National Toxicology Program

Board of Scientific Counselors

Summary Minutes from June 25, 2013

National Institute of Environmental Health Sciences

Research Triangle Park, NC

Contents

I. Frequently Used Abbreviations and Acronyms

1-BP	1-bromopropane
ACC	American Chemistry Council
BSC	Board of Scientific Counselors
DNTP	Division of the NTP
EPA	U.S. Environmental Protection Agency
FDA	U.S. Food and Drug Administration
GE	General Electric
HHS	Health and Human Services
H_2S	hydrogen sulfide
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
LAN	light at night
NAS	National Academy of Sciences
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NIOSH	National Institute of Occupational Safety and Health
NTP	National Toxicology Program
OHAT	Office of Health Assessment and Translation
ORoC	Office of the Report on Carcinogens
PWG	Pathology Working Group
QA	quality assurance
QC	quality control
RoC	Report on Carcinogens

II. Attendees

Members in Attendance:
Robert Chapin, Pfizer (by telephone)
George Corcoran, Wayne State University
David Dorman, North Carolina State University
Miguel Fernández, University of Texas Health Science Center at San Antonio
Dale Hattis, Clark University
Melissa McDiarmid, University of Maryland School of Medicine (Chair)
Richard Miller, GlaxoSmithKline
Lisa Minor, *In Vitro* Strategies
Iris Udasin, University of Medicine and Dentistry of New Jersey

***Ad Hoc* Member:**
Richard Stevens, University of Connecticut Health Center (by telephone)

Members not in Attendance:
Jack Harkema, Michigan State University
Lisa Peterson, University of Minnesota
Sonya Sobrian, Howard University

Other Federal Agency Staff:
Paul Howard, US Food and Drug Administration (FDA)
Gayle DeBord, National Institute for Occupational Safety and Health (NIOSH)

National Institute of Environmental Health Sciences (NIEHS) Staff:

Danica Andrews	Shuo Li	Katarzyna Szymanska
Linda Birnbaum	Ruth Lunn	Kris Thayer
Abee Boyles	Robin Mackar	Erik Tokar
John Bucher	Dave Malarkey	Velvet Torain
Helen Cunny	Scott Masten	Molly Vallant
Susan Elmore	Arun Pandiri	Michael Waalkes
Melissa Gentry	Katie Pelch	Nigel Walker
Ron Herbert	Andrew Rooney	Lori White
Kembra Howdeshell (by telephone)	Brian Sayers	Mary Wolfe
Kyathanahalli Janardhan	Robert Sills	Rick Woychik
Heather King	Diane Spencer	Yun Xie

Public:
Nancy Beck, American Chemistry Council (ACC)
Steven Brecher, CSS-Dynamac
Patricia Kablach Casano, General Electric (GE, by telephone)
Reshan Fernando, Research Triangle Institute International
Ernie Hood, Bridport Services
Marcus Jackson, Integrated Laboratory Systems
Katya Shmyanska, visiting veterinary student
Audrey Turley, ICF International
Michael Viana, Consolidated Safety Services-Dynamac

III. Introductions and Welcome

The National Toxicology Program (NTP) Board of Scientific Counselors (BSC) met June 25, 2013, in Rodbell Auditorium, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. Dr. Melissa McDiarmid served as chair. She welcomed everyone to the meeting and asked BSC members and other attendees to introduce themselves. She welcomed new BSC members Drs. Iris Udasin and George Corcoran. She noted that Dr. Richard Stevens from the University of Connecticut Health Center would be joining the meeting by telephone as an *ad hoc* reviewer for the draft Report on Carcinogens (RoC) concept. Dr. Lori White, BSC Designated Federal Official, mentioned that Dr. Lisa Peterson was also a new BSC member, but not able to attend. Dr. White read the conflict of interest policy statement. NTP Associate Director Dr. John Bucher welcomed the BSC members to the meeting.

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 December 2012. She reported that the NIEHS is currently in the implementation phase of the new Strategic Plan. Leadership is reviewing the

reports from the eight cross-divisional implementation-planning teams. She noted that NIEHS has hosted a series of special visitors over the past few months, including the directors of the National Institute of Diabetes and Digestive and Kidney Diseases and the National Institute of Neurological Disorders and Stroke. She mentioned that the My Air, My Health Challenge was completed in June, with a group winning the $100,000 for its design of Conscious Clothing. A new challenge, the NIEHS-National Center for Advancing Translational Sciences-University of North Carolina DREAM Toxicogenetics Challenge, was launched June 10. NIEHS is also participating in several "Big Data" initiatives, including the National Consortium for Data Science and MATCH, the Metadata Access Tool for Climate and Health, as well as several NIH initiatives.

Regarding appropriations, Dr. Birnbaum noted that sequestration became effective March 27, with NIEHS cut 5.7% in its Health and Labor appropriation. Superfund was cut 5%. The Worker Education and Training Program was cut from $10 million to $9.2 million. Moving forward into FY14, it is anticipated there will likely be no budget agreement and the federal government will operate under a series of Continuing Resolutions, which will make planning difficult. She also updated the BSC on a variety of legislative hearings and briefings over the past several months.

Dr. Birnbaum briefly summarized several recent scientific advances and publications involving NIEHS scientists from the three divisions and Office of the Director, or grantees, including findings on bisphenol A, the p53 transcription factor, arsenic exposures, traffic-related air pollution, *Ginkgo biloba* carcinogenicity, and a Tox21 update.

V. NTP Monograph on Developmental Effects and Pregnancy Outcomes Associated with Cancer Chemotherapy During Pregnancy

A. Presentation

Dr. Kembra Howdeshell, Health Scientist, Office of Health Assessment and Translation (OHAT), DNTP, participated by telephone. She briefed the BSC on the final version of the NTP Monograph. Dr. Lisa Minor chaired the BSC meeting for this topic since Dr. McDiarmid had served as the BSC liaison at the peer review of the monograph.

Dr. Howdeshell reviewed the OHAT evaluation process, and noted that the monograph is currently at one of its final steps prior to publication – presentation to the BSC regarding peer review of the draft monograph. She reviewed the background and rationale for the evaluation, and the scope and organization of the monograph, which summarizes the effects of gestational exposure to chemotherapy on pregnancy outcomes. The monograph is intended to serve as a tool for physicians and patients in making clinical decisions.

She described the peer review of the monograph conducted by an independent expert panel October 1-2, 2012, at NIEHS, including the key questions considered by the panel, which

generally agreed with the draft NTP findings on health effects associated with chemotherapy use during pregnancy.

She reported the key findings of the monograph, discussed its conclusions, and the monograph's emphasis on any decision to use chemotherapy during pregnancy be made by the patient in consultation with her health care team. She mentioned several anticipated steps moving forward, including offering the master file of data on gestationally-exposed conceptuses from the monograph as a resource, presenting the monograph at the Annual Meeting of the Teratology Society on June 25, 2013, publishing a peer-reviewed paper based on the monograph, and developing a future concept for a systematic review of the health effects of occupational exposures to cancer chemotherapeutic agents.

B. BSC Questions and Discussion

Dr. Dale Hattis asked for more information on the monograph's treatment of continuous variables. He noted that most were referred to as quantile variables, which he felt was "a sacrifice of considerable statistical power." He urged that the data be analyzed as continuous variables, despite challenges associated with that approach. Dr. Howdeshell thanked him for the suggestion and noted that the data are very challenging to interpret, particularly since many of the chemotherapy agents are used in combination with other therapies. Dr. Thayer mentioned that the topic had been discussed during the peer-review meeting. She said an example of the challenging issues is body weight, with enormous variation based on geographic areas and a span of 50 years in the data, making normal values quite variable.

Dr. Dorman said he would like to have had a clear statement in the monograph of the criteria that were used to define the associations between a health effect and chemotherapy exposure, since they did not appear to be based on a statistical analysis of the data. Specifically, he was trying to get a sense of how the group picked and chose the background rates it reported. Dr. Howdeshell said the rates reported in the document were apparent rates based on information available in the public literature. The limitations of the data were discussed by the review panel and are identified in the monograph. Dr. Howdeshell said because they had not done statistical analyses, they compared the apparent rates to general population rates, focusing on specific health outcomes that appeared to be of the highest priority in the literature reviewed.

Dr. McDiarmid said the peer-review panel spent an enormous amount of time discussing the limitations of the data. She noted that the panel was "enormously exuberant" about the comprehensive nature of the tables that were developed. There were arguments about both denominator data and numerator data over the course of the two days, and that the derived rates probably underestimated the risks. She said there had been helpful brainstorming about public health next steps, including a case report form to be circulated by journal editors, possibly leading to a registry. Dr. Howdeshell added that OHAT is considering preparing a peer-review publication on what constitutes an ideal case report.

VI. Office of Report on Carcinogens Peer Review Meeting on 1-Bromopropane and Cumene

A. Presentation

Dr. Ruth Lunn, Director, Office of the Report on Carcinogens (ORoC) briefed the BSC on the March 21-22, 2013, peer review of the draft RoC monographs for cumene and 1-bromopropane (1-BP). She briefly reviewed the process for preparation of the RoC, placing the BSC meeting on the timeline. For each substance, she provided the BSC with the following information: (1) steps in the review process, public comments, and development of the monograph; (2) the peer-review panel meeting, including membership, the charge, and the panel's recommendations (Panel Report); and (3) the NTP response to the panel's recommendations. She noted that monographs are new to the RoC process, and consist of an integrated document with the cancer assessment leading to the NTP's opinion and substance profile in one document. She described the elements leading to the ORoC's conclusions, based on level of evidence and evaluation of mechanistic data.

1-Bromopropane

Dr. Lunn reported that the panel agreed that a significant number of persons in the United States are exposed to 1-BP. The panel agreed by unanimous vote with the NTP that there is sufficient evidence of carcinogenicity from studies in experimental animals: skin tumors in male rats, large intestine tumors in female and male rats, and lung tumors in female mice. The panel also agreed by unanimous vote with the NTP's preliminary listing recommendation of 1-BP as *reasonably anticipated to be a human carcinogen.* The NTP concurred with the panel's recommendations regarding 1-BP.

Cumene

The panel agreed that a significant number of persons in the United States are exposed to cumene. The panel agreed by unanimous vote with the NTP that there is sufficient evidence of carcinogenicity from studies in experimental animals; however, they disagreed by split vote with the tumor sites contributing to sufficiency of carcinogenicity in experimental animals (lung tumors in male and female mice, liver tumors in female mice, and renal tumors in male rats). They proposed that the tumors sites were lung tumors in male and female mice and liver tumors in female mice. They proposed that renal tumors in male rats and benign nasal tumors in male and female rats provide supporting evidence for the carcinogenicity of cumene. The panel agreed by unanimous vote with the NTP's preliminary listing recommendation of cumene as *reasonably anticipated to be a human carcinogen.* The NTP concurred with the panel's recommendations regarding cumene.

B. BSC Discussion

Dr. Hattis, who served as BSC liaison to the peer review meeting, noted that the peer-review meeting commenters for both substances had advanced arguments as to why the substances should not be considered to be genetically acting carcinogens. He said that in both cases, the panel did not consider persuasive the issues raised by the commenters regarding classification.

In the case of cumene, the panel discussed various lung tumor sites as evidence of a genetic mode of action. He said it would be useful for the NTP to consider reinforcing that point, as that type of evidence is likely to become more common in the future, as genetic tools for typing mutations develop. He said changes in mutation spectra, possibly caused by selection effects, could be tested by transplantation experiments. He noted there had been extensive discussion of cumene pharmacokinetics and metabolism. A public commenter stated that 1-BP is not a direct-acting mutagen, so there should be thresholds. He noted that although public commenters had questioned the occurrence of significant exposure, the panel agreed that there were significant exposures to both 1-BP and cumene.

Regarding potential selection effects with cumene, Dr. Lunn said the ORoC searched the literature, but found very little supportive evidence. For 1-BP, she said ORoC felt there was adequate evidence of mutagenicity based upon data from closed-chamber tests, and other studies had shown the chemical to cause neurotoxicity and oxidative damage.

Dr. Corcoran asked for elaboration on the panel's split vote regarding the tumor sites in experimental animals. Dr. Lunn explained that the panel agreed that there was sufficient evidence provided by the lung and liver tumors, but was split regarding the renal tumors. The panel chose to include renal tumors as supporting evidence for NTP's conclusion of sufficient evidence of carcinogenicity from studies in experimental animal for cumene.

VII. Report of the NTP Associate Director

A. Presentation

Dr. Bucher, NTP Associate Director, briefed the BSC on recent and upcoming meetings and events, senior staff changes, a review of 2004 Roadmap accomplishments, and current and new directions for NTP.

Meetings that took place since the December 2012 BSC meeting included several events to update stakeholders on NTP's systemic review approach: (1) presentations of the OHAT systematic review approach at a National Academy of Sciences (NAS) Integrated Risk Information System (IRIS) review meeting (December 13, Irvine, CA), (2) Environmental Protection Agency IRIS stakeholder meeting (January 8, RTP), (3) exhibitor-hosted session at the Society of Toxicology (March 12, San Antonio, TX), and (4) an editorial on NTP implementation in April's *Environmental Health Perspectives*. RoC events of note were (1) presentation to the NAS committees reviewing the 12th RoC listings of styrene and formaldehyde (March 19), (2) peer reviews of the draft monographs on cumene and 1-BP (March 21-22, RTP), (3) webinar on pentachlorophenol synthesis contaminants and human cancer studies (April 11), and (4) dismissal by the DC District Court of the Styrene Information and Research Center's lawsuit challenging the listing of styrene in the 12th RoC. Upcoming meetings include the annual meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) on September 24, and NTP Technical Reports peer-review meetings on October 29, 2013 and in February 2014.

In senior staff changes, Dr. Stephen Ferguson has been hired to join the Biomolecular Screening Branch. Dr. Michelle Hooth is now Acting Chief of the Program Operations Branch, and Dr. Warren Casey was appointed the Acting Director of the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM).

Dr. Bucher described NTP accomplishments with respect to the NTP Roadmap set forth in 2004. He outlined the three main organizing principles in the document: (1) to refine traditional toxicology assays, (2) to develop rapid mechanism-based predictive screens for environmentally induced diseases, and (3) to improve the overall utility of NTP products for public health decision-making. He described many NTP accomplishments through the ensuing years related to all three organizing principles.

Dr. Bucher reviewed the timeline and progress associated with the Tox21 initiative, focusing on Phase II, during which 10,000 chemicals have been or will be screened in human nuclear receptor and related qHTS assays, in both agonist and antagonist modes. There is also a battery of assays dealing with the stress response. With those two areas covered, much of the potential toxicity (or inactivity) within the library should be characterized, allowing prioritization of future studies. He presented data showing the range of highly active compounds and those with no or very limited activity. He described the general approach to analyzing the 10,000 compounds, using connectivity networks to ultimately allow evaluation of the relevance of any response and identification of compounds of interest meriting further analysis. He outlined the major challenges and areas under development within Tox21, as well as the major questions still facing the initiative. One major challenge is to bring a higher order of cell and tissue interactions into individual cell-based assays. The NTP has accomplished many of the goals outlined in the 2004 NTP Roadmap, and the DNTP is continually working to provide new scientific opportunities and ways to address agency and public concerns, and provide answers in a more relevant time frame.

B. BSC Discussion

Dr. Hattis noted that Dr. Bucher had outlined some of the important opportunities and challenges facing NTP. He said the association maps Dr. Bucher had shown were interesting, but didn't show him enough about two aspects: how the within-cell control systems are being perturbed, and relationships between genes *in vivo*. He said it would be important to understand how stimuli in one particular cell type affect other cell types. Dr. Bucher noted that the Tox21 studies are providing data on immediate response while giving hints that can be assessed by looking in other databases such as the DrugMatix® database, which evaluates intermediate steps. Also, tumor "archeology" data can be accessed for further information on interactions. None of those methods independently allow development of predictive toxicology. The goal is to take information from known toxic substances and apply it to unknown toxic substances, through the lifespan, drawing from the various sources available. In the best of all worlds, the ability would exist to design studies that will allow specific questions to be addressed that fill in the gaps in the continuum.

Dr. Minor asked why the particular cell lines had been chosen, and whether there were any plans for moving to primary cells, due to concern that interpreting the data from the current cell lines could mislead investigators, as they are not being derived from “natural” cells. Dr. Bucher said that those were excellent points, and noted the reasons that NTP is trying to go to the multiplex assays using human RG cells. He confirmed that the assays he had shown use multiple cell types, and could result in outputs that are not always readily understandable as a result.

Dr. Paul Howard said one of the concerns is dosimetrics, i.e., how much of a test article is delivered to cells. He asked what the plans are for dealing with that issue. Dr. Bucher acknowledged that dosimetrics is a challenge, but that there are several approaches, including having the appropriate analytical chemistry on the 10K library. Dr. Howard noted that solubility and vapor pressure could affect whether the target article actually gets to the cell. Dr. Bucher said test articles in the 10K library are dissolved in dimethyl sulfoxide, but work is progressing to determine how to establish a water-soluble library. Dr. Minor asked whether kinetic solubility studies are being performed. Dr. Bucher said 15-point dose-response curves are being done that run from 5 micromolar to 100 millimolar ranges, but it is difficult to determine if the chemical is insoluble in the system.

Dr. Corcoran said the projects underway at the NTP are very impressive. Regarding Tox21, he noted that they are calling for exclusive use of human cells and human organ models, eschewing murine data. He asked if NTP agreed in concept with moving exclusively to human test system, and if yes, that NTP establish an instrument that assesses where NTP currently stands in this transition, that maps out how the NTP intends to achieve this objective, and that can provide informative and regular updates to stakeholders." Dr. Bucher said Tox21 had chosen to utilize human cells because there would be a limit on the amount of information that could be transferred from rodent models into understanding human cell biology.

Regarding the goal of improving the utility of NTP products for public health discussions and decision-making, Dr. Dorman asked whether any consideration had been given to using performance metrics such as surveys to qualitatively look at the impact of the programs. Dr. Bucher said that was a good suggestion and Dr. Mary Wolfe added that there are initiatives being planned to assess the impact of NTP projects.

Dr. Richard Miller wondered whether some of the existing public/private partnerships might be helpful in Tox21 efforts, and vice versa. Dr. Bucher noted that there had recently been data-sharing activities with the International Life Sciences Institute-Health and Environmental Sciences Institute. Most of the data generated by Tox21 would be made publicly available within 6 months to one year of data verification. He said the NIH Chemical Genomics Center is primarily in the area of translational medicine, particularly focused on drug development, and an offshoot of Tox21 is to try to bring the whole area of toxicological evaluation into the area of drug development. Dr. Birnbaum said the NTP is interested in developing public and private partnerships, and would be receptive to proposals in that area, particularly given current tight budgets. She stated that NIEHS is committed to making all of its data publicly available, as is all of NIH.

Dr. Hattis said it was a good idea to use human cells in Tox21, but expressed some concern that the kinds of human cell that are easily cultured are not necessarily the most important mediators of human responses. He felt it would be helpful to have some effort be given to using some of the more difficult-to-culture cells. Dr. Bucher noted that stem cells are one major area of the strategic goals for the NIEHS, and there is an interest in moving into the use of stem cells in the Tox21 program.

Dr. Udasin asked whether aging cells were being addressed, as they might respond differently than stem cells. She asked whether menopausal versus pre-menopausal cells were being examined in reproductive studies. Dr. Bucher replied that although aging cells are clearly important, those areas are currently beyond NTP's capabilities. Stem cells would be used to assess cells at different stages of development.

Dr. Corcoran again asked about NTP's plans for the utilization of human cell assays. Dr. Bucher said that human cells are being used whenever possible, although animal cell lines are still important in some instances. As a more genomic-based approach is used more, human cells will be used more frequently. Dr. Corcoran asked where Tox21 is quantitatively in terms of its long-range goal to use human cell-based assays almost exclusively. Dr. Bucher replied that it would be a very difficult prediction to make, but noted that in the 2007 NAS report *Toxicity Testing in the 21st Century*, it was thought to be a 20-25-year process. He felt that the process is further along already than originally projected, and is likely to accelerate. The challenge would not necessarily be the technological advances needed, but instead developing the human capacity to analyze and manipulate the huge amounts of data being generated.

Dr. McDiarmid asked about the Technical Report on cobalt. Dr. Bucher said cobalt had been on the NTP's radar for some time as a potential carcinogen based on epidemiology studies out of France. A NTP Technical Report concluded that there was evidence of carcinogenic activity of soluble cobalt sulfate heptahydrate in rodents. Toxicity considerations for metallic cobalt include speciation and solubility.

Dr. Miguel Fernández asked whether hydrogen sulfide (H_2S), a compound becoming more important due to hydraulic fracturing ("fracking"), might be considered a candidate for NTP analysis in the future. He said he is seeing more and more workers exposed to H_2S who suffer acute symptoms. He also suggested that NTP might consider looking at studies of the non-human biome, with its micro-organisms that may have effects on the human biome. Dr. Bucher noted that one of the roadmap activities is the exposome, with the human internal biome being part of that effort. He noted that H_2S had been nominated previously, particularly following the Gulf oil spill. He said that the issue of long-term exposure to very low levels of H_2S is important, and that it is a chemical of concern to the NTP.

Dr. Hattis said he was pleased to see glycidamide on the list of substances to be evaluated. Dr. Howard clarified that the draft NTP Technical Report on glycidamide would undergo peer review at a future meeting.

VIII. Contract Concepts: Quality Assessment Support

Introduction

Ms. Velvet Torain, NIEHS Contracting Officer, briefed the BSC on the guidelines for reviewing contract concepts, and the BSC's charge with regard to the contract concepts.

B. Presentation

Dr. Matthew Stout, Toxicologist, Program Operations Branch, DNTP, briefed the BSC on the Quality Assessment Support contract concept. He noted that the purpose of the contract is to conduct independent audits and inspections of testing facilities conducting NTP studies, study records, data, materials, and reports. He said the process aids the NTP in carrying out studies and generating data and reports of the highest quality. Such a contract has been in place for nearly three decades, with approximately 450 audits having been conducted over the past 9.5 years of the current contract. He described the various types of audits and inspections involved, and depicted where in the process the audits and inspections occur. He noted that the proposed changes to the current statement of work include changes to reflect the need for audit or inspection of NTP studies, data, and reports of increasing size, complexity, and diversity, as well as increasing capacity to audit information and data used in NTP's literature-based evaluations and stored in electronic databases. He said the NTP sought approval from the BSC to continue these activities using a contract mechanism.

C. BSC Questions and Discussion

Dr. Minor asked whether a lab inspection occurs prior to awarding a contract. Dr. Stout said it does, but with the use of a slightly different process for those inspections, although this contract may participate. He noted that under the quality assessment support contract, laboratory inspections are typically done for ongoing studies at laboratories already under contract. Dr. Minor asked about the anticipated increase in size and scope of current/future studies compared to the last 10 years. Dr. Stout replied that the NTP anticipates more reproductive and developmental toxicology studies, as well as immunotoxicology and neurotoxicology studies. Dr. Bucher added that more sophisticated auditing of complex data sets is needed, for example to understand cell phone radiation exposure data.

Dr. Corcoran, first discussant, stated, "quality assurance is absolutely essential," and asked Dr. Stout to elaborate on what the contractors do, such as validating spreadsheet calculations, as an example. Dr. Stout said the contractors would validate a spreadsheet if it were part of the study record. They audit factual information, both qualitative and quantitative. He noted that they sometimes audit 100% of a study's data, but for large datasets may audit a selected 10-20%. Dr. Corcoran asked if contractors do a re-execution of the model for a toxicokinetic calculation for a chemical within a report. Dr. Stout said the contractors would if the calculation were in the raw data. Dr. Corcoran asked if contractors ever validate whether the code is correct. Dr. Stout said he could not recall contractors ever doing so.

Dr. Corcoran asked whether a report is generated of all actions taken to sustain the validity of an approved report, with an appendix of specific actions. Dr. Stout replied that all findings are

documented. Although a formal response is not required, the NTP asks for a memorandum discussing how the findings were addressed. Dr. Corcoran asked if over the course of 450 audits certain things would come up repeatedly that would lead to changes in the policies and procedures for how NTP studies are done. Dr. Stout said they do, and that the inspections are very critical to ensuring that adequate records are kept. Dr. Bucher noted that this activity had been going on for 30 years, and that audit findings have contributed to laboratories refining their procedures. The audit findings have also lead to a refinement in the global statements of work from the NTP, which has led to the identification and elimination of many problems in the contracts.

Dr. Corcoran said the NTP could not exist without this type of activity, which is essential to the viability and integrity of NTP reports. He responded positively to each of the specific charge questions.

Dr. Minor, second discussant, felt that quality assurance audits are very important to ensure the quality and accuracy of data and studies. She noted that the scope of audits needs to expand to cover more emerging aspects of toxicology. She said she would "100% support moving forward with the audits."

Dr. Dorman asked about the proposed expansion of the audit activities related to ORoC and OHAT documents. He was unclear about the process by which NTP would audit those data, and stated that such audits may require different expertise than traditional Good Laboratory Practice audits. Dr. Stout replied that in the case of auditing a systematic review, auditors would check the database against the manuscripts with those data, to determine whether the data were entered accurately. He added that the expansion would not be intended to include the overall analytical assessment. Dr. Kristina Thayer agreed that quality assessment for literature-based evaluations would be complex, and that OHAT is in the process of working to refine its procedures to facilitate efficient and accurate quality assessment.

Dr. DeBord stated that NIOSH does its own quality assurance/quality control, and uses an outside auditor to audit its results. She asked whether NTP would audit the contractor to assess its performance. Dr. Stout said that was not currently planned.

Regarding scientific or clinical uses for the contract work, Dr. Fernández asked whether it referred to translational activities. Dr. Stout said that it did not in terms of the contract under consideration. Dr. Corcoran said it was important for the public to have faith in the data being generated, and that it has been validated and certified.

Dr. McDiarmid asked for a motion to approve the contact concept. Dr. Miller so moved and Dr. Corcoran seconded the motion. The BSC voted unanimously (8 yes, 0 no, 0 abstentions) to approve continuing this activity using a contract mechanism.

IX. Contract Concepts: Pathology Peer Review and Pathology Support

A. Presentation

Dr. David Malarkey, Pathology Group Leader, Cellular and Molecular Toxicology Branch, DNTP briefed the BSC on a contract concept for Pathology Peer Review and Pathology Support. The contracts would serve both DNTP and the NIEHS Division of Intramural Research. The new contracts would include the same pathology support that has been in place for 30 years, currently provided in three contracts. The purpose of the three contracts is to provide independent pathology peer review and pathology services for the studies conducted by the NTP and NIEHS intramural research, with the primary objective being to verify and generate accurate data in NTP studies. Other objectives include providing staffing, necropsy, histology, special techniques, training, and support for NTP and NIEHS investigations. These include the many types of studies conducted by NTP, such as the 2-year bioassay and 90-day studies. Dr. Malarkey alluded to the near-term expected number of studies and the amount of anticipated work. He provided an overview of the NTP pathology review process, which is comprised of four elements: Audit of Pathology Specimens (APS), Pathology Data Review (PDR), Pathology Quality Assessment (PQA) and Pathology Working Group (PWG). The NTP pathology review process is recognized internationally as a gold standard. Dr. Malarkey described each of the four elements, as well as the current workflow for NTP pathology peer review. He presented the new workflow for the review process, which will streamline and provide added flexibility, efficiency, and cost savings. This new workflow involves several changes to the current statement of work. Dr. Malarkey mentioned the NTP Non-Neoplastic Lesion Atlas, a guide for standardizing terminology in toxicologic pathology for rodents, that is a support activity aimed at improving the quality of non-neoplastic data.

B. BSC Questions and Discussion

Dr. Miller asked if there is a plan to digitize any of the slide images, either for costs savings or future ease of access. He also asked whether trainees are involved in the PWGs. Dr. Malarkey said there is a huge database of digital images from the PWGs, and that there is a very active training program.

Dr. Chapin, first BSC discussant, said the NTP has achieved a position of global leadership in conduct and interpretation of toxicological studies, through a process that has been transparent and inclusive. He felt that the proposed contract re-competitions continue the NTP tradition of scientific rigor and excellence. He said that as long as the proposed changes under the two contracts cover all of the work that needs to be done, he supports the concept.

Dr. Miller, second BSC discussant, said he was very positive regarding the concept in support of the NTP mission.

Dr. Howard asked when the Non-Neoplastic Lesion Atlas would be completed. Dr. Mark Cesta, Staff Scientist, Cellular and Molecular Toxicology Branch, DNTP replied that the atlas would be available by the end of the year.

Dr. Birnbaum thanked Dr. Malarkey and his team for working to maintain the quality of the NTP pathology work while streamlining the process.

Dr. McDiarmid asked for a motion to approve the contract concept. Dr. Miller so moved and Dr. Chapin seconded the motion. The BSC voted unanimously (8 yes, 0 no, 0 abstentions) to approve continuing this activity using a contract mechanism.

X. Systematic Review and Evidence Integration for Literature-Based Health Assessments

A. Presentation

Dr. Andrew Rooney, Deputy Director, OHAT, DNTP, briefed the BSC on recent developments related to the OHAT Approach to systematic review and evidence integration. The Approach had been initially presented to the BSC at the December 2012 meeting. Subsequently, the *Draft OHAT Approach for Systematic Review and Evidence Integration for Literature-based Health Assessments – February 2013* was released for public comment, and later, two case study protocols were released for public comment.

Dr. Rooney reviewed the seven-step draft OHAT Approach, and provided an overview of his presentation, which included: (1) major technical and scientific questions moving forward, (2) how comments have informed the issues, (3) an outline of how NTP is trying to reach resolution, (4) illustration of the initial approach with examples from case studies, and (5) discussion with the NTP BSC.

One of the major areas of question is study quality and Dr. Rooney said there seemed to be confusion for where and how the Approach addresses study quality. This aspect generated many public comments, including support for considering study quality in terms of internal validity or risk of bias, as well as the opposite view that there should be no restriction related to study quality. Other comments discussed suggested additions. Dr. Rooney described in some detail how study quality currently fits into both steps 4 and 5 of the overall approach and that study quality is evaluated in terms of both internal validity and external validity. Other major questions included the issue of excluding studies or establishing “tiers” based on quality, the issue of confidence in the body of evidence or initial confidence rating, and the issue of consideration of other relevant data, such as mechanistic data. For each of those issues, he related details showing how the public comments had informed the issue and how OHAT is trying to reach a resolution on these issues moving forward, occasionally illustrating with examples from the case studies.

XI. Systematic Review and Evidence Integration by OHAT: Next Steps

A. Presentation

Dr. Kristina Thayer, Director, OHAT, DNTP briefed the BSC on OHAT's next steps for the OHAT Approach, including plans for evaluating the performance of the new framework, new developments in data management, and the next two phases of implementing the approach – developing a framework for considering mechanistic information from other studies, and revisiting the level of concern framework.

She provided details on plans for evaluation of the systematic review framework, including several methods of tracking performance metrics. OHAT will also focus on ease of implementation, clarity of language in both the protocol and the framework, with the possibility of changing the Approach as required. OHAT will continue to consider public comments and input, and plans to hold "lessons learned" public webinars after the case studies are finished. Dr. Thayer anticipated that the case studies would be completed during the next calendar year. Ultimately, the first two case studies will result in changes to the protocol in the near term, and the protocol should largely stabilize thereafter.

Dr. Thayer provided an update on data management issues, including the fact that OHAT will continue to use DistillerSR for screening, but move to a different software tool, DRAGON, for data extraction. DRAGON, a free tool, has modules for human and animal studies, with an *in vitro* model coming soon. Moving on to the next phases of work, Dr. Thayer said that one of the major objectives is to develop the framework for considering mechanistic information – for reaching confidence ratings and hazard identification. It will be an NTP-wide collaboration, with federal partners and other stakeholders engaged in the process, along with public meetings. Another major task ahead will be to re-visit the NTP level of concern conclusions, which constitute a formal NTP opinion based on an OHAT evaluation, integrating the hazard identification label with the extent of human exposures and other factors to reach a five-level scale, including a category for insufficient data. Dr. Thayer said the system needs to have categories with better descriptions, with perhaps fewer categories, along with several other needs for updating, including potential inclusion of mechanistic data such as *in vitro* and high throughput data.

B. BSC Questions

Dr. Dorman asked whether the level-of-concern statements are intended to replace the language in Step 7 of the protocol. Dr. Thayer replied that they do not replace, but come after the completion of the hazard identification and build upon the current language, integrating the hazard identification label and exposure information. Dr. Dorman said he was still unclear about it, since exposure information contributes to the hazard identification label itself. He asked how the two elements would be dissected out. Dr. Thayer said that in the current protocol, there are no restrictions on dose levels that are included in the evaluation. Regarding public perception of risk, Dr. Bucher noted that the NTP can make policy and conclusions about health assessments, but will not do quantitative risk assessments. He observed that OHAT

evaluations are no different from the RoC evaluations, where the NTP is asked to render conclusions about the carcinogenicity of substances. In the case of OHAT evaluations, the levels-of-concern terminology has been used for several years and has gained acceptance among other agencies. Dr. Howard noted that in the levels of concern, currently there is not a "no" concern level and asked if it meant the same as "negligible" concern. Dr. Thayer said that is how "negligible" is currently interpreted.

Dr. Corcoran said he understood that the level of concern folds in the degree of confidence. Dr. Thayer confirmed that impression. He asked if there would be a separate scoring for a level of confidence for each stage of concern. Dr. Thayer noted that that was one of the elements OHAT is considering. Dr. Bucher referred Dr. Corcoran to the Step 7 diagram and explained that the low, moderate, and high levels of confidence feed into the categories of hazard identification labels. As noted earlier, concern levels are integrated from the hazard identification label and the extent of human exposure. Dr. Corcoran said it was difficult to separate the two concepts.

Dr. Fernández said he was a bit unclear as to the definition being used for "hazard," and whether it was mainly pertaining to carcinogenicity or to other hazards as well. Also, he noted that the term "insufficient data" appears to be on the level of concern scale, but he felt that it should not be on the scale itself, as there may be concern about something while acknowledging that there are insufficient data. Dr. Thayer agreed. She said the "hazard" designation in OHAT terms does not refer to carcinogenicity, since it deals with non-cancer endpoints.

Dr. Wolfe noted that written public comments had been received on this topic, and had been made available to the BSC in advance of the meeting.

C. Public Comments

Ms. Pat Casano, an environmental attorney with General Electric (GE) Corporate Environmental Programs, commented by telephone. She emphasized three points contained in her previously submitted written comments and addressed one additional issue. She also stated support for the comments provided by the American Chemistry Council (ACC).

She said GE supports OHAT's efforts to develop a structure and process for systematic review of scientific literature and other information that can be applied in a consistent manner across multiple chemicals. She said GE's view was that the draft framework is too generic, leaves too much to be decided on a chemical-by-chemical basis, and could lead to much duplication of effort. She suggested that the two case studies be combined and then used to provide additional detail that could be folded into the protocol itself. This would provide a template for the protocol, which would then address more issues and questions that arise during reviews.

Ms. Casano said GE finds it puzzling that OHAT proposes to extract data from studies before evaluating study quality. She suggested that OHAT evaluate study quality first, and then only extract data from studies determined to be of sufficient quality as to be reliable. She said studies that do not properly account for confounders should not be regarded as providing

evidence of an association between a chemical and an effect, and should be regarded as hypothesis generating.

The third aspect of the draft protocols she said her group had found “puzzling“ relates to the factors used to upgrade and downgrade the level of confidence that a body of evidence establishes the true relationship, if any, between a chemical and a human health effect. She said the distinction between factors used to upgrade and downgrade confidence seems to be artificial. She recommended that the two sets of factors be combined.

The new comment she wished to express related to the quality of *in vitro* assays, and how to incorporate information from *in vitro* assays in hazard identification and determination of dose response. She called for OHAT to provide more information in the protocols regarding the *in vitro* assays used.

Dr. Nancy Beck, Senior Director, Regulatory and Technical Affairs, ACC, presented comments on the OHAT Approach on behalf of the ACC and its Center for Advancing Risk Assessment Science and Policy. She said that although the NTP Approach is a good step forward, substantive improvements are necessary to make the approach transparent, objective, and relevant. She outlined six specific areas that the ACC suggest need improvement, along with suggestions for improvement: (1) consideration of exposure information, (2) evaluating study quality (3) mode of action as a critical component of evidence integration, (4) objectively determining confidence in the body of evidence, (5) objectively evaluating associations vs. causation, and (6) risk communication is critical.

She noted that getting systematic review right is important, as a wide range of stakeholders would likely be adopting it, so they should be engaged. She said the NTP must ensure that the Approach is grounded in science and objectively uses all of the evidence, from diverse data streams, based on its quality and relevance.

D. BSC Discussion

Dr. Dorman, first BSC discussant, said OHAT and NTP are to be applauded for trying to take the lead on this very challenging project. He felt that one of the main challenges is going to be coming up with nomenclatures that work for OHAT that may be different from those used by other organizations. He said OHAT might need to re-convene another working group to get guidance on a number of the topics being raised during the current meeting.

Dr. Hattis, second BSC discussant, agreed that OHAT and NTP are to be applauded for organizing a systematic set of procedures for review. He said they may need to add an additional step to the analysis related to the mechanisms being evaluated. He said there is a need to be able to tell a causal mechanistic story for people to be able to interpret the data properly; without a narrative the audience will imprint its own stories. He provided several examples, and suggested expanding the kinds of literature considered relevant to the evaluations.

Dr. Bucher said Dr. Hattis had described an important concept that he wanted to be considered by the entire BSC. He noted that the evaluation of empirical data with and without mechanistic understanding remains an important distinction. He wanted the BSC's thoughts on whether mode of action is a requirement for making public health decisions. Dr. Dorman said mode of action is not required; that hazard conclusions can be determined without it. Dr. Udasin agreed and added that she approved of the tiered approach. She was concerned about terminology used, particularly terms that are used in clinical settings that could be open to various interpretations. She also felt that potential conflicts of interest should be taken into account when evaluating studies.

Dr. Wolfe said that when the OHAT systematic review team was considering which hazard category names to use, they picked the ones used by the globally harmonized system, so there would be consistency with what is being used by others. Dr. Thayer agreed that the intent was to harmonize.

Dr. Dorman said that the BSC working group spent an afternoon discussing the terminology; the NTP's current use of terms is consistent with the recommendations of the working group. He also noted that in its efforts to integrate data from the three sources, human, animal and mechanistic, NTP seems to be striving to accomplish that integration by adding a Z axis to the current X and Y axes. He felt that such an approach would be a mistake at this time. It would be using the mechanistic data for the purpose of hazard identification, whereas instead, attention should be focused on trying to use mechanistic data to help inform the integration of the human and animal streams through a non-quantitative, qualitative, or narrative approach.

Dr. Thayer asked Dr. Dorman if in what he had outlined, he would try to have a confidence assessment of the mechanistic data. He replied that he would not, in that for a large number of chemicals there may be more than one putative mode of action. Dr. Rooney clarified that Dr. Dorman was recommending use of a qualitative approach for such data, but to stop there. Dr. Thayer said one of OHAT's next steps is to develop a framework for *in vitro*-type data. Dr. Dorman said his concern is that the desire to wrap the mechanistic data into the existing framework as a separate evidence stream would slow the process down unnecessarily. He cited the example of lack of data regarding the impact of risk of bias on quality assessment.

Dr. McDiarmid agreed that integration of mechanistic data is "a messy project." As a clinician, however, she felt that the proposed procedures are good, but cautioned against getting bogged down in mode of action considerations. Regarding nomenclature criticisms, she felt that it would be valuable to use existing hierarchies of evidence or study quality, but not in too strict a manner, since they come from other disciplines. She liked the approach for not labeling a study and then assigning it a particular value based on the study's label. Overall, she felt OHAT was on the right track.

Dr. Hattis said going without a mechanistic framework would limit consideration to dose or exposure levels as used in the study, precluding an ability to extend those to lower doses, which are in many cases the doses that people would be exposed to. Because projections would be

necessary for arriving at levels of concern, there would need to be a mechanistic framework for that interpretation.

Dr. McDiarmid asked Dr. Dorman to clarify his remarks, in that her impression was that he had not suggested eliminating mode of action, but that it be handled in a different way. Dr. Dorman reiterated that he felt that a hazard identification determination could be conducted absent mode of action data. He said he agreed with Dr. Hattis that mechanistic data is very informative. He was concerned that NTP is trying to develop mechanistic data as a stand-alone surrogate for hazard identification, which may be an unnecessary step at this time. Regarding the initial confidence ratings, he said randomized clinical trials typically get the highest ratings, but that they are rarely available for environmental health chemicals. He suggested the NTP consider developing its own rating approach rather than relying on existing methods in the literature, as long as it is transparent.

XII. Draft Report on Carcinogens Concept: Shift Work at Night, Light at Night, and Circadian Disruption

A. Presentation

Dr. Bucher briefly introduced the topic, which he acknowledged is an unusual area that previously has not been encountered by the NTP. He said the NTP is now looking for assistance in defining the problem and its terminology to focus efforts toward improving public health.

Dr. Lunn first briefly reviewed the RoC process, including the preparation of a draft concept document, which is the stage at which this topic stands. She then summarized the nomination history of the topic, which included light at night (LAN) having been nominated by several individuals and a 2007 conclusion by the International Agency for Research on Cancer (IARC) that "shiftwork that involves circadian disruption" is probably carcinogenic to humans. She provided more details about the LAN hypothesis and circadian disruption, which is often the result of shift work and time zone travel. She described existing human and animal studies, and provided data on the extent of potential exposure to conditions leading to circadian disruption, which is believed to be widespread. She reviewed the issues and questions involved in developing protocols to assess human cancer studies in the area, including exposure metrics, effect modifiers, and use of mechanistic data. She said the use of technical advisors would be one way to overcome some of the challenges inherent in the type of research involved, along with establishment of a website and webinars to facilitate scientific and public input.

B. Presentation

Dr. Thayer described OHAT activities on non-cancer health outcomes related to shift work at night, light at night, and circadian disruption. She said OHAT had received a nomination for "exposure to light at night" in 2011, and is currently developing a concept for presentation at a future BSC meeting. Activities to refine the focus of the concept include following the ORoC's efforts to define the scope of the issue, conducting an exploratory literature search, engaging

technical experts, releasing a Request for Information, and eventually presenting a concept to the BSC, with opportunity for public comment. She defined the issues to consider for developing the concept: (1) scope, with an inclination to focus on non-day shift work given the complexity and size of the literature, (2) how to evaluate health outcomes where a number of systemic reviews have already been conducted, and (3) when to identify and consider supporting and mechanistic data.

C. BSC Discussion

Dr. Fernández, first BSC discussant, noted that it is reasonable to conclude that a significant number of people in the United States are exposed to light at night or other exposures that may cause circadian disruption, and that as a result, effects on public health are vitally important. Although there are many studies in the area, they are all very different and not standardized. The scientific issues identified as relevant are reasonable in his view.

He discussed the issues surrounding the different types of occupational exposures to LAN, including the different types of lighting involved. He recommended that studies available on shift work be evaluated for those differences, with occupational specificity in mind, which would help identify occupations at risk and potential risk reduction strategies. He noted that it would be important to define the occupations at risk and the potential neoplasms carefully. He emphasized the importance of specificity in defining the occupations in order to avoid over-generalization.

Dr. Udasin, second BSC discussant, noted that there are at least 17 million shift workers potentially affected in the United States, and that meteorological studies have shown that artificial light pollution is a significant problem in urban areas and may disrupt circadian rhythm. She described the effects of shift work as outlined in a study by Arendt (2010) and suggested that the definition should focus on exposures that cause circadian disruption. Genetic screening might be necessary, particularly in the case of breast cancer risk, which may result in interventions in the workplace aimed at prevention. She noted that nurses would be an excellent cohort for study in that they are likely to return surveys and other study instruments. She supported the use of mechanistic studies. She also recommended that the issue of hormone replacement therapy be considered when evaluating female shift workers.

Dr. Richard Stevens, *ad hoc* reviewer, was the third discussant. He agreed that the exposures are pervasive, in that virtually everyone in the United States is exposed to light at night. It is a very different type of light, he noted, and it is emerging that genes associated with circadian rhythms involve up to 10% of the genome. Thus, the main issues are the extent to which electric lighting alters human circadian rhythmicity, and the potential health consequences pertinent to cancer risk. He noted that there are considerable mechanistic data from human studies available. Disentangling sleep from circadian disruption is very difficult, since the endogenous circadian rhythm requires dark, but not sleep. He recommended that NTP not restrict its efforts to shift workers alone.

Regarding occupational specificity, Dr. Lunn agreed that there are many studies involving nurses, with good reporting and potentially fewer occupational co-exposures than other

professions such as firefighters. She asked Dr. Stevens whether sleep deprivation might be considered a potential confounder or part of the same pathway in his studies of shift workers. He replied that they are inextricably linked, but that the distinction is very important. He noted that in studies of shift workers and breast cancer, the control women are not unexposed to LAN. He added that a few studies have suggested that blind women are at lower risk of breast cancer. The evidence for the mechanistic potential of circadian/sleep disruption to induce obesity, diabetes, and depression is emerging, with solid evidence now and a growing evidence base.

Dr. Dorman asked how the NTP is planning to address biological gradients. He noted that the questions include how much light needs to be present to disrupt a circadian rhythm, and how much change in circadian rhythm is associated with a cancerous response or other adverse health effects. Dr. Stevens replied that the experiments done with light and breast cancer were conducted by very good laboratories and published in very good journals, but are limited. He said there is clearly a dose response, with the simplest biological mechanism having to do with melatonin. There is definitely an effect in rodents. Dr. Lunn said in human studies, much would depend on the definition, and that in many cases shift work would be used as a surrogate. Dr. Dorman noted that not all light at night is necessarily the same, so that issue arises in terms of dose response; he asked how NTP would handle it. Dr. Stevens said the change in the lighting industry and the lighting environment is a practical change that matters right now. He added that different types of lighting could be used in rodent experiments to determine the varying effects. Dr. Howard elaborated, noting that many of the biological responses appear to be wavelength-dependent, which should be considered in interpretation or study design.

Dr. Hattis recommended using whatever means possible to get away from the biological complexity in such experiments toward a biologically plausible model. Dr. Stevens agreed, providing examples of how melatonin expression might be studied. Also, he described work characterizing epigenetic effects on clock genes related to shift work.

Dr. Fernández agreed that nurses would be a good population to study, but noted that they are not without confounding variables. He said that refresh rate of lights should be a consideration, along with wavelength

Dr. Miller said he supports NTP working in this area, and suggested that the work should not be limited to breast cancer or cancer alone, but should explore the other disease processes potentially linked to the issue. Dr. DeBord agreed that NIOSH would support NTP pursuing the work, especially since LAN is obviously an occupational problem. She encouraged the NTP to put together a good technical advisory group, because there would be so many confounders with whatever population is chosen to evaluate.

Dr. Minor agreed that it is a very complicated area, and concurred that it would be very important to establish an excellent advisory group.

Dr. McDiarmid summarized the discussion, stating that the BSC members endorsed the idea, but provided many caveats about how difficult it would be. It would be important to not simply look at the various endpoints and catalogue everything that has been written about them, thus

potentially losing the detail, which in this case is essential in terms of characterizing the exposures and other factors. She felt the NTP should not try to do everything, but should figure out a strategy to characterize specific factors. She said that certain occupations might be one approach. She noted that nurses are exposed to a variety of potential carcinogens beyond artificial light, and so using them for such studies would need to be carefully parsed.

Dr. Udasin clarified her comment about nurses, saying that she meant that nurses were exposed to a less overwhelming level of carcinogens compared to firefighters, not that nurses are not exposed to carcinogens at all.

XIII. Adjournment

Dr. Birnbaum and Dr. Bucher thanked the BSC and the NTP staff for their excellent contributions and hard work during the meeting. Dr. McDiarmid adjourned the meeting at 5:00 pm, June 25, 2013.

References

Arendt J. Shift work: coping with the biological clock. 2010. Occup Med (Lond). 60(1):10-20.