Summary Minutes
NTP Board of Scientific Counselors
June 17-18, 2014

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I. Frequently Used Abbreviations and Acronyms

ACC	American Chemistry Council
ADME	absorption, distribution, metabolism, and excretion
AOP	adverse outcome pathway
BPA	bisphenol A
BPAF	bisphenol AF
BPS	bisphenol S
BSC	Board of Scientific Counselors
CDC	Centers for Disease Control and Prevention
CRU`	Clinical Research Unit
DNTP	Division of the NTP
EPA	U.S. Environmental Protection Agency
ET	ethyltoluene
FDA	U.S. Food and Drug Administration
HPV	High Production Volume
HT	high throughput
ILS	Integrated Laboratory Systems, Inc.
IRIS	Integrated Risk Information System
LLNA	local lymph node assay
NICEATM	NTP Interagency Committee for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NIOSH	National Institute of Occupational Safety and Health
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OHAT	Office of Health Assessment and Translation
QSAR	quantitative structure-activity relationship
TCC	triclocarban
TK	toxicokinetics
TMB	trimethylbenzene

II. Attendees

BSC Members in Attendance:

Milton Brown, Georgetown University Medical Center
Robert Chapin, Pfizer
George Corcoran, Wayne State University
David Dorman, North Carolina State University (NCSU)
Mary Beth Genter, University of Cincinnati
Dale Hattis, Clark University
Steven Markowitz, City University of New York
Lisa Peterson, University of Minnesota (chair)
Sonya Sobrian, Howard University
Iris Udasin, University of Medicine and Dentistry of New Jersey

BSC Member not in Attendance:
Jack Harkema, Michigan State University

Other Federal Agency Staff:
Timothy Buckley, U.S. Environmental Protection Agency (EPA)
Gayle DeBord, National Institute for Occupational Safety and Health (NIOSH), BSC Liaison
Nicole Hagan, EPA
Paul Howard, U.S. Food and Drug Administration (FDA), BSC Liaison
Haluk Ozkaynak, EPA
Jon Sobus, EPA
John Wambaugh, EPA

National Institute of Environmental Health Sciences (NIEHS) Staff:

Scott Auerbach	Barry McIntyre	Vicki Sutherland
Mamta Behl	Alex Merrick	Kyla Taylor
Linda Birnbaum	Mark Miller	Kristina Thayer
Abee Boyles	Retha Newbold	Raymond Tice
John Bucher	Richard Paules	Molly Vallant
Warren Casey	Shyamal Peddada	Suramya Waidyanatha
Jennifer Collins	Katie Pelch	Nigel Walker
Michael DeVito	Cynthia Rider	Vickie Walker
Paul Foster	Andrew Rooney	Amy Wang
Dori Germolec	Kristen Ryan	Lori White
Kembra Howdeshell	Brian Sayers	Kristine Witt
Jui-Hua Hsieh	Keith Shockley	Mary Wolfe
Robin Mackar	Robert Sills	Rick Woychik
David Malarkey	Stephanie Smith-Roe	
Scott Masten	Matthew Stout	

Public:
Patrick Allard, University of California, Los Angeles
Gregory Baker, Battelle
Chris Bartlett, SciMetrika
Neepa Chokski, Integrated Laboratory Systems, Inc. (ILS)
Reshan Fernando, Research Triangle Institute
Shuva Gupta, NCSU
Milton Hejtmancik, Battelle
Ernie Hood, Bridport Services
Brett Jones, ILS
Nicole Kleinstreuer, ILS
Joseph Manuppello, People for the Ethical Treatment of Animals (PETA)
Richard McKee, ExxonMobil Biomedical Sciences
Antonio Quinones, Gojo Industries, Inc.
Ivan Rusyn, University of North Carolina at Chapel Hill
Barney Sparrow, Battelle

Webcast Participants:
Joe Algaier, MRI Global
David Allen, ILS
Patricia Bishop, PETA
Joseph Chang, ADial
Brad Collins, NIEHS
Paul DeLeo, American Cleaning Institute
Reshan Fernando, RTI International
Kim Gaetz, ORISE/EPA
Dori Germolec, NIEHS
Steve Graves, Battelle
Robbin Guy, NIEHS
Kristina Hatlelid, U.S. Consumer Product Safety Commission
Alison Harrill, University of Arkansas
Steve Hentges, American Chemistry Council (ACC)
Cynthia Hines, NIOSH
Stephanie Holmgren, NIEHS
Linda Loretz, Personal Care Products Council
Angela Lynch, ACC
Joseph Manuppello, PETA
Anne Pilaro, FDA
Keith Shockley, NIEHS

June 17, 2014

III. Introductions and Welcome

The National Toxicology Program (NTP) Board of Scientific Counselors (BSC) met June 17-18, 2014 in Rodbell Auditorium, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC. Dr. Lisa Peterson served as chair. She welcomed everyone to the meeting and asked BSC members and other attendees to introduce themselves. Dr. Lori White, BSC Designated Federal Officer, read the conflict of interest policy statement.

IV. Report of the NIEHS/NTP Director

Dr. Linda Birnbaum, Director of NIEHS and NTP, updated the BSC on developments at NTP and NIEHS since the last BSC meeting in April 2014. She described several activities related to climate change and disaster response, and briefed the BSC on staff changes.

She reported that several pieces of legislation impacting environmental health are under consideration in Congress, but all are unlikely to pass in the near future. In science

news, she mentioned publication of a paper describing new NTP testing paradigms for reproductive and developmental toxicity, and a paper outlining a new assay for endocrine disrupting chemicals.

Dr. Birnbaum discussed developments in training and mentoring, including the 17th Annual NIEHS Biomedical Career Symposium and the Transdisciplinary Environmental Health Fellowships in Epigenetics and Stem Cell Research. She mentioned data management and technology news, including a data sharing webinar for young environmental health investigators and the development of a language standard for environmental health science. She showcased several awards and recognitions won by NIEHS staff members. Dr. Birnbaum concluded by presenting a plaque to Captain Gayle DeBord, commemorating her service as NIOSH representative to the BSC for the past four years.

Dr. Dorman asked Dr. Birnbaum whether there had been an effort to involve industry in the interdisciplinary postdoctoral fellowships. Dr. Birnbaum replied that it had not been considered, because during the three-year program, all that could be offered was working with the intramural program, the NTP, and the extramural program. She noted that the NIEHS could not use Federal funds to pay for a fellow to work in industry. Dr. Dorman said it would be useful to provide opportunities for the fellows to broaden their horizons. Dr. Birnbaum noted that opportunities are provided by programs such as career fairs.

V. Report of the NTP Associate Director

Dr. John Bucher, NTP Associate Director, briefly addressed the BSC to provide members a perspective on the NTP, the DNTP, and how the concepts to be considered by the BSC are initiated.

He stressed that the NTP is an interagency program. Although it is housed at NIEHS, there is very strong interaction with Centers for Disease Control and Prevention, NIOSH and the FDA. He noted that several divisions of NIEHS work on NTP-related programs, including the Division of Extramural Research and Training, the Division of Intramural Research, and the DNTP. He described the structure of the DNTP, including the various branches and offices that comprise the division.

He previewed several agenda items to be presented over the two days of the meeting, noting that many would be of interest to the BSC members.

VI. NTP Research Concepts: Introduction

Dr. Scott Masten, DNTP Office of Nominations and Selection, provided the BSC with background information about NTP research concepts, which are planning and communication tools designed to distill a great deal of deliberation and planning into a concise document. A research concept contains the rationale, proposed approach, significance, and expected outcome of a proposed research project to address toxicological data needs for a specific substance or issue. The concepts have sufficient detail to outline the scope, strategy, and direction of a project, but do not contain specific details on study design or protocol, or commit the NTP to carrying out the entire plan.

He described the NTP study nomination review process, as well as the process used to develop NTP research projects, which involves an iterative approach to project development, study design, performance, and interpretation. He noted that every project is different and described the scoping and problem formulation undertaken by the NTP. Part of the research concept is to present the initial choices made by the NTP project team, asking that the BSC weigh in on the clarity and justification for those choices.

He mentioned that the concept of "read-across" would be important in the day's deliberations. He defined it as the ability to infer information for a particular property or hazard endpoint from one chemical that does not have that data from another chemical that does. Read-across allows prediction based on chemical structure or biological similarity, and is a strategy for hazard assessment and data gap filling.

Dr. Masten provided background information for each of the four NTP research concepts to be addressed during the meeting: bisphenol S and derivatives, triclocarban, C9 alkylbenzenes, and xylenes.

He presented a schematic based on the NTP Roadmap that depicted the overall NTP research and testing framework. It incorporates novel and alternative testing approaches, and is ultimately designed to yield useful information for public health decision-making.

He outlined the process for review and discussion of each of the research concepts.

VII. NTP Research Concept: Bisphenol S

A. Presentation

Dr. Vicki Sutherland, DNTP Toxicology Branch, briefed the BSC on the NTP research concept on bisphenol S (BPS). She noted that bisphenol A (BPA) and bisphenol AF (BPAF), structurally similar chemicals, are currently being evaluated by the NTP. The

NTP is now considering evaluation of BPS and derivatives, as several are being used as commercial replacements for BPA and BPAF. BPS was nominated by the EPA and the NIEHS based on its potential endocrine activity, limited toxicological testing, and high probability for human exposure. BPS and derivatives are used in a variety of consumer products and applications. They can be found in food, indoor dust, sediment, and paper and paper products such as toilet paper, cashier receipts, and currency.

In Tox21 screening, BPS has been found to be an estrogen agonist and is active in two estrogen receptor assays. Limited animal data have shown a variety of potential effects of exposure. Knowledge gaps include limited developmental, reproductive or endocrine evaluations, no metabolism information, no chronic exposure data, and no immunotoxicity, neurotoxicity or carcinogenicity data.

The specific aims of the project include characterizing the dose-response effects of BPS, assessing metabolism of BPS and derivatives, determining the need for chronic toxicology studies, and comparing and contrasting BPS *in vivo* and *in vitro* data with other analogues and derivatives to build a knowledge base of bisphenol chemicals. The proposed approach is comprised of three phases (1) leveraging initial NTP efforts, characterization of rodent ADME (absorption, distribution, metabolism, and excretion)/TK (toxicokinetics), and *in vivo* toxicity evaluation of BPS in rodents; (2) completing further *in vivo* toxicity evaluations in rodents; and (3) doing additional studies as needed, and comparing and contrasting *in vitro* and *in vivo* data on selected analogues.

B. BSC Discussion

Dr. Chapin asked why there were two different kinds of *in vivo* study designs; one using rats, and one using mice. He asked what the added benefit of adult mouse studies would be. Dr. Sutherland said perinatal studies are easiest to conduct in the rat, and the mouse studies would allow evaluation of an adult model.

Dr. Sobrian asked about the potential effect of the NTP change of rat strains. Dr. Sutherland noted that the change of strains had actually taken place in 2008-2009, so the studies would take place in the same strain of animals in use for the past five years. Also, the BPAF program is being conducted in the current strain, allowing direct comparison with BPS and access to a good historical database for the strain.

Dr. Corcoran inquired about how the Phase 3 *in vitro* studies would relate to Tox21, and how they might be extrapolated to human relevance. Dr. Sutherland said they would compare the high throughput (HT) data to the already-existing *in vitro* data, as well as including specific *in vitro* assays for estrogen receptors and any other missing areas,

and then compare those results to the *in vivo* data to allow extrapolations to the human data.

Dr. Brown asked whether a chemical approach had been considered. Dr. Masten replied that ADME studies looking at potential reactive metabolites are part of the concept. Dr. Dorman noted that there might be opportunities to look at physicochemical properties and structural alerts to predict metabolism, allowing selection of which derivatives might be suitable for formal ADME studies. Dr. Sutherland said that could be considered during the study design phase. Dr. Dorman commented that some of the endpoints such as hepatotoxicity and nephrotoxicity had been triggered by the quantitative structure-activity relationship (QSAR) studies, and wanted to ensure they would be included in Phases 1 and 2. Dr. Sutherland said that the perinatal studies in rodents would incorporate each of those endpoints, with further assessment as needed in those studies.

Dr. Genter asked if there was any information on dose or relative potency of BPS compared to BPA. Dr. Sutherland said the NTP has not yet considered dose levels.

Dr. Sobrian asked in how many products BPS might potentially be used to replace BPA. Dr. Sutherland noted that BPS is already used on its own in polyether sulfone, as an alternative to polycarbonate. It has primarily been used as a replacement for BPA in thermal paper. Dr. Howard said BPS and BPA are used in quite different types of materials, and the manufacturing process largely determines how much leaching takes place.

Dr. Chapin asked whether a read-across algorithm would be used. Dr. Sutherland said there is no specific algorithm set up for the BPS program, but it would be advantageous to develop one for the bisphenol class of chemicals, to avoid having to test each individual analog by itself. Dr. Chapin recommended that creation of a read-across algorithm should be an internal NTP goal over the next two years.

Dr. Hattis felt that the stability of the sulfone linkage is a key issue. If the linkage breaks significantly *in vivo*, the metabolites would need to be considered.

Dr. Peterson acknowledged receipt of written comments from People for the Ethical Treatment of Animals (PETA).

Dr. Chapin, first discussant, said he could think of no better compound and class of compounds to fit the mission and goals of the NTP than BPS. His only concern was that immunotoxicity is adequately addressed early in the study. He supported of all of the parameters of the project, and rated the overall significance of the project as high. Dr. Sutherland clarified that immunotoxicity endpoints would be included in the earlier studies to determine whether further immunotoxicity studies would be warranted.

Dr. Sobrian, second discussant, asked how the information from the 2014 BPA studies would be used to inform the aims of the BPS study. Dr. Sutherland replied that that information was primarily used to help determine how to set up some of the proposed studies. Dr. Sobrian asked how it will be determined which BPS derivatives will be tested. Dr. Sutherland said that the preliminary studies would be important, in that some derivatives may show causes for concern and be taken forward for additional studies. Dr. Sobrian said she agreed that the project is high priority.

Dr. Corcoran, third discussant, felt that the project is clearly within NTP's mission and goals. He said the clarity in the proposal was high, and the stated strategy and approach were perfect. He asked whether given limited resources, it might not be more practical to focus on high-value toxicological targets rather than comprehensive testing. Dr. Bucher said for most chemical classes, that would be an appropriate approach, but with the wide variety of effects reported within this particular class, it would not be appropriate to put anything on a second tier, because the question of health outcomes is still very important. Dr. Corcoran asked how NTP is moving toward Tox21 in terms of addressing endpoints such as these. Dr. Bucher said the Tox21 update presentations scheduled for the next day would clarify that. Dr. Corcoran said moving forward with the project had his full support, as a high priority.

Dr. Peterson summarized the BSC discussion, noting that the BSC deemed the project very important, with a high priority.

VIII. NTP Research Concept: Triclocarban

A. Presentation

Dr. Sutherland briefed the BSC on the research concept on triclocarban (TCC). TCC is an antibacterial chemical found in health and skin care products. Its use is growing as a replacement for triclosan, which is currently being evaluated by the NTP for potential carcinogenicity and endocrine-related health effects. It is primarily found in bar soaps and was nominated by NIEHS for toxicological evaluation due to its use in consumer products, high level of human exposure, and potential endocrine activity. Routes of exposure to TCC are primarily dermal, though it is also detected in wastewater.

Dr. Sutherland reviewed the Tox21 data and the available *in vivo* data on TCC. Knowledge gaps include limited developmental and reproductive public data, limited information on endocrine effects of TCC, limited chronic exposure data available for public review, no comprehensive ADME/TK profile, no immunotoxicity or neurotoxicity data, and limited carcinogenicity data.

The research project would include three phases (1) *in vivo* toxicity evaluation of oral administration of TCC in rodents, including a perinatal oral exposure dose range finding study in rats, a short-term adult oral exposure toxicity study in mice, and ADME/TK characterization; (2) a subchronic oral study, including a perinatal exposure window to assess the potential for reproductive and developmental toxicities in rats, a developmental immunotoxicology evaluation, and an adult mouse 90-day toxicity study, and (3) additional studies as needed.

B. BSC Discussion

Dr. Dorman asked whether any *in vitro* to *in vivo* extrapolation had been attempted in the Tox21 TCC studies. Dr. Sutherland said it would be done before design of further studies would go forward. Dr. Dorman noted that there is a rich database of TCC studies. Dr. Sutherland said the database is not publicly available and typically contains older studies that may not address the current questions. Dr. Dorman asked if the full suite of studies would still be conducted if the database were publicly available. Dr. Sutherland replied that it would depend on whether it addressed the endocrine activity concerns. Dr. Dorman noted that the proposed studies go well beyond just endocrine endpoints, and wondered whether the older studies would inform the proposed studies, such as the short-term oral exposure study. Dr. Sutherland said that the short-term study would be used to help establish doses for the longer-term studies, and for the inclusion of the endpoints of concern.

Dr. Markowitz noted that the presentation had shown a decrease in TCC use in the past 5-10 years, and asked if it is still being used. Dr. Sutherland said it is still be used, but there are suggestions that its use has decreased due to concerns and the FDA's request for additional information.

Dr. Udasin asked about human oral exposure to TCC among food handlers washing their hands, and if that exposure scenario has been evaluated. Dr. Sutherland said she had not found any specific studies, but noted the use of antibacterial hand washes by health care workers in hospital settings.

Dr. Corcoran asked about the proposed FDA rulemaking regarding TCC in the marketplace. He said he saw barriers for its continued use as it has been. First, it would need to achieve the "generally accepted as effective" standard. Failing that, it would probably no longer be included in bar soap. If that were to happen, he asked if the NTP group would still retain its high degree of enthusiasm for the project. Dr. Sutherland noted that the product has been in use for a very long time, and endocrine activity has not been assessed. If that were still deemed important, the NTP would want to move forward. If that were given a lower priority and an FDA ruling ceased use of TCC, the project's priority could be decreased. Dr. Corcoran asked about the potential

for TCC magnifying bacterial resistance. Dr. Sutherland replied that it is a big concern with any of the residue-producing antibacterials. Dr. Corcoran asked how challenges involving studies of dermal exposure in animals might be overcome. Dr. Sutherland said dermal exposure in perinatal studies is not typically done; the majority of studies were conducted in adult animals where licking of pups is not an issue, and licking of specific areas can be prevented. Therefore, oral perinatal studies, including an oral to dermal bridging study, would be considered

Dr. Birnbaum asked Dr. Sutherland to elaborate on the study she had cited involving radiolabeled TCC in humans. Dr. Sutherland said the single-dose absorption study had been done in males the 1970s.

Dr. Corcoran asked about the presence of TCC in toothpaste. Dr. Sutherland said that to her knowledge it is not being used currently in toothpaste. Dr. Howard noted that triclosan is approved for use in one brand of toothpaste.

Dr. Chapin asked whether the intent is to perform endocrine activity studies early in the process, and then later to study the healthy aging of endocrine systems in early exposed animals. Dr. Sutherland confirmed the order of studies.

Dr. Dorman asked about the fact that Phase 1 dermal exposure studies were not included. Dr. Sutherland said study designs were not complete yet, but plans for an oral to dermal extrapolation study are included.

Dr. Peterson acknowledged receipt of written comments from the American Cleaning Institute and PETA.

Dr. Corcoran, first discussant, said that the project has merit and fits the mission and goals of the NTP. He felt Dr. Sutherland's presentation was very clear, and that there is a valid rationale for moving forward. He felt that the proposed strategy was sound, but that it might be reconsidered based on whether or not TCC remains a "generally accepted as effective" agent. Should it fail to meet that threshold, questions should be asked about devoting resources to the project. He noted inherent challenges to the studies, particularly in dermal murine studies, given that that is the primary route of exposure. He said it appears that industry is already moving to using much lower TCC concentrations, which may dampen enthusiasm for the project. Although it is already present in most humans, the question becomes how important those levels are. He rated the overall public health significance of the project as moderate, and said that if TCC were found not to be generally accepted as effective, his enthusiasm would drop to low.

Dr. Brown, second discussant, said the proposed project has very high merit, and the key significant issues were clearly identified. He considered the overall significance and

priority for the project high, given that exposures are still high regardless of production trends. He also felt that the public health impact could be high, and that preemptive studies should still be conducted to increase understanding of the TCC.

Dr. Dorman, third discussant, said the proposed studies meet the mission of the NTP and would broaden the toxicologic database available for TCC. He noted the availability of a fairly broad database from hazard assessments that have already been done, although they may not be publicly available, including a 3-generation reproduction study and acute toxicity studies in rodents. He said at times it was not clear what the major driver was for the broad suite of endpoints being considered by NTP. He questioned whether part of the intent was to extrapolate from Tox21 studies to *in vivo* studies to basically validate those findings. Similarly, with the availability of radiolabeled studies in humans, the data gap in pharmacokinetic or ADME studies was unclear. He wondered why immunotoxicity had been singled out in Phases 1 and 2 over other endpoints. He felt the project needed more consideration of the data that are already available and of the potential challenges and potential pitfalls, which were not identified in the concept. He rated the overall significance as low to moderate, largely because of the missing rationale for the full suite of studies and the need for more clarification of the major drivers behind the proposed approach. He noted that both public commenters had discussed studies not cited by the NTP, and thought they may be worth reviewing for their potential to influence the suite of studies the NTP wants to pursue.

Dr. Sutherland noted that the majority of studies cited were older and did not include endocrine activity, warranting a better evaluation of that endpoint. For the ADME/TK evaluation, studies bridging the oral and dermal studies are needed. She noted there had been some indication of potential immunotoxicological effects in the literature, leading to a particular interest in that endpoint. Dr. Sutherland said there is keen interest in the potential endocrine activity of TCC, and the available studies were conducted before that was a significant research area. Dr. Howard said FDA had dealt with the same issue with triclosan, i.e., data gaps resulting from the lack of availability of information in certain areas due to proprietary considerations, which is an unfortunate aspect of regulatory science. Dr. Dorman said he understood that issue and would have more enthusiasm for the project if there were further discussion among the agencies to potentially avoid having to redo some of the existing studies.

Dr. Genter noted that TCC had been shown to be an aryl hydrocarbon receptor agonist, with the potential immunotoxicological effects rendering assessment of perinatal exposure important.

Dr. Udasin, fourth discussant, said that establishing a dose-response relationship with oral exposure to TCC is consistent with the mission and goals of the NTP. She added that she was concerned about the issue of limited resources. She wondered whether

there is any current perinatal exposure, and said it would be important to discover how many people are using TCC and whether it lingers in the environment. With the apparently decreasing exposure, she said she would rate the project as moderate priority.

Dr. Masten said TCC is contained in bar soaps, especially those advertised as being deodorant soaps. He said exposure might have decreased slightly, but TCC is still in widespread use. Dr. Udasin asked how long TCC remains in urine. Dr. Sutherland replied that TCC's plasma half-life was approximately three hours.

Dr. Dorman noted that if there is truly antibacterial activity with TCC, its potential effect on gut flora should be examined. Dr. Birnbaum agreed that microbiome exposure should be assessed with this and other similar agents. Dr. Howard noted that TCC's antimicrobial activity is still an open question. Dr. Bucher said the issue of the microbiome is a big concern for toxicology in general, because the potential for changing microflora in rodent toxicity studies has not been evaluated. Dr. Nigel Walker noted that there are periodic meetings with the FDA to keep apprised of current NIEHS/NTP projects of interest, and this project would be included.

Dr. Peterson summarized the comments of the BSC, stating that there was a range of priorities expressed for the project.

IX. NTP Research Concept: C9 Alkylbenzenes

A. Presentation

Dr. Brian Sayers, DNTP Toxicology Branch, briefed the BSC on C9 alkylbenzenes, a class of aromatic hydrocarbons with 9 carbons that occur naturally in crude oil and are produced from the naphtha fraction of crude oil. They are used as a gasoline-blending stream and also as solvents. Humans are exposed through the inhalation and oral routes, with exposures occurring both in the ambient environment and in occupational settings. The commercial C9 fraction contains 75-90% trimethylbenzene (TMB)/ethyltoluene (ET) isomers, with the amount of each isomer within the C9 fraction varying due to variations in petroleum source and processing. Interest in this research concept was spurred by NTP identification of cumene, another alkylbenzene, as a carcinogen in rodents. The C9 aromatic hydrocarbon fraction was tested via a Toxic Substance Control Act Section 4 Test Rule promulgated in 1985. Dr. Sayers described the results of those studies. He noted that, among other findings, the results showed motor activity effects in male rats, which are common in alkylbenzene exposures.

The TMBs are currently being assessed through EPA's Integrated Risk Information System (IRIS) program. One ET isomer was assessed in 2009 by the EPA High

Production Volume (HPV) Challenge program. Dr. Sayers described the EPA assessments in more detail. He said that limitations in C9 alkylbenzene data sets make it difficult to establish safe exposure levels, for all members of the class.

Test material selection and inhalation exposure study design will be challenging, with the key being formulation of the appropriate testing strategy. Specific aims are to (1) determine the appropriate C9 alkylbenzene test agents for toxicity and carcinogenicity studies, (2) evaluate the toxicity and carcinogenicity of up to two test agents, (3) conduct comparative short-term inhalation toxicity studies on several isomers, and (4) assess *in vitro* screening of C9 alkylbenzenes. The phases are not designed to be sequential and will run in parallel. The proposed studies will use both mixed preparations and individual isomers.

B. BSC Discussion

Dr. Genter asked whether the usual human exposure was to the TMB and/or ET isomers individually, or as part of complex mixtures. Dr. Sayers said in terms of human occupational solvent exposure, exposure would primarily be to mixtures. He added that there is potential exposure to both, depending on how they degrade in the environment.

Dr. Sobrian asked why there was a focus on inhalation, as there is some dermal contact, particularly given that the inhalation studies are expensive. Dr. Sayers agreed that there is potential for dermal exposure in the occupational setting, but that inhalation was likely to be the most relevant route of exposure based on volatility of the chemicals. Dr. Sobrian asked why they would not start with the *in vitro* test, and then judge which endpoints to pursue from that. Dr. Sayers said that the typical testing program is a funnel approach, starting big and then becoming more specific as the testing program progresses. The chronic study was selected based on EPA's identification of data gaps. He noted that endpoints could be added as they may become relevant throughout the testing program. Dr. Walker noted that the current Tox21 assays are not really applicable with this project. Dr. Sobrian asked if NTP was aware of any data on specific endpoints where there was a difference between individual isomers compared to the entire mixture. Dr. Sayers said he was unaware of any such data.

C. Public Comments

Dr. Peterson acknowledged receipt of written comments from PETA, the Cumene Panel of the ACC, the Hydrocarbon Solvents Panel of the ACC, and the American Petroleum Institute.

Joseph Manuppello, representing PETA, noted that two of the TMB isomers have been registered for Registration, Evaluation, Authorisation and Restriction of Chemicals [REACH], and are the subjects of recent prenatal developmental toxicity studies.

Dr. Richard McKee, ExxonMobil Biomedical Sciences, representing the Hydrocarbon Solvents Panel of the ACC, noted that EPA had already identified a number of the TMB isomers for consideration. In commerce, most of the molecules are actually part of a gasoline-blending stream, and are a complex mixture of various C9 molecules. Thus, most of the exposures are to the complex mixtures. He said that some time ago the EPA had instituted a test rule program looking at complex mixtures containing C9 molecules. When the test rule program was begun, 90-day and 12-month repeated inhalation studies were already available. Since then, other inhalation studies have been conducted on TMB, ET isomers, and cumene, along with oral studies of two of the TMB isomers and one of the ET isomers. He described some of the findings of those studies, covering developmental, subchronic, and reproductive toxicity. With those data in mind, he said his group feels that a lot of the needed testing has already been done, and that hazard characterization for isomers that have not been tested could be reasonably done by read-across from the existing data. Regarding the previous inhalation studies of cumene and naphthalene, he felt that the important question to emerge is on human relevance, and the cause of the observed respiratory tumors. Dr. McKee said the real research question is not getting more hazard characterization data, because enough is already available, but understanding the existing data, and how should they be used in a regulatory context.

D. BSC Discussion

Dr. Hattis, first discussant, said there is considerable merit to studying this class of compounds, due in part to high exposure in the general public to gasoline and gasoline components. He recommended first focusing on carcinogenicity and mutagenicity; there is no obviously active functional group in this class of chemicals, but there is the possibility of metabolism producing mutagenic intermediates. Cumene is an example of that possibility. The second focus should be on reproductive and developmental endpoints. The most sensitive endpoint is inhibition of fetal growth, so the relative potency of the isomers for inhibiting fetal growth should first be determined. If there is a wide difference in potency, the more potent components should be focused on for further analysis. Overall, he felt that the project is worth prioritization, but some restructuring of the assessments should be done. Dr. Hattis gave the project a medium-to-high priority, depending on the initial results.

Dr. Sayers agreed that evaluating the potential for reactive metabolites is important, and metabolic assessment via *in vitro* assays is being considered, such as use of a

metabolically active liver cell line being evaluated by the NTP Biomolecular Screening Branch. He said reproductive and developmental endpoints need to be considered, and those endpoints would be incorporated depending on the test agents that are studied.

Dr. Genter, second discussant, said there is merit in understanding some of the data gaps because there is such widespread human exposure. The presentation clarified how certain goals would be accomplished. She was glad the *in vitro* assessment is being included, but felt that some *in vivo* data would still be needed to understand the dose and metabolites in liver and lung, which can be very different. She questioned whether anything could be learned from cumene, through read-across. She said her enthusiasm is moderate, but could go up or down based on initial findings.

Dr. Sayers said other data generated on alkylbenzenes could be useful for comparisons, but that read-across for carcinogenicity would be difficult given that no definitive pattern has been seen among the alkylbenzenes. Dr. Bucher agreed, and noted that EPA's initial assessment of cumene was that there was low probability that it was a carcinogen, but data have shown that it is a carcinogen.

Dr. Sobrian, third discussant, rated the merit of the project as moderate. She asked what the read-across value might be, depending on whether the study goes forward using the mixtures or the individual isomers. Dr. Masten said Dr. Sobrian had just articulated part of the rationale for why NTP is conducting this inquiry. He said there is value in read-across, but that probably no one would characterize all C9 alkylbenzenes carcinogenic to the nose and the lung in rodents, as cumene is. He said the difficult choice is whether to do chronic inhalation studies in one or two of the most abundant isomers, or whether it is acceptable to combine some sets of isomers together. Dr. Genter concurred that the issue is what the relevant exposure combinations or mixtures are. Dr. Walker added that the issue is relevant in the context of other activities underway at the NTP related to mixtures versus a component-based approach.

Dr. Peterson summarized the discussion, stating that the BSC gave the project a moderate priority.

X. NTP Research Concept:

A. Presentation

Dr. Matthew Stout, DNTP Program Operations Branch, briefed the BSC on the draft NTP research concept for xylenes. Xylenes are alkylated monoaromatic hydrocarbons produced in high volume and found in all environmental media. Xylenes have been nominated for NTP testing on multiple occasions by different organizations for a variety of endpoints. Although NTP has conducted prechronic and chronic toxicity and

carcinogenicity studies via oral gavage, significant data gaps remain. Seventy percent of the more than 8 billion pounds annually produced in the US is used in production of ethylbenzenes and individual isomers, with 30% used in solvents, paints, and coatings. The primary route of human exposure is inhalation. Dr. Stout presented data on prior NTP carcinogenicity studies of benzene, toluene, ethylbenzene, xylenes, and related compounds. He noted that compounds structurally similar to xylenes have been found to be positive for carcinogenicity via inhalation exposure, but that xylenes themselves have not been tested for carcinogenicity via inhalation. Also, the database has limited information on reproductive and developmental toxicity, and on neurotoxicity in developing animals. The purity and presence of ethylbenzene in the test agent will be a key issue. The proposal is to test a high purity mixture of the three xylene isomers. Route of exposure and choice of endpoints are also key issues, with the strategy being to generate data on multiple endpoints under the same exposure scenario, allowing the ability to make direct dose-response comparisons. The project's specific aims include obtaining a high purity mixture of the three xylene isomers free of ethylbenzene for use as a test agent, evaluating subchronic and chronic toxicity and carcinogenicity of xylenes following whole body inhalation exposure, and evaluating the developmental, reproductive, and neurotoxicity of xylenes following whole body inhalation exposure.

B. BSC Discussion

Dr. Hattis asked for confirmation that there was a case where there was a negative result for carcinogenesis via gavage, but a positive result by inhalation. Dr. Stout noted that ethylbenzene had made up a large fraction of the test agent used in the xylene gavage studies, which did not result in carcinogenicity, but when given by inhalation, it was found to be carcinogenic. He said that if the test agent chosen contained ethylbenzene, it would probably be carcinogenic via inhalation. He said there are several agents that have tested negative by gavage, but positive by inhalation.

Dr. Dorman said that one of the huge challenges is that there are many co-exposures with xylenes. Obtaining data for purified xylene is not really relevant to human exposures, as they always involve mixtures. He questioned the value, from a public health perspective, of testing a very pure form of xylene to which no one would ever be exposed. Dr. Stout said if xylene included 15-20% ethylbenzene by inhalation, it would be likely to be carcinogenic at exposure concentrations similar to those that may be selected for testing of xylenes. There would be a large fraction of a known carcinogen. He noted that very pure xylene isomers are widely available and relatively inexpensive, which implies they are high production volume. Thus, people are likely exposed to pure xylene isomers. Dr. Stout said there is a need to understand if xylenes are contributing to the effects, separate from ethylbenzenes. Dr. Dorman said exposure to pure xylene isomers is likely to occur in an occupational setting, rather than a general environmental

setting. Dr. Stout noted that many of the xylenes, both mixtures and pure isomers, might be produced for use in solvents and other applications. Thus, people may be exposed to both xylene mixtures and pure isomers. Both commercial and occupational exposures to xylenes occur.

Dr. Markowitz noted that there was no information provided about the breakdown in potential consumer exposure, in terms of individual isomers versus mixtures. Dr. Stout said he did not have that information. He said that for practical reasons, the NTP strategy is to look at a mixture of the three xylene isomers. He said the NTP had been unable to find any commercial products that did not also contain ethylbenzene. Dr. Howard mentioned that commonly used paint thinner, which is xylene-based, does contain ethylbenzene; this is probably the most prevalent consumer exposure other than gasoline. Dr. Udasin questioned whether the xylene used in pathology labs is a mixture with ethylbenzene. Dr. Stout confirmed that it is.

C. Public Comment

Dr. Peterson acknowledged written comments from the Toluene and Xylenes Panel of the ACC.

Dr. McKee, representing the Toluene and Xylene Panel of the ACC, provided oral comments. He said he found it confusing that the core assumption in the proposed concept is that the three xylene isomers could be considered to be equivalent, with a test of a mixture of the three then being representative of the hazards of any of the individual isomers. This is opposed to the assumption underlying the TMB concept, in which the individual components were thought to be so different that they should be tested individually, precluding testing of mixtures.

Dr. McKee addressed both the proposals for prechronic testing and for chronic testing and questioned the justification of the inhalation carcinogenicity study, pointing out that in the prior NTP gavage studies, the technical grade xylene containing ethylbenzene did not produce tumors. Thus, the justification for a pure xylene carcinogenesis study is a belief that the ethylbenzene was somehow obscuring the effects of the xylene in the previous study, which would seem unlikely if the ethylbenzene by itself produced tumors in the animals. The other possibility would be a portal of entry issue, which aligns with his previous comment that many of the alkylbenzenes are respiratory irritants in rodents. He questioned whether such agents that produce respiratory tumors in rodents following prolonged inhalation exposure really represent a risk to humans.

Regarding the proposed non-cancer endpoints, he cited a number of previous tests that had been conducted, and was unsure what additional testing in animals would add to already existing knowledge. Data gaps should be addressed using the read-across

strategy. He added that it was his understanding that the individual isomers are primarily used as chemical intermediates, so to the extent there is exposure, it would be occupational, whereas the technical grade xylene is used as a solvent. He said exposure to xylene in gasoline vapor is unlikely.

D. BSC Discussion

Dr. Genter, first discussant, said she believed the project has merit given the lack of an inhalation study specifically to look at respiratory tract effects. The question for her was whether it should be conducted with a relevant mixture including ethylbenzene or on the purified xylenes mixture. It was unclear to her what the mixture would be. She noted that there appear to be no standard, 2-generation reproductive toxicity or developmental neurotoxicity studies, so those gaps should be filled through inhalation studies. She asked to see information about possible differences in the intermediate metabolism of the xylene isomers prior to conjugation, and how those might compare to ethylbenzene metabolites. She rated her enthusiasm for the project as moderate-to-high.

Dr. Stout agreed that determining the composition of the test article is a challenging issue. He said it would be challenging to create a mixture that contained ethylbenzene at a low enough level as to not be carcinogenic.

Dr. Markowitz, second discussant, said he would give the project high priority, because the mixture is produced at a rate of one billion pounds per year. He said the likelihood is high of workers and consumers being exposed.

Dr. Hattis, third discussant, said he had limited enthusiasm for the carcinogenesis portion of the project, given the negative results from the gavage assay. He felt the emphasis for the chemical should be shifted to developmental toxicity and reproductive effects. Overall he rated the priority as moderate-to-low, regardless of high exposure.

Dr. Dorman noted that there is a huge database available for technical grade xylene and questioned where the stated data gap exists. He asked if there is any evidence to suggest that the technical grade mixture would under-predict the toxicity of purified xylene. He thought it would be relevant to test the two types of compounds comparatively to see if the difference had any effect on endpoints. Dr. Stout acknowledged the existing databases, but said data gaps remain, particularly in carcinogenicity and developmental neurotoxicity information. He said the idea is to assess whether the xylenes represent a hazard in and of themselves. Dr. Dorman suggested including technical grade material along with the pure material in the study design, so that any evidence of interactions between xylene and ethylbenzene could be determined.

Dr. Peterson noted that the BSC had differing rankings for the project, but overall ranked it as having a moderate priority.

Dr. Bucher commented that the nomination had been with NTP for some time, and that staff had been struggling with the same issues brought forth by the BSC.

X. Office of Health Assessment and Translation (OHAT) Concepts

A. Presentation: Introduction

Dr. Kristina Thayer, DNTP OHAT, introduced the two OHAT concepts that would be presented to the BSC. She provided the BSC with a brief background about OHAT, which is part of the NTP's assessment and translation capabilities, along with the Office of the Report on Carcinogens. OHAT produces literature reviews, workshops, and research projects, with an emphasis on translation research.

Dr. Thayer described the OHAT process for bringing concepts to the BSC. She noted that at this point methods for the proposed projects are not fully developed, allowing for public and BSC input.

B. Presentation: NIEHS-EPA Collaborative Project to Improve Characterization of Personal Care Product and Home Exposures

Kyla Taylor, DNTP OHAT, briefed the BSC on the collaborative project between the NIEHS and the EPA to improve characterization of personal care product and home exposures. The research needs tied to the project emerged in Theme 2, Goal 3 of the 2013 NIEHS Strategic Plan and the 2011 NIEHS Mixtures Workshop. The motivation for the project stemmed from NIEHS's interest in evaluating the NIEHS Sister Study personal care product questionnaire as an exposure assessment tool.

The collaboration with the EPA allows the project to expand its scope through extensive and repeated collection of exposure information, including additional exposure information from EPA collection tools. Objective 1 will be to evaluate the utility of the two questionnaire instruments from the Sister Study and questionnaire from the Rudel 2011 study of food packaging and processed food. Objective 2 will be to inform and evaluate models designed to predict exposure to chemicals in the environment, and to demonstrate and evaluate novel methods for chemical exposure measurement. The proposed approach involves a 10-day pilot study of 10-15 women using the Clinical Research Unit's (CRU) sample registry. Participants would take the three questionnaires, and blood and urine samples would be collected. An EPA field team member would visit the participants during the morning of each sampling day, to collect the urine samples and house dust samples. Results from the pilot would be used to inform a larger exposure study.

C. BSC Discussion

Dr. Udasin asked about the process of becoming a registrant at the CRU. Ms. Taylor said the registrants are people willing to come to the CRU and participate, and that they are paid. They are not meant to be representative of society as a whole, she added. Dr. Birnbaum added that there is a large study at the CRU called the Environmental Polymorphism Registry, which has recruited between 12,000 and 16,000 people from the local area, with as many as 15 active protocols running at a time. Ms. Taylor said the subjects for the pilot study would likely be some of the Registry participants and some who are recruited especially for the study.

Dr. Hattis noted that the samples to be collected would cover a range of time, and that uses of different products would also be applicable to different time periods. He asked whether the differential time factor would be treated in the analysis. Ms. Taylor noted that the questionnaire covers 12 months, but that the daily diary and biomonitoring would only be 10 days. She said the questionnaire does ask seasonal questions, and the daily diary and biomonitoring would be used to assess correlation, if any, between reported and documented uses of personal care products.

Dr. Howard asked about the 25-40 year-old age range planned for the pilot study. He suggested it might be more effective to narrow the age range. Ms. Taylor said the range was chosen for reproductive years, partially to explore endocrine disrupting chemicals exposure. However, she said a narrower range would be considered when designing the study.

Dr. Chapin asked if the intent of the study is to see how good the questionnaires are by selecting a few people who have taken the questionnaires and then analyzing them and their surroundings, to see how well the questionnaires recapitulate what the analysis numbers show. Ms. Taylor said the pilot phase is about feasibility, and that there would not be the ability to reach valid conclusions with such a small sample, although interesting patterns may emerge. Assuming a larger sample size, the intent would be to see if the questionnaires could be used as surrogates for biomonitoring data. Dr. Chapin asked if there were plans to modify the questionnaires if they do not do a great job of mimicking the biological data. Ms. Taylor said there are no such plans, as the questionnaires are already in use in the Sister Study. Dr. Thayer said the results of the project might inform future survey tools.

Dr. Timothy Buckley, EPA Division of Human Exposure and Atmospheric Sciences, added that EPA sees this as fertile ground for designing strategies to assess exposures in personal care products, as well as models that have been developed. He said the kinetic connections made would be a very important outcome, helping to provide a mass balance accounting of chemicals in personal care products.

Dr. Hattis said that an almost equally important outcome for assessing exposures would be assessing uncertainty in the individual exposure estimations.

Dr. Dorman suggested that another experimental endpoint to consider would be to assess whether self-reporting of product use is subject to recall bias. Ms. Taylor agreed that would be of interest. Dr. Howard asked about the positive control in the study for assessing exposure. Dr. Buckley said that in a strict sense there is no positive control, but the intent of the design is to assess variability in exposure to chemicals in the consumer products. He felt that was consistent with the design of a pilot study. Dr. John Wambaugh, EPA, added that assessment of chemicals such as parabens could serve as positive controls in the sense that much is known about their sources of exposure.

Dr. Thayer asked Dr. Buckley whether there would be ingredient lists for the products. Dr. Buckley said there would be detailed information about the consumer products purchased for use within the home, as well as other sources of the data about the ingredients and contaminants in the products.

Dr. Chapin noted EPA's use of the questionnaires along with exposure measurements. He said the combination brings the EPA's analytic abilities together with the questionnaire methodologies, seeking correlations between questionnaire responses and biomonitoring observations and analysis. Dr. Bucher said a huge amount of information is gained by integrating the two types of tools.

Dr. Markowitz noted that two of the questionnaires cover a 12-month period, and said that part of the intent would be to see how well they match up with the short-term environmental exposure measurements.

Dr. Udasin, first discussant, said the project has much merit, with the potential to help scientists who want to use the questionnaires for cancer research, or as a surrogate for biomonitoring. She considered the scope of the problem clearly defined, but felt the age range should be narrowed, because with such a small sample size, the subjects should be as uniform as possible. She assumed that the pilot size of 10-15 subjects was related to cost. She said the public health impact of the work would be very high, and that it is good for the agencies to work together to study patterns of human behavior without the need to study animals. Ms. Taylor confirmed that cost and feasibility were the main reasons for the sample size. Dr. Birnbaum added that to have a study with more than 9 subjects, Office of Management and Budget approval, which can take 6-9 months, must be obtained for the survey instrument.

Dr. Markowitz, second discussant, said he was reasonably enthusiastic about the project, approving of the idea of combining questionnaires with biomonitoring data. He

understood that this is a pilot project, and that it would inform larger sample size. He assumed the pilot would help focus the ultimate objectives. He suggested that comparable pesticide studies and nutritional studies would help inform methods. He said his priority for the project would be a moderate, but supported the collaboration.

Dr. Buckley agreed that the project would need more focus, which would come with time as the project is developed further. He said one of the attractions of the project is its longitudinal nature. Dr. Birnbaum questioned whether the study is actually longitudinal, being only 10 days. She said that the Sister Study was more traditionally longitudinal. Dr. Michael DeVito, DNTP Laboratory, suggested that in this case, "longitudinal" should be thought of in a different way, in that some of the chemicals have very short half-lives, some of 24 hours or less. Thus, over 10 days, 10 half-lives would be measured, which is considerable.

Dr. Chapin, third discussant, said he also approved of multiple agencies working together on a project, but he struggled to find a firm rationale and a problem statement the project would attempt to solve. He could not assess the value of OHAT's contribution to the project. He said his enthusiasm is high in that the project may lead to improved questionnaires in the future, but he is concerned about the lack of clarity regarding outcomes.

Dr. Thayer said OHAT sees the value in improving questionnaires and other tools. Dr. Bucher added that such improvements would also improve systematic reviews. Dr. Chapin asked how far beyond the three questionnaires those improvements might extend. Dr. Thayer said there is much redundancy in some of the other questionnaires, with overlapping questions.

Dr. Dorman noted that many survey instruments look at frequency but do not look at amount, and asked if there had been any thought to adding a mechanism to quantify amount of exposure. Ms. Taylor said the collaborators are considering that.

Dr. Udasin said she felt enthusiastic about the project, but would like to see more information about the study design, including phrasing of questions. Dr. Birnbaum reminded the BSC that the project is still in its early stages, and is a work in progress. She said the ultimate question for the BSC is whether it is worth pursuing, and that her impression is that the BSC feels that it is, but it needs much more work.

Dr. Peterson felt the BSC had medium-to-high enthusiasm for the project, with the highest priority element being the interagency interactions, and with the hope that the pilot study will yield useful information.

Dr. Peterson adjourned the meeting for the day at 4:30 PM.

June 18, 2014

Dr. Peterson reconvened the meeting and asked BSC members and other attendees to introduce themselves. Dr. White read the conflict of interest policy statement.

XI. Tox21 Update

A. Presentation: Introduction

Dr. Raymond Tice, DNTP Biomolecular Screening Branch, updated the BSC on the Tox21 program. The program arose from the 2004 NTP Vision and Roadmap for the 21st Century, which identified the need for a major initiative to develop a high throughput screening program with the goals (1) to prioritize chemicals for further in-depth toxicological evaluation; (2) to identify mechanisms of toxicity (characterize toxicity pathways, facilitate cross-species extrapolation, provide input to models for low-dose extrapolation); and (3) to develop predictive models for *in vivo* biological response in humans.

He reviewed the timeline, goals, and organizational structure of Tox21. He described the three phases of Tox21. Phase I, which lasted from 2005-2010, was the proof of principle stage. Phase II, which ran from 2011-2014, involves expanded compound screening, including the 10K Compound Library and the 1000 Genomes Toxicity Screening Project. Tox21 Phase III, which began in 2013, focuses on improving biological coverage and relevance.

B. BSC Discussion

Dr. Chapin commented on how amazing the capability of testing hundreds of thousands of chemicals is.

Dr. Sobrian asked how Phases I and II will inform Phase III. Dr. Tice said the program is moving into high-content screening with multiple measures in the same cell type rather than HT only, and that the data generated in Phases I and II are being used to identify the most relevant assays, the compounds of special interest, and the concentrations to be used. He noted that because it is known what the 10K compounds do in different assays, it is now possible to develop a tool to assess correlations across compounds, looking for similarity of biological and chemical patterns. The biological data would then be applied to the transcriptomics data to help identify pathways. Dr. Bucher noted that Phases I and II dealt with developing huge technological capabilities and approaches, all of which will be applicable to the multiplexed materials that will come out of Phase III.

Dr. Hattis said he had analyzed some of the Phase I data several years ago, and found poor correlation between dose and effect. He said he was pleased that now attention is being paid to communication among cells. He cited the Jaworska (2013) paper on adverse outcome pathways (AOPs) as an example. Dr. Tice described current efforts involving zebrafish and *C. elegans* as involving cellular interactions, although they do not have the throughput to look at large chemical space and to bin compounds. However, prioritization going from simplicity to complexity is important, using some filters along the way to ensure being in the right biological space. He said that no single system would be sufficient, but the totality of the data is important.

C. Presentation: Tox21 Phase III: The S1500 Genes HT Transcriptomics Project

Dr. Richard Paules, DNTP Biomolecular Screening Branch, presented the BSC with a progress report on the Tox21 Phase III S1500 Genes Project HT Transcriptomics Project. The project operates under the hypothesis that following exposures, alterations in the transcriptome in cells and human tissues, as well as model organisms, can provide linkage between chemicals and human toxicity and/or disease outcomes. The need is for a rapid and low-cost method to measure such alterations in large numbers. At this time, whole transcriptome technologies are prohibitively expensive for HT applications. Therefore, it will be necessary to focus on a subset of genes to use in a rapid, low-cost technology suitable for HT studies.

Dr. Paules described several efforts that preceded the S1500 Project, including the HT Transcriptomics Workshop in 2013, the expression-based Connectivity Map Project from the Broad Institute that led to the development of the HT L1000 Landmark Genes Project, and the Library of Integrated Network-based Cellular Signatures [LINCS] NIH Common Fund Project. Tox21 efforts have focused on identifying a “sentinel” set of approximately 1500 genes to be used in a HT assay. This will focus efforts on a human gene set first, providing linkage with Tox21 HT screening efforts utilizing human cell systems to focus on human health. Robust bioinformatic modules are being developed, utilizing two large rat toxicogenomics data sets for training and testing the algorithms. Once a bioinformatics approach is developed, it will be applied to human Affymetrix data in the Gene Expression Omnibus public data repository of *the* National Center for Biotechnology Information. The training data set being used is the Toxicogenomics Genomics-assisted Toxicity Evaluation System [TG-GATES] rat liver data and the test data set being used is the independent DrugMatrix rat data. “Extrapolatability” will be a vital component of the program, referring to the ability to infer or impute, with some accuracy, the expression changes in all genes from those observed in the reduced set of sentinel genes. Overall, the S1500 gene set should have the attributes of capturing maximal biological diversity, capturing those genes with maximal co-expression,

ensuring maximal biological pathway coverage, inclusion of important toxicity and disease related genes, and inclusion of the L1000 Landmark gene set as a component of the S1500 genes.

D. BSC Discussion

Dr. Dorman asked about the effect on imputation of randomly selecting 1500 genes, as opposed to using an expert-guided approach. Dr. Paules said that had not yet been done, but it is something the group would be doing in the future. Dr. Dorman asked about oversight of the project, given that it is being conducted at multiple locations and agencies. Dr. Bucher said there is an NTP Executive Committee with representatives from the four agencies involved. He noted that the complexity of the projects makes it difficult for one body to oversee the entire program. He said public outreach is an important component of transparency, and Dr. Tice has made presentations on Tox21 globally. Dr. Dorman asked about the role of the BSC, assuming that it is more "for your information" than direct scientific oversight. Dr. Bucher said the BSC could not possibly be provided with sufficient information ahead of meetings to be able to answer charge questions about the development of the program. He said he sees the BSC's role as bringing a perspective to reviewing the broad strokes of the program, providing important comments, or suggesting other opportunities or approaches. Dr. Birnbaum agreed that the BSC's role is "big picture." Dr. Tice added that he and his counterparts at the other agencies all report to their superiors, and have advisory groups to help manage the program. He said there exists both a formal internal structure and a formal structure for acquiring external guidance and support from the management groups.

Dr. Hattis said it was his impression that the ultimate goal is to be able to predict *in vivo* potency and modes of action, and that the selection of the subset of genes to be studied should be influenced by how well it contributes to the more distant goal. Dr. Paules replied that by using the two robust rat data sets, the team has the ability to examine chemical classes, mode of action, and biological adverse endpoints.

Dr. Sobrian, first discussant, asked why genes from rats, zebrafish, and *C. elegans* are being studied, when the goal is to assess effects in humans, and how the information will relate to public health in humans. Dr. Paules said it is being looked at on two levels. First, the robust rat data are being used to assess the success of the algorithms. The real reason for looking across multiple species is that it has been difficult to extrapolate from other species to humans, and by understanding the biology and the pathways, and how similar the pathways are to humans, extrapolation to human biology will be improved. The ultimate goal is a better understanding of impacts on human health, but all of the tools mentioned in the project will continue to be used to generate data that will ultimately inform human health protection.

Dr. Chapin, second discussant, appreciated the structure of Dr. Paules' presentation, which made an overwhelming topic much more approachable. He felt the project represents a sensible reduction in complexity, is an appropriate use of resources across the involved agencies, is exactly what NTP should be doing, and should have a high priority. He discussed the importance of phenotypic anchoring and the necessity for the ultimate answers being human-based. Dr. Paules said all scientists are forced to use systems that have limitations, and that it is important to acknowledge those limitations in this and all projects.

Dr. Dorman, third discussant, said the sequence to be employed was somewhat unclear in extrapolating from the other species to humans. He asked if the 1500 genes number would go across multiple species and explore multiple chemical spaces. Dr. Paules responded that the 1500 genes sets would be unique to each species, but would be derived from the same algorithm, with the same goal in mind.

Dr. Brown, fourth discussant, said it was a "big data" problem and perfect for a government-scale undertaking. He suggested that it would be important to identify and use a training set of molecules, as well as a test set, to validate the approach to finding early toxicity signals. Dr. Brown compared it to the use of comparative molecular field analysis in drug discovery, in which models are generated based on differences in large numbers of structurally similar compounds. Dr. Tice noted that Tox21 is using training sets and tests sets. Dr. Brown said the advantage of employing such methods is the ability to generate predictive values from actual values, and that the correlative value will help understand the overall value of the test set, showing where the gaps might be in the model. Dr. Brown recommended that the changes seen in transcripts should be related to proteomic changes. He suggested there should be more clarity on the criteria for the selection or identification of compounds to be included in the project.

XII. NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) Adverse Outcome Pathways and Skin Sensitization Testing

A. Presentation

Dr. Warren Casey, NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), briefed the BSC on current initiatives involving AOPs and skin sensitization testing. An AOP is defined by the Organisation for Economic Co-operation and Development (OECD) as "an analytical construct that describes a sequential chain of causally linked events at different levels of biological organisation that leads to an adverse health or ecotoxicological effect." AOPs are the

central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning.

Dr. Casey described the background and history of AOPs, and provided examples of AOPs currently listed in the OECD Wiki. He discussed how AOPs are utilized as part of integrated testing and decision strategies. He provided details on the skin sensitization AOP, which is well defined and the first and thus far only AOP testing procedure to be validated and approved by OECD. It is highly probable that *in vitro* skin sensitization testing can replace live animal testing, sparing considerable animal use, while providing equal protection.

Dr. Casey discussed the potential uses of Bayesian networks, a method of arriving at statistical likelihood based on partial information. He said they provide a coherent probabilistic framework for reasoning and guiding decisions on the classification of a substance or the need for additional testing. He described the work of Dr. Joanna Jaworska, Proctor and Gamble, who has developed a Bayesian approach specifically for skin sensitization (Jaworska et al., 2013). He noted that many regulators are not accustomed to probabilistic approaches, preferring certainty. That is an obstacle that will need to be overcome to allow widespread use of the Bayesian AOP approach. Limited availability of software and lack of transparency in the software have also been challenges, but now NICEATM has worked with other groups to develop and publish open source software, which is now publicly available (Pirone et al., 2014).

Dr. Casey described a pilot project being used to validate an integrated decision and testing strategy using a variety of computational approaches to classify chemicals as sensitizers or non-sensitizers, using the local lymph node assay (LLNA) as the reference.

He mentioned a workshop scheduled for September 2014, titled *AOPs: From Research to Regulation.*

B. BSC Discussion

Dr. Peterson acknowledged written comments from PETA.

Dr. Hattis said the AOP diagram in the 2013 Jaworska paper confused him, due to the arrows going in seemingly inappropriate directions. Dr. Nicole Kleinstreuer, Integrated Laboratory Systems (ILS), explained that the direction of the arrows follows a convention used in Bayesian networks.

Dr. Udasin asked about the use of AOPs in the occupational health setting. Dr. Casey said that part of the validation process would be to determine which tests would provide enough information to make regulatory decisions.

Dr. Brown, first discussant, asked Dr. Casey about the criteria that would be used to select components. Dr. Casey replied that the primary criterion is that they are validated. Dr. Brown asked how the criteria would be used to guide decisions regarding unknown compounds. Dr. Casey said QSAR models would be used to help provide some initial information to guide which *in vitro* tests might be used. Dr. Brown said in this case it would be important to develop the right testing strategy to test the Bayesian analysis, perhaps to use a training set. Dr. Casey agreed and said it would definitely be part of the validation process. He added that the biggest challenge is having enough reference chemicals in the validation for training and testing sets.

XIII. Draft NTP Concept: Inflammation-based Atherosclerosis Associated with Environmental Chemicals

A. Presentation

Dr. Andrew Rooney, OHAT, presented the draft NTP concept on Inflammation-based Atherosclerosis Associated with Environmental Exposures.

The proposed project is related to Goal 1 of the NIEHS Strategic Plan. He presented background information on chronic inflammation's contribution to multiple health effects, with a focus on atherosclerosis. He noted that inflammation is a cellular response to chemical damage, physical damage, or infection that can be part of a healthy restorative process, or contribute to adverse health outcomes when the response becomes chronic. The extent to which environmental exposures ultimately lead to adverse health effects through an inflammatory pathway remains unclear, despite growing evidence for a role of the environment in a wide range of diseases that involve inflammation.

The proposed approach is to restrict the evaluation to a single health effect, atherosclerosis, allowing direct comparison of supporting or opposing evidence. Key issues are selecting inflammatory markers associated with atherosclerosis and addressing the extent to which conclusions can be integrated across environmental agents. The overall objective is to evaluate the evidence that environmental substances contribute to inflammation that leads to atherosclerosis. An additional objective is to evaluate the evidence for specific biomarkers of environmentally induced inflammation linked to atherosclerosis.

A scoping-level literature search shows that the atherosclerosis literature is of manageable size for review. The methods for the proposed evaluation are complementary. There will be a literature-based evaluation using the OHAT approach to systematic review and evidence integration, and development of an AOP depicting the environmental influences on inflammation-based atherosclerosis.

The project may inform the selection of biomarkers in future studies of environmentally induced inflammation and evaluate the utility of an AOP as part of a dual approach for assessing a literature-based environmental health question. Lessons learned in evaluating the role of environmentally induced inflammation for atherosclerosis can potentially inform the evaluation of inflammation on a wider range of health effects

B. BSC Discussion

Dr. Brown asked what strategies would be used to select the best assays for assessing atherosclerosis. Dr. Rooney replied that the choices would largely be guided by consulting technical experts. Dr. Brown asked if a mathematical assessment were being considered. Dr. Rooney said yes.

Dr. Corcoran asked if the AOP would be put on a wiki page to obtain crowdsourced information. Dr. Rooney said he was not sure if the NTP would follow the OECD model, but that the project and the AOP would be posted on NTP webpages for public comment and the team is interacting with the OECD about the AOP.

Dr. Markowitz, first discussant, said he was still somewhat unclear about the information flow in the project, in that there did not seem to be intent to explore the relation between biomarkers and atherosclerosis. Also, he said it appears that the intent is to select a subset of biomarkers to explore their relationships with environmental exposures. Dr. Rooney said atherosclerosis was selected because of the established role for inflammation in development of the disease, and therefore the evaluation would not be focused on proving that relationship. He clarified that the intent is to cast a “wide net” and include any inflammatory biomarkers with a potential link to atherosclerosis because there is no way to know which inflammatory marker might be a critical pathway for environmental substances. He explained that data from known pathways by which infectious agents contribute to atherosclerosis may help develop the AOP, but environmental substances will be the primary focus. Regarding the acute versus chronic inflammation, Dr. Markowitz asked whether the acute inflammation could be differentiated from chronic inflammation in the project. Dr. Rooney agreed that separating acute and chronic inflammation might be a challenge and the ability to analyze the different phases of inflammation would depend on available data. Dr. Markowitz rated the project as having a high level of significance.

Dr. Dorman, second discussant, expressed concern with some of the language in the write-up regarding inflammatory biomarkers and disease states. He said that at some point, certain markers will need to be stated as being associated with atherosclerosis, and then environmental agents would be causally linked with that subset of biomarkers. He questioned the choice of atherosclerosis as the topic for a “mode of action” systematic review, as it is so complicated, particularly with comorbidities. He noted that

many of the animal models do not replicate the pathology associated with atherosclerosis in humans. He suggested looking at a simpler disease state such as respiratory sensitization or another type of disease state in which comorbidities would not be quite as important. He rated his overall enthusiasm for the project as moderate. Regarding the question of causality, Dr. Bucher said the expectation is that the project will fall short of being able to establish causality between any particular environmental agent and atherosclerosis. However, it will establish a degree of confidence in the existing data, and set the stage for studying a disease that NTP has not contributed to in any significant way in the past, but is hugely important from a societal standpoint. Dr. Rooney said atherosclerosis was chosen because of the clear inflammatory link. He agreed that there are challenges regarding comorbidity. The human and animal evidence streams would be evaluated separately, and, if possible, overlapping inflammatory markers would allow direct comparison between animal and human. Dr. Dorman emphasized the importance of phenotypic anchoring for what would be called an atherosclerotic lesion in humans and animals.

Dr. Udasin, third discussant, approved of the project, and said she considered it to be extremely clinically relevant. She applauded NTP for addressing a health effect with such broad relevance. She agreed there was concern about the issue of comorbidities; however, she looked forward to seeing more detail on how that issue would be addressed.

Dr. Genter, fourth discussant, appreciated Dr. Casey's AOP presentation, which she said set the stage nicely for Dr. Rooney's talk. She said she had high enthusiasm for the project.

Dr. Peterson said that overall the BSC expressed support for the project.

XIV. Adjournment

Dr. Birnbaum thanked the BSC for its input. Dr. Bucher thanked the NTP staff members for their work in preparing the extensive materials for the BSC meeting.

Dr. Peterson adjourned the BSC meeting at 12:05 PM, June 18, 2014.