National Toxicology Program
Board of Scientific Counselors

April 13, 2011

National Institute of Environmental Health Sciences
Research Triangle Park, NC

Contents

I. Frequently Used Abbreviations and Acronyms

ADME	absorption, distribution, metabolism, and excretion
BSC	Board of Scientific Counselors
CC	current control
CDER	Center for Drug Evaluation and Research
CERHR	Center for the Evaluation of Risks to Human Reproduction
DERT	Division of Extramural Research and Training
DIR	Division of Intramural Research
ENM	engineered nanomaterials
EPA	U.S. Environmental Protection Agency
FDA	U.S. Food and Drug Administration
HC	historical control
HHS	Health and Human Services
ICCVAM	Interagency Coordinating Committee on Validation of Alternative Methods
ICH	International Conference on Harmonisation
IOM	Institute of Medicine
LLNA	local lymph node assay
MOG	modified one-generation
NCATS	National Center for Advancing Translational Sciences
NCCT	National Center for Computational Toxicology
NCEH	National Center for Environmental Health
NCI	National Cancer Institute
NCGC	NIH Chemical Genomics Center
NCTR	National Center for Toxicological Research
NICHD	National Institute of Child Health and Development
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
OSHA	Occupational Safety and Health Administration
RA	retinoic acid
RDA	recommended daily allowance
RP	retinyl palmitate
RoC	Report on Carcinogens
UL	Tolerable Upper Intake Level

II. Attendees

Members in Attendance:
David Eastmond, University of California (Chair)
Elaine Faustman, University of Washington
Dana Loomis, University of Nebraska Medical Center
Stephen Looney, Medical College of Georgia (via telephone)
Melissa McDiarmid, University of Maryland School of Medicine
Richard Miller, GlaxoSmithKline
Mitzi Nagarkatti, University of South Carolina School of Medicine
Ruthann Rudel, Silent Spring Institute
James Sherley, Boston Biomedical Research Institute
Gina Solomon, Natural Resources Defense Council
Justin Teeguarden, Pacific Northwest National Laboratory (via telephone)

Members not in Attendance:
Janan Eppig, The Jackson Laboratory

Pending Board Members:
Nicholas Jewell, University of California Berkeley
Judith Zelikoff, New York University School of Medicine

Ad Hoc Members:
Robert Chapin, Pfizer Global Research and Development (via telephone)

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

National Institute of Environmental Health Sciences Staff

Danica M. K. Andrews
Scott Auerbach
David Balshaw
Mamta Behl
Linda Birnbaum
Jack Bishop
Chad Blystone
Abee Boyles
John Bucher
Matthew Burr
Rajendra Chhabra
Michael Cunningham
Helen Cunny
Michael DeVito
June Dunnick
Susan Elmore
Paul Foster
John French
Dori Germolec
Robbin Guy
Jean Harry
Wanda Holliday
Ernie Hood
Kembra Howdeshell
Paul Jung
Edward Kang
Grace Kissling
JoAnn Lewis
Ruth Lunn
Robin Mackar
David Malarkey
Scott Masten
Elizabeth Maull
Barry McIntyre
B. Alex Merrick
Ellen Moul
Shyamal Peddada
Wei Qu
J. Scott Redman
Cynthia Rider
Robert Sills
Cynthia Smith
Diane Spencer
William Stokes
Christina Teng
Raymond Tice
Molly Vallant
Michael Waalkes
Suramya Waidyanatha
Nigel Walker
Vickie Walker
Chris Weis
Lori White
Kristine Witt
Mary Wolfe
Richard Woychik

Public
Gary Burleson, BRT
Tai Guo, Virginia Commonwealth University
Courtney Granville, Battelle
Milton Hejtmancik, Battelle
Marcus Jackson, ILS
John Menkedick, Battelle
Richard Morris, SRA International
Ivan Rusyn, University of North Carolina at Chapel Hill (UNC)

III. Introductions and Welcome

The National Toxicology Program (NTP) Board of Scientific Counselors (BSC) met April 13, 2011, in Rodbell Auditorium, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC. Dr. David Eastmond served as chair. He 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 and noted that Drs. Dana Loomis, Melissa McDiarmid, and Richard Miller are now full voting members of the BSC.

IV. Report of the NTP Director

A. Presentation

Dr. Linda Birnbaum, Director of NIEHS and NTP, welcomed attendees to the meeting.

In staff developments, she noted the recent addition of Dr. Rick Woychik, former CEO of the Jackson Laboratory, Bar Harbor, ME, as NIEHS Deputy Director, and the appointment of Dr. Gwen Collman as Director of the NIEHS Division of Extramural Training and Research (DERT). She said the selection of a new Associate Director for management has been made, and the process for finalizing and announcing the selection is underway. Searches continue for Scientific Director and Clinical Director.

Regarding NIEHS/NTP FY 2009-2012 appropriations, she noted that the recent potential government shutdown had been averted, and that the government would be on a Continuing Resolution (CR) for the rest of the year. The latest figure for NIH for the FY2011 CR, following the budget agreement, is $30,924,544,000, a decrease of roughly $320,000,000. The NIEHS appropriation for that period is now $683,725,000, a reduction of approximately 1%. Superfund is funded at $79,054,000, just a 0.2% cut. She said the current cuts are a better outcome than had been expected, but that further cuts are to be anticipated.

She noted personnel changes at NIH, the intention to merge the National Institute on Drug Abuse and National Institute on Alcohol Abuse and Alcoholism, and the plan to transform the National Center for Research Resources (NCRR) to the National Center for Advancing Translational Sciences (NCATS). She updated the BSC on her recent Congressional testimony activities.

Dr. Birnbaum recounted several recent NIEHS/NTP highlights, including a significant increase in research activity at the Clinical Research Unit, with almost 20 active protocols in progress. She mentioned several activities related to Environmental Justice, including a community forum held in West Louisville, KY, sessions at the American Public Health Association meeting, and a White House Environmental Justice Forum.

Regarding the status of the NIEHS/NTP reorganization, making the NTP a separate division of NIEHS, documents were signed by the HHS Secretary in February and the **Federal Register** notice was still pending as of the BSC meeting date. She provided details on the new NIEHS Strategic Plan and encouraged BSC members to participate and contribute their ideas. Dr. Birnbaum concluded her remarks by thanking retiring BSC member Dr. James Sherley for his service to the BSC and to NTP.

B. BSC Discussion

Dr. Elaine Faustman asked for more information on the new NCATS center. Dr. Birnbaum said the focus of the center will be to move drugs into the marketplace more quickly, i.e., translation from bench to bedside, whereas NIEHS' focus is more bench to public health and public policy. She noted that NIEHS is actively informing NIH about its ongoing, long-standing relationships with the FDA, EPA and CDC, and efforts to keep the regulatory agenda moving forward.

V. Report of the NTP Associate Director

A. Presentation

NTP Associate Director Dr. John Bucher updated the BSC on NTP activities since the last BSC meeting.

One highlight was the NTP Workshop: Role of Environmental Chemicals in the Development of Diabetes and Obesity, which was held January 11-13, 2011, in Raleigh, NC. The goals of the workshop were to evaluate the literature, provide input to NTP and NIEHS for development of a research agenda, and bring together diverse expertise to consider the relevant evidence. At the workshop, general support emerged for (1) plausibility of the "obesogen" hypothesis, (2) linkage of Type 2 diabetes to certain chemical exposures, (3) common mechanistic basis for certain chemical classes, (4)

utilization of Tox21 approaches to identify substances of potential interest, and (5) refinement of endpoints examined using high throughput screening approaches.

Dr. Bucher reported on the Interagency Coordinating Committee on Validation of Alternative Methods (ICCVAM) Best Practices for Regulatory Safety Testing Workshops, held in Bethesda, MD, January 19-20, 2011. He summarized the various activities conducted at the meeting, which focused on methods for assessing the potential for chemically induced eye injuries and methods for assessing the potential for chemically induced allergic contact dermatitis.

He also briefed the BSC on the conclusions of the January 26, 2011 peer review of NTP draft Technical Reports (TRs) on kava kava extract, retinoic acid/retinyl palmitate (RA/RP), methyl *trans*-styryl ketone, styrene-acrylonitrile trimer, and α,β-thujone. He asked Dr. Mitzi Nagarkatti, who had served as BSC liaison to the peer review meeting, for comments. She said Dr. Bucher had summarized the meeting's results well, noting that the panel had changed language in just two of the five reviews, agreeing with the draft language in the other three. Dr. Faustman asked for elaboration on the finding regarding RP. Dr. Paul Howard, FDA/ Nation Center for Toxicological Research (NCTR) explained that the RP and the control cream were both found to induce skin cancer in the presence of UV light, or simulated sunlight as used in the assays. The addition of RP was found to induce the cancers more rapidly and to increase multiplicity compared to the control cream alone.

Dr. Bucher elaborated on the impending reorganization of NTP as a separate division within NIEHS. He said the change was in recognition of the unique mission of NTP, the unique way it carries out research as an NIEHS intramural program, and the unique training requirements and capabilities of its staff, necessitating a unique place on the NIEHS organizational chart. He emphasized that the change applies only to the NIEHS portion of NTP. He shared the new NTP organizational chart with a Division Office, two Deputy Division Directors, five branches, and five offices. He noted the Center for the Evaluation of Risks to Human Reproduction (CERHR) has been renamed the Office of Health Assessment and Translation (OHAT), because the title "CERHR" was no longer sufficiently descriptive of the analysis role the office fills. OHAT encompasses an expanded role grounded in reproduction and development assessments but also considering a broader range of human health effects. The change was announced at the Society of Toxicology (SOT) 2011 annual meeting and will be the subject of an editorial in the May edition of *Environmental Health Perspectives*. NTP and NIEHS were very active at the SOT annual meeting, with more than 100 staff presentations. Also at SOT, the International Cooperation on Alternative Test Methods welcomed a new member, the Korean Center for the Validation of Alternative Methods.

Dr. Bucher also mentioned the draft TRs peer review meeting that took place April 5, 2011, which reviewed reports on senna, acrylamide, *Aloe vera*, and combinations of AIDS therapeutics. He noted that the panel's recommendations on the conclusions for those draft reports would be presented at the July BSC meeting.

B. BSC Discussion

Dr. Mark Toraason questioned why the word "risk" had been removed in the new title for CERHR, and about the continued use of the "levels of concern" metric. Dr. Bucher replied that "risk" is not in OHAT's name to alleviate confusion and reflect the fact that it does not carry out risk assessment activities. He said the NTP currently plans to retain the "levels of concern" scale as its method for communicating findings. Dr. Howard spoke in support of the CERHR name change, and appreciated the work of its staff in reaching out to the regulatory agencies concerning activities such as the diabetes and obesity workshop and the folate workshop to be proposed later in the meeting.

VI. Contract Concept: Potential for Environmental and Therapeutic Agents to Induce Immunotoxicity (ACTION)

A. Presentation

Ms. JoAnn Lewis of the NIEHS Office of Acquisitions reviewed the guidelines for BSC action regarding the discussion of research concepts. She asked the BSC to review the concept for its overall value and for its scientific relevance to fulfill the program's goal of protecting public health. The specific areas to consider are scientific, technical, and programmatic significance, availability of the technology and other resources necessary to achieve the required goals, extent to which there are identified, practical scientific or clinical uses for the anticipated results, and, where pertinent, adequacy of the methodology to be used to perform the activity. Discussions, she said, should be limited to a review of the general purpose, scope, goal, and optional approaches to pursue the overall program objective. She noted that the meeting would be closed to the public should the discussions turn to the development or selection of the details of the project or the request for proposal, such as specific technical approaches, protocol, statement of work, data formats or product specifications. Should the meeting be closed, it is to protect the free exchange of the BSC members' opinions and to avoid premature release of details of the proposed contract project or request for proposal.

Dr. Dori Germolec, Immunology Discipline Leader in the NTP Toxicology Branch, presented the contract concept. The purposes of the contract, which is a recompetition of a testing program that has existed in NTP for more than 25 years, are to: (1) develop and validate methods to evaluate modulation of immune function, (2) evaluate the immunomodulatory potential of agents of concern using a tiered testing panel, and (3)

conduct investigative studies to define cellular and molecular events associated with modulation of immune function.

Dr. Germolec said the original validated testing panel was published in 1988 and has remained largely unchanged. Since the testing panel was validated, more than 100 compounds have been evaluated for their ability to induce immunosuppression or allergic hypersensitivity. The last recompetition of the contract added evaluation of immunotoxicity following developmental exposures and assessment of chemical modulation of autoimmune disease. The testing contract has been at Virginia Commonwealth University since the early 1980s, with immunotoxicology studies also having been conducted at the IIT Research Institute, as well as in-house studies at NIEHS.

Under the current contract, four screening or range-finding studies are conducted annually, assessing immunotoxicity and the dose at which the substance modulates the immune system. A positive finding in those studies generates a definitive or full protocol study, going into more depth on the specific targets involved. Two definitive studies are conducted annually. Two developmental immunotoxicology studies, two hypersensitivity studies, and one autoimmunity study per year are also conducted. The distribution of the studies can be modified to accommodate programmatic needs.

The basic tiered testing panel used for the screening studies consists of 28-day studies testing immunopathology, clinical pathology, cell-mediated immunity, humoral-mediated immunity, non-specific immunity, and cell quantification. The definitive studies assessing immunomodulatory effects in more depth examine a number of endpoints, including humoral- and cell-mediated immunity, a number of non-specific immune endpoints, evaluation of bone marrow if it is found to be a target, and host resistance assays. Hypersensitivity studies use the local lymph node assay (LLNA), the mouse ear swelling test, and can include optional endpoints in case of a positive result, including cell quantification in a draining lymph node and cytokine mRNAs.

Under the current contract, Dr. Germolec reported, mouse models for lupus, diabetes, and various autoimmune skin and renal diseases are used. Endpoints vary by model, but generally include quantification of autoantibodies, serum immunoglobulin levels, protein and glucose in urine, and histology. She added a description of the various investigative studies currently being pursued under the contract.

Although the relative goals and testing panel will be the same under the new contract, there are some proposed changes to the current statement of work. To bring immunotoxicity testing in line with other NTP studies, all studies will be required to be performed under Good Laboratory Practice (GLP) standards. There will be an increased focus on developmental immunotoxicity studies conducted in conjunction with

NTP's Modified One-Generation (MOG) Studies. There will also be increased partnership with ICCVAM, to assist with validation and use of alternative methods for hypersensitivity testing.

Ultimately, said Dr. Germolec, the purpose of the contract is to develop methods, to evaluate immune system toxicity using an established test panel, and to conduct investigative studies into the mechanisms associated with modulation of immune function. Recompetition will continue to meet those goals with some modification to reflect changing needs within the program, through increased flexibility, focus on developmental studies, and validation of alternative methods.

B. BSC Questions and Discussion

Dr. Howard asked for clarification regarding the use of alternative methods; he perceived she was actually discussing alternative *endpoints*. Dr. Germolec agreed that in the case of the LLNA, alternative endpoints were being considered, but said the group is also interested in true alternative methods that have been proposed, such as *in vitro* cell lines, in order to help ICCVAM increase its databases on specific proposed alternative methods.

Dr. Sherley asked how findings that may be outside of the contract's focus would be dealt with. Dr. Germolec said such information flows both ways—from the wider NTP program to the immunotoxicity testing program, and vice versa.

Dr. Gina Solomon wondered about the relatively small number of chemicals going through the program annually. Dr. Germolec explained that there is not a large backlog, but the flow is steady. There are options in the current contract to increase capacity when necessary, which has been done several times based on programmatic needs. Also, she said, it takes considerable time to get an individual chemical through the testing program, since five individual 28-day studies involved.

Dr. Nagarkatti, first lead reviewer, said she agreed with the added flexibility in the proposed contract, and the inclusion of developmental toxicity studies, as well as the use of alternative methods or endpoints to detect changes. She stressed the importance of the concept of the fetal basis of adult disease. She cited recent studies showing the transgenerational impact of exposures to certain environmental chemicals or drugs, such as with diethylstilbestrol. She expressed concern about over-reliance on the LLNA. She recommended the addition of some molecular tools, the ability to look at genetic and epigenetic targets, and the inclusion of oral exposure studies as well as dermal. She also recommended the inclusion of host resistance assays to assess viral infections and cancers as well as bacterial infections, and examinations of T helper cells and dendritic cells.

Dr. Judith Zelikoff, second lead reviewer, emphasized the importance of the program from a translational perspective, as the information emerging on immunotoxicity relates to a great many diseases. She said it is "an outstanding example of something that can lead to dramatic effects in terms of protecting human health." She approved of the increased emphasis on investigation of developmental immunotoxicity. She pointed out that understanding how immune system responses relate to public health diseases will allow the development of more targeted treatments and intervention strategies. She also advocated the increased focus on hyperreactivity and autoimmune disorders. She noted that the chosen exposure route in studies should not be one of convenience, but should be selected based on relevancy.

Dr. Germolec said she appreciated the suggestion to add molecular and genetic endpoints. She said it is not realistic at this time to incorporate those endpoints into the routine testing panel because the endpoints are not yet sufficiently developed to know how predictive they would be in terms of responses or disease outcomes. She agreed with the concept of including assays addressing T helper and dendritic cells, but noted that the methods are not available to routinely include them in screening. She respectfully disagreed with the suggestion of more routine use of host resistance studies, particularly from an animal welfare standpoint, as such studies tend to be very animal-intensive. She said the program does focus on translatability, and always makes an effort to look at relevant routes of exposure.

Dr. Faustman moved to approve the concept and Dr. Nagarkatti seconded the motion. The BSC voted unanimously (8 yes, 0 no, 0 abstentions) to approve continuing this activity using a contract mechanism.

VII. NTP's Modified One-Generation Reproduction Study Design

A. Presentation

Dr. Paul Foster, Chief of the NIEHS Toxicology Branch, presented the Modified One-Generation (MOG) reproduction study design to the BSC. *Ad hoc* reviewer Dr. Robert Chapin, Pfizer Global Research and Development, joined the session by telephone.

To establish the appropriate context for his description of the MOG study design, Dr. Foster briefly reviewed the reproductive cycle. Any of the functions in the cycle could be the target for a chemical exposure to produce an adverse effect on reproduction. He described the studies outlined by the International Conference on Harmonisation of Guidelines (ICH), which breaks up the reproductive cycle into three segments. The first testing study segment focuses on fertility and early embryonic development. The second segment is the Embryo-fetal Development Study, with assessment just prior to parturition. That study does not allow exposure to a number of critical developmental

processes, several of which occur late in gestation in the rat. Therefore, the Environmental Protection Agency (EPA)/Organisation for Economic Co-operation and Development (OECD) Prenatal Development Toxicity Study extends the dosing period to just prior to term. The third ICH segment is the Pre- and Postnatal Development Study, which has exposure from implantation through to the end of lactation, with assessment of the animals during growth and development and when they are sexually mature. These studies are designed for intentional exposures, such as pharmaceuticals.

For unintentional exposures, one study would be used, currently the EPA/OECD Multigeneration Reproductive Study, which has exposure throughout the reproductive cycle. It is known as a two-generation study, with two breeding generations. After 10 weeks of pre-breeding exposure in the dams, the offspring are eventually culled, and the discarded animals are not examined further. At weaning, there is a second cull from four males and four females to one male and one female from each litter. Then in the second generation, the process is repeated. Dr. Foster described for the BSC the kinetics of rat spermatogenesis, illustrating the need for the 10-week pre-breeding exposure strategy,

He used a graph to depict the effect of screening more animals than just one pup per litter on power curves for detection of offspring abnormalities – with just one pup, detection of a 10% incidence was just 4.7%, but with four pups, detection rose to 86.5%. Thus, by retaining animals that would have been discarded and examining them at a later stage, power to detect potential reproductive tract malformations exhibited postnatally is greatly enhanced.

Dr. Foster outlined several recent NTP study design developments that have led up to the current proposal for the MOG Study:

- Following several NTP workshops, the NTP changed its default exposure paradigm to include exposure during pregnancy and early life in rat carcinogenicity studies.
- The NTP has conducted “perinatal cancer bioassays” in the past, but they required special justification. The new default is to undertake such a study unless there is a scientific reason not to do so.
- Before doing so, preliminary dose-range finding studies would normally be required to ascertain dose levels, using perinatal exposure.

The preliminary dose-range study would ascertain dose levels to allow dams to carry their offspring to term, deliver them, successfully raise them to weaning, and allow determination of target organ toxicity in adults. This type of study design could easily be adapted to provide toxicity information on a range of other endpoints, and maximize the use of the animals already produced.

The MOG study is characterized by continuous exposure from implantation through sexual maturity, and examines the majority of animals produced. It employs the concept of using various interchangeable "cassettes" incorporated from other standard regulatory studies, based on the NTP nomination. The first cohort of offspring is designed to provide information on target organ toxicity, but also can be used to evaluate other endpoints. The second cohort evaluates the potential for prenatal developmental toxicity. The third cohort may be used to evaluate breeding and littering for potential examination of the subsequent generation. All F_1 animals after postnatal day 4 are taken to adulthood for pathology examination. Two of these cohorts are used to examine functional effects on reproduction, i.e., fertility and fecundity. All three of the classical segments are incorporated in the MOG study design.

To illustrate the utility of the MOG design, Dr. Foster showed the example of tetrachloroazobenzene rat studies. Under the older paradigm, at least seven studies would have been required. With MOG, just a pilot, the MOG and the 2-year perinatal toxicity/cancer study would have been necessary.

He noted that OECD is developing a new protocol, the extended one-generation reproduction study, to be used for all chemicals to replace the multigeneration reproduction study. It is being developed to address the testing requirements of the European Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) law, which requires the use of increased testing for product registration, but with pressure at the same time to reduce animal use. He said a problem with the OECD approach is its use of internal triggers, which have significant limitations and drawbacks. For example, it provides for only two weeks of prenatal exposure, which leaves many germ cell types unexposed and it is underpowered for specific end points in some respects. He noted the triggers for F_1 breeding, pointing out some of their inherent limitations and impracticalities. He described the advantages of the NTP approach (1) emphasis on F_1 animals, (2) retention of the 10-week prebreeding exposure, and (3) the ability to assess function and reproductive structure in the same animals.

Ultimately, advantages of the NTP MOG over the OECD design include (1) flexible design, (2) robust datasets, (3) 10-week prebreed exposure, (4) lack of "internal" triggers, (5) pre-natal developmental toxicity information, and (6) sub-chronic toxicity information, including clinical pathology.

In conclusion, Dr. Foster said the proposed NTP MOG is a robust evaluation of reproductive and developmental toxicity that maximizes the utility of the animals already produced and available for study. It also reduces the overall number of animals employed compared to other protocols. The number of animals used is comparable to the proposed OECD design but yields much more information on developmental

outcomes. The MOG will facilitate NTP's requirement for information on subchronic toxicity and dose setting before embarking on a rat perinatal carcinogenesis study. It will allow refinement of toxicity study designs, will replace certain other standard toxicity studies by folding them into the proposed protocol, and will reduce overall animal use.

B. BSC Discussion

Dr. Chapin, first lead reviewer, prefaced his comments with a question to Dr. Foster about similar experiments that had been undertaken in the 1980s with limited success. Dr. Foster recollected that there were three publications on cancer outcomes, one of which showed positive results for prenatal exposures compared to a normal design, one did not, and one had mixed results. He pointed out that using a perinatal design, additional safety factors for childhood cancers were unnecessary, given the exposures *in utero* and in early life. Thus, the exposures more realistically reflect what women of childbearing age and their offspring might experience.

Dr. Chapin said he absolutely approved of the whole concept, which places NTP in the forefront of creative and thoughtful approaches to safety assessment. He noted that with the fact that adult functions other than just the endocrine system (e.g., blood pressure and insulin response) can be affected by prenatal exposures, many additional endpoints should be examined. He deemed the MOG design a "golden opportunity," and recommended that non-lethal assessments of immunotoxicity and neurotoxicity be routinely performed with all compounds.

He said that the study design's "one horse carries all" approach creates a risk for differential sensitivity of different organ systems, with the possibility that a dose range that works well for showing effects in female reproduction could be too high for showing effects on embryo/fetal development, or that the internal levels in females at a given dose level could be much more or less than in males. He noted that while such discontinuities do happen, they are fortunately rare, and while the hazard exists, the risk of damage would be rare, and the upside of such a finding would be a clear delineation of the more sensitive process.

Regarding suggestions for improvements in the paradigm to further decrease animal use, Dr. Chapin felt that there are likely to be limits to reducing animal use given the litters being produced. He suggested that the immunotoxicological and neurotoxicological endpoints to be developed be non-lethal, allowing the use of the same cohort of animals to be used for those additional tests.

Dr. Zelikoff asked Dr. Foster about the issue of litter bias, which she said was historically why only one male and female from a given litter had typically been used. Dr. Foster replied that it would be relatively easy to control for any such bias statistically;

the NTP had been doing so for many years already. She asked why mice had not been added. Dr. Foster said rats were still being used because they are the standard species for reproductive and developmental toxicity studies. The NTP had considered using its inbred strain of mice, but studies were limited by poor fertility and small litter size. Dr. Birnbaum added there have been discussions about the possibility of using outbred mice or multiple mouse strains. Dr. Zelikoff asked if sex is taken into account when culling animals. Dr. Foster said it is, to equalize potential sex bias. Dr. Zelikoff also recommended that the program use validated and defined outcomes and methods when investigating behavioral endpoints. Dr. Foster agreed that all studies would need to be done with validated methods, which was one reason for using cassettes taken from other standard studies the NTP routinely uses.

Dr. Nagarkatti asked about the proposed pilot perinatal study. Dr. Foster elaborated that it would use a small number of animals, looking at gross endpoints, including placental transfer and lactational transfer from dam to pup.

Dr. Toraasen asked about the level of experience with the proposed method, the effect on dosimetry for chronic studies, and whether there had been any dialog with OECD on harmonization. Dr. Foster said there had been dialog, particularly with the EPA. NTP had conducted approximately six 90-day perinatal studies, but none of the one-generation studies using every component. Each of the cassettes has been performed, but there has been no single stand-alone study using all of them, although there are several proposals to do so. He described some of the methods used in past studies to adjust dosimetry, and some of the different options available depending on the information at hand with any given compound.

Dr. Howard commented that there had been considerable interest in the study design at the FDA following presentations by Dr. Foster.

Dr. Faustman, second lead reviewer, considered the initiative very significant and gave it her highest rating. She said it is very important and has the potential to make a significant impact on how reproductive and developmental toxicity assessments are conducted. She added the proposal provides an avenue for reduction of animals used during the reproductive testing protocols but also looks in detail at the experimental design to insure that animals are used in a manner that would provide the maximum amount of toxicologically relevant information for these endpoints by reviewing what is already known about the biology of spermatogenesis, etc. Regarding the clarity and validity of the rationale for the proposal, she stated it wisely examines a variety of experimental designs for evaluating reproductive endpoints and then proposes modifications that are consistent with biology. She was particularly pleased to see the proposed use of 10 weeks of exposure prior to the mating of the F_1 animals and the inclusion of toxicokinetic information.

Dr. Faustman asked for additional details on (1) the subchronic cohort aspect of the design, (2) cassettes, particularly the triggers for different cassettes of test batteries that could be inserted in the proposed NTP design, (3) how decisions would be reached to change or insert additional tests, (4) how NTP would address the potentially "underpowered" neurotoxicity cohort if that would be considered as one of the endpoints addressed in the cassette approach, (5) the use of statistics in general in the design, and (6) the dose metric that would be used, especially as it relates to peak concentrations or cumulative concentrations.

Dr. Faustman found the proposal to be "of great merit in addressing NTP goals in providing information that will be relevant and transferable to humans." Regarding statistical power, she does not approve of the three-dose group plus control approach, and suggested that the proposed design include more dosing groups to generate more quantitative information.

Dr. Foster thanked the BSC reviewers for their remarks, and suggested that a major review article about the proposed study design might be a good way to move it forward and address many of the reviewers' comments.

Ms. Ruthann Rudel asked about the decision on dosing the animals, suggesting that it might be useful to consider starting dosing earlier, pre-mating, to take epigenetic effects into account. She also suggested incorporating premature senescence as a way to increase the sensitivity of the model. Dr. Foster replied that he was aware of only one study design routinely used to measure reproductive senescence – the NTP's reproductive assessment by continuous breeding, that is still an option for specific nominations. With regard to adding additional end points he noted that the NTP scientists had been sensitive to the possibility of adding extra end points that "if there are too many ornaments on the Christmas tree, it's going to fall over." He said the approach has to be practical and pragmatic, with good decisions about what elements would be taken forward.

Dr. Eastmond summarized the discussion, stating the BSC is very supportive of the initiative.

VIII. New Statistical Methods for Analyzing the National Toxicology Program's 2-year Cancer Bioassay Data

A. Presentation

Dr. Shyamal Peddada, NIEHS Biostatistics Branch, briefed the BSC on three proposed new statistical methods for analyzing NTP 2-year cancer bioassays. He pointed out that decisions made regarding carcinogenicity of a chemical are based on a variety of

information, not just p-values. However, it is desirable to develop methods that increase the statistical power of the data while controlling the rate of false positives.

When looking at the testing paradigm, three questions arise: Is there a dose-related trend relative to the concurrent control group? Is there a dose-related trend relative to the historical controls? How do the historical controls compare to the concurrent controls? Thus the three comparisons of interest emerge—dose groups vs. concurrent controls (CCs), dose groups vs. historical controls (HCs), and CCs vs. HCs. The first two encompass the trend test and pairwise comparisons of individual dose groups vs. controls.

When comparing dose groups with CCs, the current methodology used is the Poly-3 trend test, which accounts for survival differences among dose groups by using Poly-3 statistical correction to the sample size. The Poly-3 trend test is intrinsically ideal when the dose trend is linear, but it often loses power for non-linear trends. Thus, an alternative is needed, as increasing numbers of non-linear trends are observed. A new trend test is needed that (1) controls the false positive rate as well as the Poly-3, (2) is as powerful as the Poly-3 for linear trend, (3) has greater power than the Poly-3 for non-linear monotonic trends, and (4) does not make complicated modeling or other assumptions

Dr. Peddada said to meet these needs, the Max-Iso-Poly-3 trend test was proposed. It consists of two components—an isotonic regression-based test (T1) (Williams-type test) and a Poly-3 trend test (T2). T1 modeling assumes that no complicated model is used to describe the dose-response relationship and uses mathematical inequalities to describe monotonicity. It accounts for survival differences among dose groups through Poly-3 corrections to sample size, with no additional assumptions and it is designed to be computationally simple. Dr. Peddada described the isotonic regression concept in more detail, and related several simulation studies comparing outcomes from the Max-Iso-Poly-3 and the NTP Poly-3 trend tests. The Max-Iso-Poly-3 test did not show higher false positive rates in any of the hundreds of simulations run, and it is no more liberal than the Poly-3 test. In most cases, the proposed test is more highly powered than the Poly-3, but in some instances the reverse is true—there is at most a 10% loss in power. Also, as desired, there is greatly increased power for non-linear monotonic trends—as much as 66%.

For pairwise comparisons of individual dose groups vs. CCs, the current NTP strategy would remain, but the Poly-3 test for two groups would be replaced by the Max-Iso-Poly-3 test for two groups. Dr. Peddada showed an example of a test of isoprene in female rats, assessing mammary gland fibroadenomas. The Poly-3 p-value was not significant, but applying the Max-Iso-Poly-3 generated a highly significant trend test p-value. Pairwise comparisons with controls were all highly significant as well.

Dr. Peddada described two possible strategies for implementing the use of HC data. The first would be a single global comparison, comparing dose groups with all controls (CCs and HCs) together, while acknowledging variability among HCs. The other strategy would employ three separate comparisons: dose groups with CCs, dose groups with HCs, and CCs with HCs. The latter strategy is preferred by NTP. Under the current NTP strategy, dose groups are formally compared with CCs using the Poly-3 test, while informally, dose groups are compared with HCs and CCs are compared with HCs, both using a historical control range. There are, however, several problems with the historical control range parameter.

Therefore, the proposed strategy has three components (1) comparison of dose groups with CCs using the Max-Iso-Poly-3 test, (2) comparison of dose groups with HCs, also using the Max-Iso-Poly-3 test, modified to account for within- and between-group variability of historical controls, and (3) comparison of CCs with HCs using a Z-test, which accounts for within- and between-group variability of historical controls.

Dr. Peddada noted that it was important to understand that in this strategy all controls are assumed to be from a common homogenous population, although there may be variability among the controls. Under standard NTP practice, all controls are matched in terms of a variety of characteristics such as sex, species and strain, tumor type, and other characteristics. Pair wise comparisons of individual dose groups with HCs are carried out according to the NTP current strategy, but using the Max-Iso-Poly-3 test. To illustrate the outcomes of the comparisons, he showed data for tetralin in male rats and androstenedione in female rats.

The proposed HC tests, he explained, offer several advantages: (1) no complicated models are used, (2) no more assumptions are made than what is currently being assumed by NTP, (3) they are applicable even when the number of HCs is as small as one group, (4) there is better control of false positive rates, and (5) they are powerful for rare tumors.

B. BSC Questions and Discussion

Dr. Toraasen asked about the assumptions made by NTP that substances are carcinogenic. Dr. Peddada replied that no assumption is made about the shape of the response, and that the null hypothesis would be lack of carcinogenic activity. Dr. Toraasen expressed concern that the use of p-values in the HCs could introduce a bias.

Dr. Faustman suggested that the “nearest neighbor” approach to HCs be considered, as one way to control some of the variable factors such as drift in a more statistical fashion. Dr. Peddada explained that to achieve homogeneity among the controls, they are matched for the variable factors prior to statistical analysis.

Dr. Loomis asked if there were closed form solutions for the simulations Dr. Peddada had presented. Dr. Peddada said that was not possible for those expressions. Dr. Loomis felt that the potential 10% loss of power by the Max-Iso-Poly-3 test compared to the Poly-3 test could be quite significant. He also asked about the underlying rationale for using HCs. Referring to his graph depicting power comparisons, Dr. Peddada reiterated that the use of HCs lent increased statistical power. Dr. Walker added that HCs are just one of many factors NTP considers when making calls about carcinogenicity, but are useful for evaluating low level and rare tumors.

Dr. Nagarkatti supported the concept of using HCs, but was concerned about their consistency in terms of where, when, and how they were performed. Dr. Bucher said those factors are taken into consideration, and that they are just one element among many contributing to making judgments on the carcinogenic activity of substances. Dr. Howard said there is tremendous value in using the HCs to bring the CCs into perspective, but with the considerable variability in the HCs, to make the tumor call based on that information would be "very problematic." Dr. Sherley said he felt the current approaches are excellent, and that the proposed new ones are "really scary," particularly since the HCs are not replicates. He was also concerned about the approaches to Type 1 and Type 2 errors.

Dr. Nicholas Jewell, first lead reviewer, expressed concern about the inherent assumptions about mortality using the Max-Iso-Poly-3 test. He approved of the inclusion of isotonic regression in the test and felt the test helped make up for potential problems in ascertaining the appropriate dose range. Regarding the plan for using HCs, he said although they are not typically useful in human studies, they would potentially be relevant in animals studies, adding significantly to statistical power, with a systematized and organized approach to using HCs. He was concerned about how they would be chosen, with the possibility of subjectivity creeping in. He was also concerned about asymmetrical use of HCs, in terms of how well they might resemble the CCs.

Dr. Stephen Looney, second primary reviewer, participated by telephone. Referring to the Peddada and Kissling, 2006 paper, he felt the evaluations of the Max-Iso-Poly-3 test were generally well designed and well executed, and was pleased that the issue of multiple comparisons had been formally addressed. He was concerned that there were very few non-null simulation conditions considered, and as such, was difficult to evaluate the relative performance of the Max-Iso-Poly-3 test and the Poly-3 test. He was impressed by the ability of the HC/CC comparison to control for Type 1 errors, especially compared to the range-based method. He expressed concern about the adequacy of the power in the proposed tests.

Dr. Miller, third lead reviewer, said he had been concerned about the use of HC data, but was reassured by comments during the meeting and supported the use of it in the context of it being one of many biological and statistical tools used to reach conclusions regarding carcinogenicity. He recommended the assumptions to be used be captured and clearly presented.

Responding to some of the reviewers' concerns, Dr. Peddada elaborated on the background considerations that had gone into the proposed use of the HC data.

Ms. Rudel said she was pleased to see the issues of nonlinear response and HCs being formally addressed within the proposed strategies.

Dr. Eastmond commented on concerns about Type 1 errors due to multiple comparisons. He indicated that this has been one of his concerns with the current approach, and that the proposed strategy could substantially increase the number of comparisons employed. He said it was important for NTP to be aware of this possibility in the use of multiple tests such as those proposed. Dr. Peddada agreed with that assessment.

Summarizing the discussion, Dr. Eastmond expressed BSC's opinion of considerable support for the Max-Iso-Poly-3 test, but with some reservations about the use of HC data.

VIII. NTP Research Concept: Nanomaterials Exposure Assessment

A. Presentation

Dr. Charles Geraci, NIOSH/CDC, briefed the BSC on the proposal to continue the research effort to assess occupational exposures to nanomaterials that began in FY 2008.

Materials are known to take on new and different properties at the nano scale. These materials, known as Engineered Nanomaterials (ENMs) are being used or developed for use in a wide variety of businesses and industries. The initial investigation was undertaken due to the rapid development and commercialization of ENMs and initial toxicology findings for select ENMs that raised human health concerns. The current challenges are to (1) identify nanomaterials that pose a possible risk to human health, (2) evaluate the nature and extent of exposures, (3) prioritize nanomaterials for testing, and (4) develop a method to remain current with the development of new materials.

The strategy to meet those challenges was to develop a partnership concept between NIOSH and NIEHS/NTP, to conduct material and market surveillance, and to develop

information on human exposures. Several objectives are embodied within the proposed research concept: (1) identify ENMs to evaluate, (2) identify and solicit participation from sites, (3) identify workers with potential exposures, (4) characterize exposures and the ENMs involved, (5) develop a worker exposure profile, (6) provide input to NTP for candidate ENMs and develop background for NIOSH health effects study, and (7) develop risk management guidance.

To accomplish those objectives, NIOSH staff will seek to gain access to nanomaterial sites, deploy a team of industrial hygienists to conduct on-site exposure assessments, focus on initial ENM exposure studies (with detailed studies possible later), apply existing techniques and develop new ones to evaluate worker exposure, and characterize workplace exposure to selected ENMs.

Several nanoparticles are already under investigation, mainly via the inhalation and dermal routes of exposure. Target organs include the lung, skin, brain, and cardiovascular system. Endpoints of interest have been inflammation, oxidant stress, fibrosis, and translocation. To help guide and prioritize recent efforts, researchers have concentrated on a list of ENMs generated by the OECD, which includes some of the better-known, higher production volume ENMs such as single- and multi-walled carbon nanotubes, fullerenes, and titanium dioxide, as well as newer, emerging ENMs such as nanoclays and nano crystalline cellulose. In total, there are approximately 2,453 ENMs available from 155 suppliers.

Dr. Geraci pointed out that the nanotechnology workforce is moving from the laboratory to the shop floor as the materials grow in commercial production and importance. ENMs account for six million jobs worldwide, with an increasing need for technically skilled workers anticipated in research and development and in manufacturing. The nanotechnology workforce and workplaces will be quite diverse as the industry progresses, as will be the scenarios involved in exposure assessment. To evaluate exposures, multiple metrics are in use, with both process and area measurements, as well as personal exposure measurements. Dr. Geraci shared several examples of workplaces and exposure assessment methodologies. NIOSH field investigations have ranged from university research labs to high-volume production facilities.

Thus far in the program, there have been 18 visits to 15 separate sites, with a total of 14 nanomaterials evaluated. The proposed extension of the program from FY 2011-13 would encompass exposure studies at an additional 12 sites, and would expand the number of ENMs evaluated and extend the investigations down the ENM product life cycle.

At this point, NIOSH has evaluated only a small portion of the rapidly growing nanomaterial market; however, workplace exposures do occur. Exposure limits are still

being developed; for example, NIOSH recently issued recommendations for titanium dioxide and carbon nanotubes. Although mass is still the primary metric for exposure, additional metrics need to be explored, such as fiber count for carbon nanotubes. Additional confirmatory methods are also needed. It has been shown that conventional engineering controls can be effective in eliminating workplace exposures.

B. BSC Questions and Discussion

Dr. Nagarkatti asked whether NIOSH would also examine the toxicity of some of the nano-encapsulated drugs. Dr. Geraci said NIOSH does not do drug safety assessments, but they would examine some of the planned or proposed carriers used as delivery systems, as well as looking at the safe manufacture of delivery vehicles or drug particles themselves.

Dr. Birnbaum asked whether the researchers would also be looking at surface area as a metric. Dr. Geraci said they had and would continue to, e.g., evaluating the utility of measuring surface area of a complex aerosol.

Dr. Zelikoff asked about the importance in the program of dermal exposures. Dr. Geraci said NIOSH is looking at how they might extend some of the dermal work done in the past on other types of compounds such as pesticides and metals into ENMs, so dermal exposures will be part of the long-range research plan. The protective equipment research lab in Pittsburgh, PA has also done extensive work on protective garments and respirators to protect against ENM exposures.

Ms. Rudel, first lead reviewer, said the research seemed quite important, but the scope of the proposal seemed quite modest in relation to the great need, information gaps, and potential threat posed by ENMs. In addition to working with established tools, there is a need to develop innovative measurement methods to characterize important aspects of ENMs for health effects. She hoped the administration, which she said is supportive of commercialization, would be equally enthusiastic about safety evaluation.

Dr. Zelikoff, second lead reviewer, felt the validity and rationale for the concept had been well presented. The first four stated goals of the program (identifying sites and companies, identifying affected worker populations, characterizing occupational exposures and evaluating patterns of exposure, and examining the chemical and morphological properties of airborne ENMs), from the NTP/toxicology perspective, were appropriate for assessing toxicity. She said the other two stated goals (characterizing engineering control techniques and providing appropriate risk management recommendations) were a bit premature and did not fit very well with NTP's mission and goals. She noted that nanomaterials with shells could have different toxicity than those with just the core exposure, and wondered if shell materials would also be examined.

She recommended that certain parallel toxicity studies be conducted. Overall, she gave the proposal a moderate rating, explaining that the proposal was too broad in some areas but too narrow in others. She recommended "some inclusion of toxicity or health endpoints that could be performed along with or subsequent to the identification and evaluation of occupational exposures in facilities."

Dr. Geraci noted that the body of nanotoxicology literature has increased dramatically in the last four to five years, so there is a growing body of information available, which the researchers are using to prioritize materials on which to focus their efforts. The technology is moving so quickly that it's important to work with companies to take a prudent, protective, proactive approach, even if the health and safety information is not complete. He agreed that surface charge is an important parameter. He said that on the national level, the health, safety and environmental impacts of ENMs are in fact being extensively considered and explored, particularly within the National Nanotechnology Initiative legislation.

Dr. Loomis said it is "clear that there is a compelling rationale for doing this work." He likened the rapid diffusion of nano products to the rapid diffusion of asbestos in the 20th century and agreed with earlier comments that the scope of the project is modest. He recommended more robust investigation of worker exposure.

Dr. Solomon asked whether there had been any assessment of the effective protection of personal protective equipment such as Tyvek suits, gloves and respirators from dermal exposures. She also wondered whether there is a need to investigate areas downwind of ENM manufacturing facilities for proliferation of the materials into the local environment.

Dr. Nagarkatti asked about the range of sites to be investigated. Dr. Geraci said the number of sites using ENMs is much greater than the number that produces them, and many of those sites modify the materials. However, NIOSH has an understanding of the highest-volume uses and applications, and has plans to launch some broader industry-wide or commerce-wide studies of classes of materials across multiple industries. Regarding effective personal protection, he said some of the early NIOSH research in the area was on aerosol exposures and on the efficacy of high efficiency particulate filters, which proved efficient for particles as small as 10 nanometers in diameter. NIOSH research on protective garments would be published soon.

Dr. Zelikoff applauded the NIOSH's efforts to measure personal exposures in various settings, adding that such data would be important to help establish no observed adverse effect levels and lowest observed adverse effect levels.

Dr. Bucher explained why the concept had been brought for review by the BSC. There will not be a uniform workforce to evaluate from an epidemiology standpoint, but workers are potentially being exposed to ENMs with little information on the potential effects. This would lead to animal studies, which are challenging in terms of recreating worker exposures realistically. Thus, he said, the comments by the BSC would be helpful in determining the most appropriate approach and resources to be devoted to the problem. Dr. Birnbaum added that this concept and the collaboration with NIOSH is just one part of a much larger NIEHS nanosafety effort.

Regarding the issue of mitigation, Dr. Walker said it is an important element to be taken into account when prioritizing what materials to assess and in what settings.

Dr. Eastmond summarized the BSC's opinions, stating that the BSC members were quite supportive of the initiative, but there was some concern it was not moving fast enough or involving some of the NTP's core strengths. There was an understanding, however, that those limitations would be overcome moving forward.

IX. NTP Research Concept: Biospecimen Repository and Analysis Capabilities to Support NTP Exposure Assessment Projects

A. Presentation

Dr. Scott Masten, Director of the NTP Office of Nomination and Selection, said the NTP had historically conducted human exposure assessments, using them to guide decisions in selection and design of toxicology studies and to provide human health context for comparison with animal studies and characterization of workplace exposures, employing both direct and indirect measures.

Prior and ongoing exposure projects have consisted of general population biomonitoring, workplace exposure characterizations, and small, targeted population studies. Dr. Masten provided several examples of past projects, highlighting work conducted in collaboration with the CDC's National Center for Environmental Health (NCEH) and with NIOSH, as well as small population studies being conducted by the NIEHS Clinical Research Unit.

He recounted several issues that lead NTP to believe it needs to do more in this area including (1) limitations of existing human exposure information such as incomplete information on combined exposures and common mixtures and difficulty in tracking emerging substances of concern; (2) institutional focus on developmental basis of adult disease, such as (a) the growing recognition of the potential influence of early life exposures on cancer, asthma, obesity, diabetes, and neurological development, and (b)

the new HHS or cross-government department initiatives, such as the President's Children's Environmental Health and Childhood Obesity Task Forces; (3) the National Research Council Committee on Human and Environmental Exposure Science in the 21st Century; and follow up on research recommendations from two NTP workshops on, (a) diabetes and obesity (January 2011) and (b) chemical mixtures (September 2011)

The NTP aims to develop a mechanism for accessing resources of banked human biospecimens that could be used to rapidly address focused questions of relevance to NTP programs. These questions are anticipated to emerge from expanded NTP literature analysis activities and high throughput Tox21 screening activities. There is a need to perform targeted analyses of agents of emerging public health concern, to answer questions about the routes of exposures, common co-exposures, and whether particular exposures warrant further NTP evaluations. The NTP also wishes to address specific hypotheses regarding exposure-health outcome associations through targeted analyses of human biospecimens.

Dr. Masten explained that to establish the desired resource, the NTP proposes to identify relevant existing resources to incorporate into a virtual network of biospecimen repositories. The NTP will engage with NCEH and other federal partners regarding availability of biospecimens. It will also inventory existing biospecimen repositories assembled as part of ongoing or past research supported by NIEHS or National Institute of Child Health and Development extramural grants programs. Thus far 70 biospecimen banks collected and maintained via NIEHS grants have been catalogued. Other NIH-supported extramural investigators will also be contacted regarding other biospecimen resources they may possess. Sample analysis will be conducted through contract chemistry capabilities or interagency agreements with federal partners.

Other existing NTP capabilities and potential partnership options include the NIEHS Clinical Research Unit, physical biospecimens stored within the NTP Archives, and analytical chemistry capabilities from NTP chemistry support contracts, the FDA/NCTR Analytic Chemistry Unit, and the CDC/NCEH Division of Laboratory Sciences.

Delineating the envisioned scope of the project, Dr. Masten emphasized the desire for flexibility, to allow response to new trends or needs. The effort is proposed to be fairly modest, encompassing up to several projects per year, with specific project designs to be based on the hypotheses to be addressed. The focus is to be on general population exposures, particularly men and women in their prime reproductive years and in children. The biospecimens to be collected will be primarily peripheral blood and urine, cord blood, amniotic fluid, breast milk, saliva, hair, nails, and buccal and PBMC cells. The types of studies sought will be cross-sectional or longitudinal human population-based, with a preference for studies in which repeated sampling has taken place, and which have also used other exposure assessment methods, such as questionnaires.

The types of analyses to be pursued will be primarily for the parent chemicals or metabolites associated with exposures of interest, with the potential for conducting biomarker discovery/validation.

Dr. Masten provided several examples of biospecimen banks that have been identified as candidates for inclusion in the project. He concluded by summarizing the significance and expected outcome of the project, which would (1) enhance the NTP's exposure assessment capabilities, (2) provide an additional set of tools useful for putting findings from toxicology studies and literature analysis activities into human health context, (3) gain access to resources that contain repeated measures of blood and urine from men and women of childbearing age, women who are trying to become pregnant, and women during pregnancy and lactation, and (4) improve NTP's ability to identify environmental exposures that are most worthy of public attention.

B. BSC Questions and Discussion

Dr. Faustman asked about consideration of using advanced analytical methods or bioinformatic approaches to query archived analytical data files, as these may also be obtainable. This would allow probing many different exposures of interest in large studies without conducting repeated analyses or long-term storage of the physical biospecimens. Dr. Masten said he found the prospect exciting, although it is not specifically mentioned in the concept document.

Dr. Solomon asked about the interagency agreement with CDC that had been in place ten years ago, but apparently is no longer active. Dr. Masten explained that much of the work was methods development, and eventually carried on with the CDC's own funding. Dr. Bucher added that the success of the original programs had attracted increased funding from Congress for large-scale biomonitoring programs.

Dr. Miller asked whether genomics assessment was being considered. Dr. Masten replied it would, but the issues would be resource allocation and what type of analyses would be needed to answer particular questions.

Dr. Solomon, first lead reviewer, was very supportive of the concept, calling it "high on merit but a bit thin on nuts and bolts." She felt the clarity and validity of the rationale was clear and such a repository would have a huge role in contributing to exposure science and informing toxicity testing. She noted the potential public health impact of the project could be very high, filling a missing link. A repository located at the NTP could address late biomarkers of exposure and start to inform biomarkers of effect. She was unclear about the scope of the program and how many samples would be stored at NTP versus within the networks, and how the agreements for access would work. She

wondered about the niche to be filled by the on-site repository. She concurred with the suggestion for use of time-of-flight mass spectrometry as an analytical tool.

Dr. Loomis, second lead reviewer, said his enthusiasm for the proposal was quite high, agreeing with previous comments. The rationale was well stated, but the proposal fell short in explaining the program itself and what it will do that is different. He felt it was important to NTP's goals, potentially providing important information about public exposures to substances of concern. It supports evidence-based selection of substances for testing and helps move toward the goal of making toxicology a predictive science. He characterized the potential public health impact of the proposal as "very high" and felt the scope of the proposal was high on merit but short on mechanics. The significance and impact of the work would be maximized to the extent that the banked specimens can be related to or are statistically representative of actual human populations that can be identified. He was also concerned about the quality of the other data that could be linked to the specimens. A database with both population-based human exposure information and other information such as medical records or disease biomarkers would clearly have huge power to characterize exposures and associations with health outcomes. Although enthusiastic about the project overall, he reminded NTP that this type of approach can never fully replace traditional exposure assessment approaches.

Ms. Rudel, third lead reviewer, agreed with the previous comments, and was pleased to see the rationale, as she had called for a program like this previously. She wanted to see it clarified in the rationale that having the exposure data would help the NTP prioritize chemicals for testing, and that added understanding about exposure would help to formulate level of concern statements based on toxicological studies. Thus, it would help determine what is tested and how the results are interpreted. She agreed that the scope was a bit lacking in clarity.

Dr. McDiarmid, fourth primary reviewer, said she had more concerns than the other reviewers, although the concerns were more focused on the written document than the idea itself. She understood previously that there was more emphasis on a physical repository, but now understood that the starting point would be the virtual repository, for which she had much higher enthusiasm. She felt that the examples given as reasons to start a physical repository were not good. She recommended further discussions with NCEH, as they have much more experience in this area. She was disappointed that there was no mention of the necessary demographic epidemiologic documentation, which caused her concern that perhaps the concept had not been adequately thought through. She was also concerned that there was no mention of clinician involvement. Based on those concerns, she expressed moderate enthusiasm overall for the proposal.

Dr. Masten explained that the concept was more of a “pre-concept” designed to gauge the BSC’s level of support for the activity. He said the NTP did not envision competing for access to precious samples and he agreed with comments about the importance of having ancillary demographic data available along with samples, although that would not be necessary in all scenarios.

Dr. Birnbaum commented that NIEHS already has a biorepository associated with the NTP Archives. She agreed with the ideas expressed about partnering with sister agencies, and reminded the BSC that that is something NIEHS and NTP do frequently.

Dr. Nagarkatti asked whether DNA, RNA and proteins would be separated from the samples, given the extensive analyses planned. Dr. Bucher replied that those types of questions would be addressed when specific projects were developed.

Dr. Zelikoff suggested that nasal swabs be added to the list of samples to be collected and that incentives be created for investigators to contribute their precious samples. She also cautioned that variations in the quality of sample storage would have to be taken into account.

Dr. Eastmond summarized the discussion, stating that the BSC was generally supportive of the proposal’s general direction, although the proposal itself is at a very early stage. He reiterated the suggestions for partnering with other agencies, establishing relationships with the investigators to gain access to the samples, and adding the documentation of demographics and epidemiological information, as well as involving clinicians.

X. CERHR Workshop Concept: Clarifying Potential Health Effects of Excess Folic Acid Intake

A. Presentation

Dr. Abee Boyles, NTP postdoctoral fellow, briefed the BSC on the proposed workshop concept, which was developed in conjunction with the NIH Office of Dietary Supplements (ODS). She reminded the BSC members that folate, a water-soluble B-complex vitamin, is an essential nutrient to human health. It is required for nucleic acid synthesis and as a methyl donor. It is acquired via food intake and from folic acid supplementation in foods and vitamins. It is known to prevent neural tube defects, and based on that knowledge, folic acid has been added to the US food supply since 1998. That has been a public health success story, as neural tube defects have declined significantly in the United States and elsewhere since the dietary supplementation went into effect.

In 1998 the Institute of Medicine (IOM) evaluated all B-vitamins and set dietary reference intakes. For women of reproductive age, they recommended 400 µg per day of folic acid in addition to folate from a varied diet. For pregnant women, the recommendation rose to 600 µg per day. IOM also set a Tolerable Upper Intake Level (UL) of 1000 µg per day of folic acid alone. That figure was based on 30 studies, one of which was from 1990, with the rest being from 1961 or earlier. Thus, none of the studies were conducted after folic acid supplementation was added to the food supply.

Dr. Boyles showed a graph depicting folic acid intake since supplementation began, from the National Health and Nutrition Examination Survey (2001-2004). It showed that 72% of the population is still at a folic acid intake of less than 400 µg per day, whereas 4% of the population is above the 1000 µg per day level. She said it is difficult to get to the UL based on fortified foods and ready-to-eat cereals fortified with folic acid; supplements are obviously the source of the higher levels.

CERHR became interested in these possible effects of excess intake as a result of several studies that have appeared in the literature recently implicating high levels of folic acid intake in adverse health effects. One found an association between folic acid treatment and colorectal adenomas and other cancers. Another study in Norway found an increase in cancer mortality in the group treated with folate/B-12. Another cause for concern is the ready availability of folic acid supplements (typically 800 µg), which are inexpensive and are being marketed to non-pregnant women and men. Prenatal vitamins also often contain 800-1000 µg of folic acid.

Based on the review of the available literature, CERHR has identified several potential health effects to be assessed in the proposed workshop. In adults, there is possible association with cardiovascular disease, cancer incidence and progression, neurological and psychiatric disorders, and immunological dysfunction. *In utero* exposures have been implicated in respiratory effects and methylation changes, with possible epigenetic effects.

CERHR proposes to hold a State of the Science Workshop, to be co-sponsored by the NIH ODS. CERHR has held discussion for several months with the CDC, FDA, and the IOM Food and Nutrition Board. The proposal is to (1) review the published literature on folic acid intake above the recommended daily allowance (RDA) and human health effects, (2) develop a background document with input from ODS, CDC, and expert participants, (3) convene a two-day workshop in Bethesda, MD in March 2012, (4) hold plenary sessions and breakout groups at the workshop, and (5) publish a report containing the background document and a summary of the experts' conclusions from the workshop.

The goals of the workshop are to (1) clarify the state of the human literature for evaluating potential effects of folic acid intake above the RDA, (2) examine the support or sufficiency of the animal and *in vitro* literature for evaluating effects applicable to humans, and (3) identify data gaps for future research to inform conclusions about potential human health effects.

The workshop will be open to the public and include invited experts in diverse fields such as epidemiology, nutrition, toxicology, and clinical medicine (e.g., pediatricians, oncologists, and obstetricians), along with scientists from the model systems community to explore animal models of essential nutrients and *in vitro* models of folate metabolism.

Dr. Boyles emphasized that the workshop will *not* be designed to result in an NTP "level of concern" conclusion regarding excess folic acid exposure and health, nor will it make recommendations regarding fortification or supplement use.

B. BSC Discussion

Dr. Faustman, first lead reviewer, felt that the proposal was important, addressing an emerging issue that is already affecting public policy. The concept has the potential to identify significant data gaps and provide significant direction to areas of nutrition and toxicology research, as well as being of practical benefit. The proposed workshop matches directly with NTP's stated goals of strengthening the science base for toxicology. She said it was particularly important for such stakeholders as FDA and the general public, and is therefore both timely and significant. Due to the importance of supplementation and new questions about its impact, the proposal has high significance and needs to move forward. She recommended that CERHR invite members of the IOM Upper Limits Committee to attend the workshop.

Dr. Sherley, second lead reviewer, said his perspective on the proposal was somewhat different than that of his colleagues. He felt that the presentation at the meeting had changed his opinion to a certain extent, but shared his prior impressions for the record. Citing data showing that average daily US intake of folic acid was only about half of the RDA, he said his main problem with the concept was that there seemed to be no compelling reports of related health effects presented. He questioned the idea of "a workshop to review the health effects research literature for a diet-supplemented, natural, body metabolite and nutrient for which there is little basis to consider it to be even potentially hazardous" and further questioned the conjecture that folate is a formal growth regulator. He noted the background document lacked the customary exposure data, which should have been easily acquired, and metabolism reviews, which were contained in the reference materials. He found these oversights "somewhat troubling" and questioned the need for the expressed level of concern even for the small percentage of the population taking much higher doses of folic acid than the RDA. He

expressed other detailed misgivings, and rated the overall significance and public health impact of the proposal as low. He felt "too broad a literature search is proposed given that it seems that no confident indication of associated health effects is likely to be demonstrable." He supported a more careful review of the literature to see if the hypothesis about excessive folic acid intake is truly supported.

Dr. McDiarmid, third lead reviewer, had high enthusiasm for the concept due to the large potential public health impact. She agreed that a more careful review of the literature was in order, but did not wish to quibble about NTP's chosen mechanism. She pointed out the possibility of adverse effects associated with excess intake was "not on the radar screen" when supplementation was implemented, and so found it appropriate to explore the idea through the proposed concept.

Dr. Faustman made the point that negative literature has also impacted public health decisions in terms of supporting an important avenue for prevention.

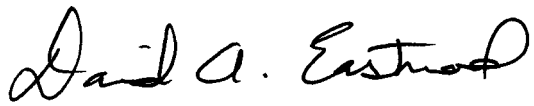
Dr. Howard praised Dr. Boyles and CERHR for the job they had done in reaching out to the regulatory agencies that face this issue. He likened this situation to the role CERHR and NTP played in the bisphenol A debate, calling the proposed workshop "an excellent opportunity." He said CERHR had provided a huge service in the bisphenol A story by compiling and vetting the relevant literature. The issue of the appropriateness of the literature became a major element in the BPA debate, he recalled, and felt that this was an excellent opportunity for CERHR and NTP to do the same thing—to gather experts together to vet the literature and conclude what stands up as good science.

Dr. Eastmond noted some difference of opinion among the BSC members, but said his impression was that the majority of the BSC would support going forward with the workshop concept, since it addresses an important public health concern.

XI. Adjournment

Drs. Bucher and Birnbaum thanked the staff and the BSC members for their hard work and Dr. Eastmond adjourned the meeting.

These summary minutes have been read and approved by the Chair of the National Toxicology Program (NTP) Board of Scientific Counselors.



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Dr. David Eastmond

Chair, NTP Board of Scientific Counselors

Date: ____July 11, 2011_____