considered information from the public and from ICCVAM agencies on relevant activities and completed the first draft, which was made available in May 2007. Public comments on the first draft were requested and a public town meeting attended by over 90 interested stakeholders was held on the NIH Campus in Bethesda, MD on June 11, 2007. At the June 12, 2007 SACATM meeting, SACATM and the public provided further comments on the draft plan. ICCVAM is presently incorporating these comments into the final plan to circulate to the agencies for their concurrence before forwarding to congress by November 15, 2007. Dr. Stokes said the report addresses four key challenges: (1) to identify priority areas and conduct and facilitate activities in these areas, (2) to identify research initiatives expected to support the future development of innovative alternative test methods, (3) to foster acceptance and proper use of alternative test methods through outreach and communication, and (4) to develop partnerships and strengthen interactions with stakeholders in order to facilitate meaningful progress. Additional information is provided in the references, a glossary of acronyms, and several appendices.

Dr. Stokes said the introduction discusses the agency's mandates to protect human and animal health and the environment and the U.S. laws required for consideration of alternative methods. Chapter one describes the current and planned activities for the priority test methods regarding their potential impact on (1) reducing, refining or replacing animal use for testing, (2) the potential of the method to provide improved prediction of adverse health or environmental effects and (3) the applicability to multiple agencies. The current priority areas include ocular toxicity, acute toxicity, biologics/vaccines testing, dermal toxicity, immunotoxicity, endocrine disruptor testing, pyrogen testing and chronic and carcinogenicity testing. Two other important areas of interest are neurotoxicity and reproductive and developmental toxicity testing. As an example, ocular toxicity is a high priority, because it is required by multiple regulatory agencies to provide precautions to consumers and workers to avoid exposure to chemicals and substances that are hazardous to the eye. He outlined the planned activities that include improving non-animal methods that detect permanent eye damage, assessing methods that detect reversible eye damage, and reviewing routine use of systemic analgesics and topical anesthetics for reducing pain and distress.

Chapter two identifies research that may lead to future innovative alternative test methods. ICCVAM will monitor the 11 agencies that have R&D programs for potential methods for use in regulatory testing. Those programs that have been identified as potentially applicable are high throughput screening, the use of lower species such as *C. elegans* and zebra fish, computational toxicology, biomarkers of toxicity, nanomaterial testing strategies, and expanded toxicology databases.

Chapter three discusses how to foster acceptance and appropriate use of alternative test methods. ICCVAM provides guidance on adequate validation study design to increase the likelihood that these studies generate the requisite information to allow agencies to make decisions on how the method can be used to meet regulatory requirements. ICCVAM will take the advantage of the expertise in the scientific community to provide advice on the validity of new test methods and foster discussion by convening public peer review meetings.

Chapter four addresses the development of partnerships and the strengthening of interactions with ICCVAM stakeholders by focusing on development of effective interactions with stakeholders to stimulate alternative test methods research, development, translation and validation. ICCVAM will seek partnerships to best utilize existing resources to (1) maximize efficiency and minimize duplication of effort, (2) ensure an early exchange of information when groups are working on the same area of toxicity test methods, and (3) facilitate national and international recognition, acceptance, and implementation of scientifically valid test methods.

Dr. Stokes then briefly outlined the activities at the town meeting held on June 11, 2007 moderated by Dr. Robert Scala, a former member of the NTP BSC. Following presentations on the process and an overview of the plan, representatives of four organizations and one independent consultant made public comments.

Primary points raised in the comments were:

- The plan has deliverables with timelines.
- There is a focus on the replacement rather than reduction and refinement of animal use.
- ICCVAM should assume a greater leadership role in the development and validation of alternative test methods and provide more analysis on how assay types are prioritized.
- ICCVAM should devise a plan to address the lengthy research and development process and suggest ways to expedite the acceptance of new and revised methods
- ICCVAM should enhance collaboration with the European and Japanese Centers for Validation of Alternative Methods and accept those methods endorsed by ESAC, the advisory committee for ECVAM, to eliminate duplications.
- Develop and use a web-based scorecard to track progress of ICCVAM test method evaluations.
- ICCVAM should be involved in the process of reviewing the U.S. Department of Agriculture's notices for vaccines.

Dr. Stokes explained why ICCVAM does not automatically endorse recommendations from European advisory committees and said ECVAM-approved protocols may only be applicable to the EU classification system. The EU process as reviewed by ESAC does not include public and stakeholder participation.

At the June 12, 2007 SACATM meeting, the committee commented on the plan and recommended the following issues be addressed in the plan:

- ICCVAM should identify two to three high priority areas and provide a detailed plan for their implementation.
- Provide a description of the roles and responsibilities of ICCVAM, NICEATM, and individual agencies
- Provide a table of methods previously reviewed and the subsequent agency actions.
- ICCVAM agencies should fully embrace the three Rs.
- ICCVAM should exert leadership and ensure actualization of these methods into regulatory testing frameworks.

In summary, ICCVAM and NICEATM hope to further reduce animal use when scientifically feasible, reduce or eliminate pain and distress for animals, and continue protection of public health and the environment. Dr. Stokes acknowledged the many participants who helped prepare the draft plan. NICEATM will consider SACATM's and the public's comments and incorporate those comments that are relevant to make a more effective plan for the future.

B. Public Comments

Dr. Catherine Willett, PETA, thanked the NTP for the opportunity to comment on the NICEATM five-year plan. Her organization has a different view of the accomplishments of ICCVAM. She said that ICCVAM has become a major obstacle to the development and use of alternative methods in the United States in spite of the progress in other countries. ICCVAM has repeatedly spent its resources on duplicative studies that have been validated in Europe and received endorsement by ECVAM. For example, ICCVAM recently held a peer review group meeting on pyrogenicity methods when nearly a year ago ECVAM endorsed the validity of five *in vitro* cell-based tests for pyrogenicity. The ICCVAM expert panel, which recently reviewed these methods, failed to support even their minimal use calling instead for extensive additional testing. Whatever plan ICCVAM proposes, it must include an expedited process for reciprocal acceptance and endorsement of European validation studies of specific methods. It is disappointing that ICCVAM is spending a large percentage of its limited resources on assays that have been approved by an international regulatory board of scientists.

She said ICCVAM has become a bottleneck rather than a facilitator and has demonstrated a lack of initiative in identifying and promoting alternative methods. She is extremely disappointed that ICCVAM did not capitalize on methods for estimating acute oral toxicity testing that were discussed at an ICCVAM-sponsored international workshop in 2000. The expert panel recommended that the methods were acceptable for immediate use to set starting doses and it was estimated that within two or three years of additional validation, these methods could replace all animal, short-term testing for acute toxicity. Nevertheless, seven years later, ICCVAM is still considering the methods as a possible reduction measure. ICCVAM must find a way to become more proactive and take the lead in method development.

She felt that the draft 5-year plan lacks direction and commitment to a coherent process and fails to achieve its objectives. PETA urged the BSC to ensure that NICEATM and

ICCVAM articulate a coherent plan for developing *in vitro* test methods and include the comments from SACATM and the public in the plan.

Ms. Sue Leary, Alternatives Research and Development Foundation, said Dr. Stokes did a fair job of presenting the public comments that ICCVAM received at the town meeting and the detailed critique of the five-year plan from SACATM. She noted that he seems aware of the work still needed to finalize the plan. She postulates that as a result of NTP's realignment, NICEATM might have an opportunity to become closer aligned with the NTP program office, and that this might allow NICEATM to take a stronger leadership role. She thought NTP needs ICCVAM and NICEATM, in an advisory capacity for directing further research into alternative methods.

C. BSC Discussion

Dr. Sikka said he did not believe NICEATM and ICCVAM could accomplish all the goals outlined in the five-year plan and recommended including a timeline by which certain tasks would be completed. Dr. Stokes said ICCVAM would prepare a detailed chart of the planned activities and their targeted dates for completion that will be available on the website.

Dr. Crump said this is an important area, and he appreciated the comments from the public speakers. He agreed that the standards for accepting new testing approaches must be achievable.

Dr. Mirsalis agreed with Dr. Crump. He said three decades ago it was thought that *in vitro* gene toxicology assays would replace animal studies for cancer testing, but now we know that is not possible. It is appropriate to use *in vitro* assays as a screen, but animal studies are still needed to confirm *in vitro* findings and some animal testing will never be eliminated. He said there has been some reluctance to adopt *in vitro* assays for early screening and to give these tests the gold seal of approval especially if there is a 5-10% false negative rate because some chemicals will be erroneously designated negative when they are true hazards. It is important that these *in vitro* tests be used and once databases are constructed, scientists will be more confident of the reliability of the tests.

Dr. Riviere noted that the focus is on replacing rather than reducing or refining animal tests. He agreed with Dr. Mirsalis that the number of animals used for testing could be reduced, and reducing or refining animal use should be the focus rather than replacing animal tests with *in vitro* assays.

Dr. Stokes said the ultimate goal is to develop test methods that do not or rarely involve animals, but for some endpoints this goal will take a long time to achieve.

VIII. Other Business

The BSC had no additional business to discuss.

Dr. Bucher thanked Dr. McCarver for chairing the meeting and keeping it focused. He appreciated the BSC discussions on the research concepts and noted that NTP would

consider them carefully. He concluded by noting how invaluable the BSC's input is to the NTP.

The meeting was adjourned at 4:15 PM.