

**Summary Minutes**

**NTP Board of Scientific Counselors**

**June 29, 2017**

## Table of Contents

I. Frequently Used Abbreviations and Acronyms .....	2
II. Attendees .....	4
III. Introductions and Welcome .....	5
IV. Report of the NIEHS/NTP Director.....	5
V. Beyond Single Chemicals: Progress and Challenges in Evaluating Toxicity of Real World Exposures.....	7
A. Strategies for Studying Combined Exposures and Mixtures .....	7
B. Where the Rubber Meets the Road: Update on the NTP Crumb Rubber Research Program.....	13
C. Screening for Biological Activities of Concern in Consumer Products .....	16
VI. Report of the NTP Associate Director.....	19
VII. Transgenerational Inheritance of Health Effects: A State of the Science Evaluation.....	20
VIII. A Roadmap for the Implementation of New Approaches for Safety Testing..	22
IX. Adjournment .....	24

## I. Frequently Used Abbreviations and Acronyms

ACC	American Chemistry Council
ADME	absorption, distribution, metabolism, excretion
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
BMD	benchmark dose
BPA	bisphenol A
BSC	Board of Scientific Counselors
CDC	Centers for Disease Control and Prevention
CERHR	Center for the Evaluations of Risks to Human Reproduction
CHEAR	Children's Health Exposure Analysis Resource
CNVRGE	Cancer Network and enVironmental Exposure Research Agenda
CoC	chemical of concern
CPSC	Consumer Product Safety Commission
CRU	NIEHS Clinical Research Unit
DERT	Division of Extramural Research and Training
DIR	Division of Intramural Research
DNTP	Division of the NTP
EFSA	European Food Safety Agency
EPA	U.S. Environmental Protection Agency
EPA IRIS	EPA Integrated Risk Information System
ER	estrogen receptor
EWG	Environmental Working Group
FDA	U.S. Food and Drug Administration
GC/MS	gas chromatography/mass spectroscopy
GHS	Globally Harmonized System of Classification and Labeling of Chemicals
HAAs	haloacetic acids
HAWC	Health Assessment Workplace Collaborative
HHS	Department of Health and Human Services
HTS	high throughput screening
IAA	interagency agreement
IARC	International Agency for Research on Cancer
ICs	NIH Institutes and Centers
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICH	International Conference on Harmonisation

ILS	Integrated Laboratory Systems, Inc.
IPCS	International Programme on Chemical Safety
IVIVE	<i>in vitro</i> to <i>in vivo</i> extrapolation
LAN	light at night
LC/MS	liquid chromatography/mass spectroscopy
LoC	level of concern
MeSH	Medical Subject Headings
MWF	metalworking fluid
NAS	National Academy of Sciences
NCATS	National Center for Advancing Translational Sciences
NCTR	National Center for Toxicological Research
NHANES	National Health and Nutrition Examination Survey
NICEATM	NTP Interagency Committee for the Evaluation of Alternative Toxicological Methods
NIH	National Institutes of Health
NIOSH	National Institute of Occupational Safety and Health
NOEL	no observed effect level
NORA	National Occupational Research Agenda
NTP	National Toxicology Program
ODS	Office of Dietary Supplements
OECD	Organisation for Economic Co-operation and Development
OEHHA	California Office of Environmental Health and Hazard Assessment
OHAT	Office of Health Assessment and Translation
OLRP	Office of Liaison, Policy, and Review
OMB	Office of Management and Budget
ONS	Office of Nomination and Selection
ORoC	Office of the Report on Carcinogens
PACs	polycyclic aromatic compounds
PAHs	polycyclic aromatic hydrocarbons
PCRM	Physicians Committee for Responsible Medicine
PECO/PICO	<u>p</u> opulation, <u>i</u> ntervention or <u>e</u> xposure, <u>c</u> ontrol or <u>c</u> omparator, and <u>o</u> utcomes of interest
PFAS	perfluorinated alkylated substances
PFC	perfluorinated chemicals
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
QSARs	quantitative structure-activity relationship models

REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RoC	Report on Carcinogens
RFR	radiofrequency radiation
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SAR	structure-activity relationship
SES	socioeconomic status
SOT	Society of Toxicology
SSS	Social and Scientific Systems, Inc.
SWIFT	Sciome Workbench for Interactive Computer-Facilitated Text Mining
TEM	transmission electron microscopy
TK	toxicokinetics
UL	tolerable upper intake level
WHO	World Health Organization

## II. Attendees

### Members in Attendance:

Cynthia Afshari, Amgen  
Norman Barlow, Johnson & Johnson  
Paul Brandt-Rauf, University of Illinois at Chicago  
Myrtle Davis, National Cancer Institute  
Mary Beth Genter, University of Cincinnati (on phone)  
Daniel Kass, Vital Strategies  
Steven Markowitz, City University of New York  
Kenneth McMartin, Louisiana State University Health Sciences Center (chair)  
Kenneth Ramos, Arizona Health Sciences Center  
Jennifer Sass, Natural Resources Defense Council  
Donald Stump, WIL Research

### Other Federal Agency Staff:

Goncarlo Gamboa, FDA, BSC liaison  
Abraham Tobia, FDA  
Elizabeth Whelan, National Institute for Occupational Safety and Health (NIOSH), BSC Liaison (on phone)

### National Institute of Environmental Health Sciences (NIEHS) Staff:

Brandy Beverly	Virginia Guidry	Cynthia Rider
Beth Bowden	Robbin Guy	Kristen Ryan
Windy Boyd	William Gwinn	Kelly Shipkowski
Linda Birnbaum	Beruk Kiros	Keith Shockley
Abee Boyles	Grace Kissling	Robert Sills
John Bucher	Ruth Lunn	Diane Spencer
Warren Casey	Dave Malarkey	Matthew Stout
Natasha Catlin	Scott Masten	Vicki Sutherland

Helen Cunny  
Sally Darney  
June Dunnick  
Anika Dzierlenga  
Paul Foster  
Rachel Frawley  
Dori Germolec

Elizabeth Maull  
Barry McIntyre  
Suril Mehta  
Dan Morgan  
Esra Mutlu  
Rick Paules  
Georgia Roberts

Raymond Tice  
Suramya Waidyanatha  
Nigel Walker  
Vickie Walker  
Amy Wang  
Mary Wolfe  
Rick Woychik

**Public:**

Megan Amos, PCRM (on phone)  
Reshan Fernando, RTI International  
Stephanie Fox-Rawlings, National Center for Health Research (by phone)  
Ernie Hood, Bridport Services  
Carol Kwiatowski, TEDX  
Steven Levine, Monsanto  
Olga Naidenko, Environmental Working Group  
Joel Tenney, Israel Chemicals  
Brian Woolsey, B-Logic Professionals

### III. Introductions and Welcome

The National Toxicology Program (NTP) Board of Scientific Counselors (BSC) convened June 29, 2017, in Rodbell Auditorium, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC. Dr. Kenneth McMartin served as chair.

He welcomed everyone to the meeting and asked BSC members and other attendees to introduce themselves. Dr. Mary Wolfe, BSC Designated Federal Official, read the conflict of interest policy statement. NTP Associate Director Dr. John Bucher welcomed everyone to the meeting.

### IV. Report of the NIEHS/NTP Director

Dr. Linda Birnbaum, Director of NIEHS and NTP, briefed the BSC on recent developments at NTP and NIEHS.

She began with a report regarding appropriations. In Fiscal Year 2017, the NIEHS budget increased by approximately \$22.7 million. NIH received a 6.2 percent increase, while the NIEHS increase was 2.7 percent. This represents a nearly 25 percent decrease in overall buying power compared to 2003. The President's Request for FY2018 represents a 26 percent reduction for NIH. The NIEHS budget request was \$533.537 million, which is \$180.274 million or 25.07 percent less than the total amount appropriated for NIEHS in FY2017. Dr. Birnbaum noted that the proposed cuts would be devastating, and Congress appears unlikely to institute such "horrific cuts." She said she was guardedly optimistic that the budget would at least stay flat.

She described several new pieces of proposed legislation that could potentially affect NIEHS, including the Airplane Impacts Mitigation Act of 2017, the Federal Accountability in Chemical Testing Act, the Investing in Testing Act of 2017, an act mandating education and training for environmental health professionals, the Radiation Exposure Compensation Act Amendments of 2017, and the Feminine Hygiene Product Safety Act of 2017. She expressed doubt that any of the proposed laws she had described would go forward.

Turning to science advances, Dr. Birnbaum briefly summarized several recent publications by NIEHS/DNTP personnel or NIEHS grantees. First, as an example of “One NIEHS” research, she described a publication called “Associations Among Personal Care Product Use Patterns and Exogenous Hormone Use in the NIEHS Sister Study.” She also summarized a recent DNTP study, “In Silico Prediction of Physicochemical Properties of Environmental Chemicals Using Molecular Fingerprints and Machine Learning.”

In recent NIEHS news and highlights, Dr. Birnbaum mentioned the May 23 ICCVAM Public Forum, NIEHS interagency coordination on PFAS, and NIEHS involvement in WHO IPCS Systematic Review training. She described recent meetings involving epigenetics, Tox21, and BioMed21, as well as upcoming advisory group meetings and other meetings and events. She highlighted several recent examples of awards and recognition involving NTP personnel. She noted that the search process for a new DNTP Associate Director is still progressing, with four finalists. Although a candidate has been chosen, the position is currently on hold due to HHS hiring limitations.

Dr. Birnbaum thanked Dr. Genter for her years of service to the BSC.

She noted that NIEHS has begun the process of formulating a new strategic plan, and asked BSC members to complete the online survey that seeks input for the new plan.

Mr. Kass asked whether the BSC historically comments during budget phases. Dr. Birnbaum replied that although members of the BSC can express their concerns to her directly as individuals, they are not empowered to comment on the budget.

Dr. McMartin noted that BSC members Dr. James Stevens (Lilly) and Dr. Katrina Waters (Pacific Northwest National Laboratory) were unable to attend the meeting.

## V. Beyond Single Chemicals: Progress and Challenges in Evaluating Toxicity of Real World Exposures

### A. Strategies for Studying Combined Exposures and Mixtures

Dr. Cynthia Rider presented the BSC a comprehensive update about NTP's research program related to mixtures. Her presentation was divided into four areas: background information and the three mixtures research areas. Each section included an opportunity for BSC members to ask clarifying questions.

#### Section 1: Mixtures research background

Dr. Rider noted that everyone is exposed to mixtures, "and we've gotten much better at characterizing that exposure, both internally and externally."

She provided definitions for several terms related to mixtures, including defined mixture, complex mixture, whole mixture, and the exposome. Exposure to a defined mixture can be aggregate, which includes consideration of different routes, or cumulative, with the addition of multiple components. Cumulative exposure can result from dose addition, by adding chemicals at the dose level, or independent action or response addition, adding chemicals at the response level. If chemicals are dose additive, a cumulative risk assessment should be performed to protect human health.

Dr. Rider related the history of mixtures research at NTP, which began in 1978 and escalated in 2011 with the NIEHS Mixtures Workshop.

She provided information about the thought processes involved in understanding the health effects of mixtures. It begins with problem formulation; whether the process starts from an exposure, a disease, or a population. Biological similarity is considered in the decision making process, as is the quality of the available data. The whole mixture approach includes consideration of the mixture of concern, and is influenced by many sources of variation. Studies of botanical dietary supplements are an example. The component approach focuses on individual compounds within a mixture. Dioxin mixture studies are an example.

Dr. Rider noted the lessons learned from past NTP mixtures work, and the evolution in mixtures toxicology. She summarized the key issues involved in mixtures research, and aligned mixtures research with Goal 4 in the NIEHS Strategic Plan. She described the NIEHS Combined Exposures Mixtures (CEM) Working Group, which meets quarterly to discuss mixtures projects throughout NIEHS. The group is developing a logic model to guide prioritization of NIEHS mixtures efforts.



### Section 1: BSC Clarifying Questions

Dr. Ramos asked whether Dr. Rider would provide any details about the logic model she had just mentioned and where information about the logic model might be available. Dr. Rider said there is a document about the model, and that she would forward it to Dr. Ramos. Dr. Ramos asked whether the group had begun to examine antagonistic interactions in its model development. Dr. Rider replied that there had been much work already on those issues.

Dr. Markowitz asked for clarification on the bullet on Dr. Rider's slide depicting evolution in mixtures technology, "Prioritizing which chemicals to evaluate for cumulative effects viewed as more important than possible interactions." Dr. Rider replied that in the past, the thinking was that combining two chemicals together would result in synergy; however, research has discovered very few examples of detecting a greater than additive effect. Dr. Birnbaum noted that there are some clear examples of potent synergy. Dr. Rider said that an interesting point, which emerged in the 2011 workshop, was that epidemiologists and toxicologists measure additivity and interaction differently. Dr. Gamboa added that there are instances of very striking synergy that should not be overlooked. Dr. Rider assured him that interactions would not be ignored, and that the models designed to detect additivity would also detect difference from additivity.

Dr. Sass asked Dr. Rider about a point in one of her slides that indicated that chemicals with the same mechanism of action, as long as they are both below their NOEL, then the mixture would be below the NOEL. Dr. Rider explained that that would be the case if the chemicals are definitely independently acting. Dr. Sass felt that there could be overconfidence in relying on mechanism of action. Dr. Rider agreed that it is a major question in the field – how similar do chemicals have to be to apply the dose addition model versus independent action?

### Section 2: Component-based approaches

Dr. Rider described the component-based approaches to analyzing mixtures.

- They require dose-response data on all individual components and the defined mixture of interest, with uncertainty in individual component data feeding into uncertainty in cumulative assessment.
- There are relatively few examples of application, particularly with higher-order mixtures (>10 components).
- Assumptions require that chemicals adhere to a specific model of additivity, and that chemicals do not interact.
- The approach requires appropriate statistical models for determining deviation from additivity.

Dr. Rider delineated the elements involved in application of component-based methods. She provided the example of polycyclic aromatic hydrocarbons (PAHs), which represent multiple, diverse sources of exposure to complex, dynamic mixtures, with multiple possible routes of exposure, and with many known toxicities associated with some chemicals in the class. She described the history of PAHs mixture research, which expanded into evaluation of a broader range of compounds, polycyclic aromatic compounds (PACs). She summarized *in vitro* and *in vivo* research on PAC mixtures.

### Section 2: BSC Clarifying Questions

Dr. Davis noted that pharmacokinetic data were not on the list as one type of data that would be collected. She asked if it had just been left out, or if it was felt that it is unnecessary. Dr. Rider said it was agreed that it is an important consideration, since in mixtures many of the interactions are pharmacokinetic. She added that testing is currently being conducted in completely parallel assays, to identify if there is deviation from additivity. However, it is not possible to characterize the exact type of interaction and whether or not it is pharmacokinetic. Samples are being saved for possible future investigation of the question.

Dr. Afshari said that given the use of immunotoxicity studies, there may be very different rankings compared to carcinogenicity. She said she had hoped Dr. Rider would tie everything together, leveraging the cell-based assays and some of the biochemistry to help understand and reconcile the data. She asked Rider for insight on how mechanistic data would be incorporated. Dr. Rider noted that Dr. Afshari had correctly perceived that the approach has gone toward looking at patterns of activity in the *in vitro* studies, hoping that the patterns of activity would allow grouping into different activity-based groups. That would allow later evaluation of mechanism for each of the activity-based groups.

### Section 3: Whole mixture approaches

Dr. Rider said there is no single “mixture of interest” for complex mixtures, so it can be assumed that the tested mixture is representative of the mixtures of interest, and an approach can be developed to determine whether or not a tested mixture is sufficiently similar to mixtures of interest. Sufficient similarity refers to a “mixture that is very close in composition to the mixture of concern, such that differences in their components and their proportions are small.” Also, the toxicologic consequences of exposure to the two mixtures will be identical or indistinguishable.

She illustrated the concept with the example of NTP studies of botanical dietary supplements, particularly *Ginkgo biloba* extract, which has shown evidence of *in vivo* effects. She also described NTP work with black cohosh extract and *Echinacea*

*purpurea* extract. Selection of test article is extremely important in whole mixture studies. Developing whole mixture approaches, which can be broadly applied, will have a huge impact on the ability to estimate health effects from exposure to complex mixtures. Whole mixture approaches provide a bridge between toxicology and epidemiology.

### Section 3: BSC Clarifying Questions

Dr. Davis asked whether a level of confidence in the mixture being used for initial comparison is necessary. Dr. Rider said that in the case of *Ginkgo biloba* extract, there were already 2-year and 90-day bioassays, allowing comparisons. The specific question in that study related to one particular mixture that had been tested. However, the same approach could be used if a reference mixture were available. Dr. Bucher added that a gold standard ginkgo and the NIST standard were available, lending a good place to start in the absence of a cancer bioassay.

Dr. Ramos asked whether the gold standard reference ginkgo is an extracted reference standard. Dr. Rider noted that two NIST standards were used, as well as off-the-shelf ginkgo preparations that had to be broken down. Dr. Ramos asked which was in the cartoon Dr. Rider had shown. She said it was a bulk, pre-formulation extract. Dr. Ramos asked for clarification of the approach with the test article. Dr. Rider said the two-year bioassay was done first. Then, for the case study, 20 extracts and some off-the-shelf formulations were purchased, allowing multiple comparisons. She clarified that the similarity among the articles was defined by both the chemistry and the biology.

### Section 4: Systems biology approaches

Summarizing the systems biology approaches to mixtures research, Dr. Rider said it begins with the problem formulation phase, when it is decided what belongs in a cumulative health assessment. Considering biological similarity, there has been a shifting paradigm going beyond the standard practice of only performing cumulative risk assessments on chemicals that share a molecular initiating event. The science has moved forward rapidly, particularly since the 2008 National Research Council report on phthalates, which recommended including not only phthalates but also other chemicals that disrupt androgen signaling. Thus, the new paradigm includes mixtures research from the disease-first perspective, identifying exposures that could potentially contribute to disease development. Adverse outcome pathways are used as a framework for predicting chemicals that may converge at the pathway or tissue level to cumulatively contribute to disease development. For example, the Cancer Network and enVironmental Exposure Research Agenda (CNVRGE) project begins with the Hallmarks of Cancer pathways and identifies environmental exposures that target each

pathway in order to develop and test hypotheses about mixtures of chemicals that are not complete carcinogens but could contribute to cancer development.

In conclusion, Dr. Rider said, “We are tackling big mixtures questions, we are using the latest toxicology tools, and our efforts are concentrated in areas that will provide data to inform the [development of the three mixtures research approaches].”

### Oral Public Comment

Dr. Olga Naidenko spoke on behalf of the Environmental Working Group (EWG), a Washington, D.C.-based research and advocacy organization. She noted that the EWG had nominated experimental evaluation of the hypothesis proposed by the Halifax Project to the NTP, and expressed her group’s continued support for NTP’s mixtures research. She observed that NTP’s research work is tremendously important to both the general public and regulatory agencies. She said that in terms of carcinogenicity risk assessment on the state and federal levels, NTP research helps groups like hers to see how the public can be protected.

### BSC Discussion

Dr. Barlow, first BSC discussant, said that NTP mixtures research has made great progress, but many great challenges remain. Variability in the mixtures is one major challenge, inherently in the three approaches Dr. Rider outlined, and in exploring real-world exposures. With the many different stressors people have, the variability is compounded. Looking at the projects NTP has going on in this area, it is “getting a handle on understanding the challenges and being able to deal with them,” he said. He wondered how NTP would continue in mixtures research in all three approaches given limited resources. He thought that since other regulatory agencies are working on mixtures, perhaps there is a path in allowing them to cover certain areas while concentrating on others. He felt that NTP is addressing and helping define the highest-priority questions. He said that the systems biology approach is interesting, but difficult. The whole mixtures approach seemed to be where the best progress has been made and where the focus should be going forward, he noted. He said that in that approach, it is important to take other factors into account, such as pharmaceutical exposures or tobacco use. He approved of the projects that have been selected thus far, and recommended continuing work in the botanical space. On the whole, since the 2011 workshop, “great progress has been made,” he said. “The NTP is rising to this challenging area, and needs to continue to push in there.”

Dr. Sass, second BSC discussant, said that the NTP continues to go down the important paths in this challenging area. She expressed concern that there is too much focus on mechanism of action. She said she was impressed that NTP had pursued the

challenge laid out in the 2008 NRC report on phthalates. She noted that it is really important for NTP to keep exposure in mind, as that is often the biggest question at hand. She felt that biomonitoring data is relevant and should be used as much as possible, such as data on house dust exposure and drinking water. She reiterated the importance of not spending too much time focused on mechanism of action, despite the concentration of regulatory agencies in that area. She recommended focusing on the kinds of mixtures that people are exposed to and that consumers think about.

Dr. Rider thanked the BSC discussants for their support and comments.

Dr. Brandt-Rauf complimented Dr. Rider on her presentation on mixtures research. He asked how mixtures could be regulated, controlled to actually improve health. He asked which approach would be most effective to get to that point. He noted that mixtures have emergent, unexpected properties, which could change the quality of the exposures and the quality of the impacts.

Dr. Ramos said he was also impressed with the presentation, and that on the whole, the mixtures program is well-focused and asking the right questions. He described PAHs mixture work that he had done 20 years ago, which concentrated on the additive model, and actually discovered strong interactions that actually protected from toxicity. He recommended that in future presentations Dr. Rider spend more time looking at the complexity of the chemical and biological interactions to try to begin to make better predictive assessments. He also suggested that an external scientific advisory board be established to bring outside perspective to the program, to help avoid rigidity of thinking. He mentioned that Superfund colleagues would be a good resource, as they also deal with mixtures. He felt that the best bet for gaining meaningful insight into outcomes from complex mixtures would emerge from the systems biology approach, because it is the most unsupervised of the approaches. He said more effort should be made to develop the systems approach.

Dr. Markowitz noted that mixtures such as ginkgo tend to be homogenous and source-based, and asked whether NTP had considered using biomonitoring results in such cases, as they are human-based mixtures from various sources. Dr. Rider replied that it had been a recommendation from the 2011 meeting, and had been much discussed since.

Dr. Afshari found the presentation enlightening. She echoed Dr. Ramos's endorsement of the systems biology approach.

Dr. Gamboa mentioned that the selection of cell lines used in *in vitro* work is very important. Dr. Rider said that is an NTP priority.

Dr. Kass said that in preparation for the meeting, he had read the 2012 European Commission assessment of the state of mixtures research and where it should be going. He asked Dr. Rider to comment on whether the NTP's approach converges or differs with that report's conclusions. Dr. Rider replied that the thinking has converged. "I definitely think we are on the same page," she noted.

Dr. Sass explained that her concern about mechanism is a regulatory concern, not so much a science concern. Dr. Nigel Walker replied that the research is both targeted and untargeted, with the strategy having evolved from being purely targeted. He said the key is the problem formulation stage, where the key decisions are made about what approach to use. Dr. Rider added that there is agreement that the risk assessment framework is too rigid, and the problem becomes where the line is drawn.

Dr. McMartin summarized his sense of the board's sentiments. He said that the BSC felt that NTP is addressing the key questions in the mixtures area, and that great progress has been made. He noted mixed reactions to the approaches, with some members urging continuation of all three, but others feeling that one approach should be favored in this time of limited budgetary resources, with some recommending emphasis on the systems biology approach, and others favoring the whole mixture approach. He reiterated the board's recommendation of use of biomonitoring exposure data to help prioritize the research. Also, the board felt that pharmacokinetics should be more actively considered, which can be "a hidden minefield." The board suggested an external advisory group for the program.

## B. Where the Rubber Meets the Road: Update on the NTP Crumb Rubber Research Program

Dr. Georgia Roberts updated the BSC on the NTP crumb rubber research program.

She provided background information about the public health concerns for playing sports on synthetic turf fields, which now number more than 12,000 in the U.S. In 2015, the California Office of Environmental Health Hazard Assessment nominated synthetic turf/crumb rubber to the NTP for testing. NTP presented a preliminary approach to the BSC in June, 2016. Dr. Roberts outlined progress in the research program since then. Chemical characterization, *in vitro* characterization, and *in vivo* feasibility studies are currently ongoing. The focus of the research is to determine what conditions in an experimental setting have the potential to result in systemic exposure to crumb rubber constituents. Possible routes of exposure include dermal uptake, ingestion, and inhalation.

Dr. Roberts described the chemical characterization studies, which are nearly complete, in more detail. Volatile and semivolatile organic compounds were found to comprise

approximately 0.0007%, while inorganics comprised approximately 8-9%, with metals accounting for 3%. The *in vitro* assays, also nearly complete, exposed human cell lines to conditioned media for 24 or 72 hours. Cytotoxic effects were observed in keratinocytes and alveolar epithelial cells when exposed to conditioned media. The *in vivo* work is still ongoing. Based on feasibility work performed, oral gavage, dosed feed and mixed bedding 14-day studies were conducted in female mice. The focus of these studies is to understand the potential for systemic exposure based on chemical analysis of biological samples (e.g. blood or urine) or evidence of biological effects.

Data release of the completed crumb rubber research is anticipated in late 2017 or early 2018.

### BSC Clarifying Questions

Dr. Afshari asked Dr. Roberts to reconcile the *in vitro* data, which showed considerable cytotoxicity, with the chemical characterization. Dr. Roberts replied that her group is working to explain the discrepancy before moving forward. She said it is possible that proteins in the cell culture media might play a role.

Dr. Davis asked about absorption in the materials, whether they were actually picking up other elements as they are incubated. Dr. Roberts said her group has not yet looked at that question.

Dr. Gamboa commented that the GC/MS method of detection may not have detected non-volatile elements that could have played a role in the cytotoxicity. Dr. Roberts said that LC/MS analysis was also done, but few additional peaks were seen.

### Oral Public Comment

Dr. Stephanie Fox-Rawlings of the National Center for Health Research commented by phone. She said her group is “concerned that there is insufficient high-quality research data to determine the exact risk of recycled crumb rubber for playgrounds and athletic fields.” She noted that scientific evidence suggests that crumb rubber may not be safe when used on playground and playing field surfaces, and described some of the suspected adverse health effects. She called the research on the health risks of recycled tire rubber “insufficient and inadequate,” and encouraged the NTP and its stakeholders to uphold the scientific integrity of its studies so that they address the gaps in the current literature. She urged the NTP to keep several research design issues in mind as it moves forward, and stressed that the NTP should be transparent about evidence indicating safety concerns.

### BSC Discussion

Dr. Genter, first discussant, noted that much work is underway on crumb rubber, and that some of the ideas the BSC had discussed in the June 2016 meeting had been implemented. She said she was encouraged by the methods used in the chemical extraction procedures, and was encouraged by the results showing no materials being released with water as the solvent. She felt that the NTP is appropriately focusing on the exposure question. Since there could potentially be lifetime exposures starting at very young ages, if NTP confirms that there is in fact exposure by some route, it would be appropriate to examine early life exposures, she noted. She felt that the research group is on target for its original time frame, and is addressing the critical questions. She approved of the outreach work with stakeholders that has taken place, including the website.

Dr. Ramos, second discussant, said he was encouraged by the progress in the project to date, but was disappointed that there is still much to do to achieve the late 2017 deadline, which may be overly ambitious. Regarding the work that has already been done, he assumed that the extraction work was designed to replicate the human experience. Thus, seeing GC/MS data at 100°C did not seem relevant to the human experience. He recommended that time be spent deliberating about how to replicate the conditions to make them more realistic, so that the data generated will be more relevant. He discussed the importance of three specific variables to the outcome of the project: age, sex, and genetics. He found it “refreshing to see something go from concept to data,” and said he looked forward to the rest of the story when it becomes available.

Dr. Roberts explained the temperature choices used in the experiments. The 100°C used in the chemical extraction was designed to be a worst case scenario. The 60°C temperature used in the cell culture work was relevant to conditions in the field. She noted that the first phase of testing concentrated on issues of exposure.

Dr. Ramos pointed out that the *in vitro* data raises many interesting questions. Dr. Roberts noted that there are discrepancies compared to what was seen in the chemical characterization, and there would be work to follow up on the results.

Dr. Davis asked whether her impression is correct that the purpose in the extraction process was to extract everything that could potentially be leached from the materials, not that there was an effort to simulate a real-world situation where leaching would occur. Dr. Roberts said that is correct, and that the bioavailability studies would address more real-world scenarios. Dr. Davis asked whether the crumb rubber is manufactured by a universal method, or if that process is variable. Dr. Roberts replied that that question is being addressed by federal partners.



Dr. Barlow noted that “this is a great use for old tires, which are an environmental concern.” Thus, the conclusions on crumb rubber will be important. He said that all tires are different, which could affect the mixtures to be dealt with. He wondered if there are any data regarding workplace exposures at tire manufacturing facilities, which could be a good source of information. He also wondered if there are any studies ongoing obtaining samples from children who use the crumb rubber fields, and thus whether exposure information would be available. Dr. Roberts replied that the variability of tires will be considered. She thought the manufacturing exposures are being discussed with NIOSH. She mentioned that federal partners are moving toward conducting biosampling studies, but the challenge is knowing what to look for.

Dr. Whalen said that there are worker exposures in tire recycling plants, but the question is how relevant that would be to the final product found on the fields.

Dr. Nigel Walker noted that the NTP program is part of a larger program of work, involving other agencies. The intent is not to try to answer all of the many questions about crumb rubber. He pointed out that Dr. Roberts’ presentation fits with the meeting’s theme regarding mixtures.

Dr. McMartin summarized the board’s sentiments. He said the BSC was impressed with the program’s progress since the original presentation in 2016. He pointed out that the program was designed by the NTP primarily to determine whether there were significant exposures, using the chemical extraction and biological assessment methods. The board noticed the dichotomy between the chemical extraction data and the initial biological *in vitro* studies, an area that should be examined in more detail for an explanation. He noted that the project is quite important because some of the epidemiology studies await results of the NTP’s work.

### C. Screening for Biological Activities of Concern in Consumer Products

Dr. Scott Masten briefed the BSC on a proposed new project nominated to NTP by the Connecticut Department of Health. The program would be designed to use targeted screening assays to evaluate the bioactivity of physiologically relevant extracts of selected consumer products designed for use by young children.

The project is at an early, formative stage. As Dr. Masten noted, “We’ve got a plan to develop a plan, and we’re going to ask your input on it.”

He provided background information about concerns related to consumer products aimed at young children. There is uncertainty about whether existing chemical of concern (CoC) lists reflect the composition of products currently on the market, so the intent of the project would be to evaluate whether there are additional emerging

contaminants in children's products that bear biological activities of potential toxicological concern. The suggested approach involves:

- Testing of a product class by compositing across multiple brands
- Discrete testing within a class to determine the range of activity within that class
- Bioassay-based fractionation and analytic chemistry analysis to identify the chemical(s) producing the bioactivity

Dr. Masten listed the many types of products under consideration, from infant sleepwear to baby bottles, along with house dust, as well as the types of chemicals and CoCs to be targeted in the analysis. He noted that the products can be considered to be whole mixtures. He described the bioactivity-based screening approach that would be used.

He proposed development of a few pilot projects designed to answer critical questions to stimulate further innovation in the field. The program would not conduct a risk assessment, nor would it attempt a comprehensive analysis of CoCs in consumer products.

Dr. Kass asked Dr. Masten about the information he had shown about a past project that used cross-laboratory analysis. Dr. Masten said he had included that information simply to illustrate that such an analysis had been attempted before, and had survived peer review and been published.

### BSC Discussion

Dr. Kass, first discussant, said "I struggle with this." He felt that the fundamental questions being asked is important and valuable – to what extent can we count on the safety of children's consumer products, and are the chemicals of concern biologically active? He agreed that such information is currently lacking, and that today a piecemeal approach is being used, tackling the issue chemical-by-chemical, with an iterative limitation of chemical use in such products. He said that he found it difficult to draw a logical pathway between the broad intent of the nomination and how to achieve it. He noted methodologically troubling and resource-intensive aspects that would limit the generalizability of the findings, such as the dramatic heterogeneity of composition in the products. He said the project may result in incremental toxicological improvement of understanding, but would be unlikely to apply directly to the real world of manufacturing and commerce. "I'm struck by the problem of interpretability with this," he observed. He said that if the NTP believes there is value in creating a plan in this area, it should do so, but it should focus on interpretability and demand for resources. If it does not look good on those fronts, on balance the program would be a distraction rather than a program of value to NTP's mission.

Dr. Markowitz, second discussant, said that when he first saw the nomination, his immediate reaction was to question whether useful public health information could be produced. He felt that it was an impossible request. He said the science is not far enough along to provide information that would actually be useful. He also wondered whether Tox21 is far enough along to provide information that can be used for personal decision making. He observed that perhaps a smaller “bite” of the program could be undertaken, skipping over the exposure stage and seeing whether the constituents of a particular product have already been studied under Tox21. “To me, this is an entire, complicated, and ambitious research program that NTP can’t undertake,” he concluded.

Dr. Afshari, third discussant, felt that the scope of the proposal is undefined. She said that the approach of only looking at biological activity of certain chemicals in the products does an overall disservice in terms of public health applicability. On the other hand, she noted, it is an opportunity to speak about the strength of NTP and where the current state of the science is. If a specific product were to be the focus, the program would not be very different from the crumb rubber initiative. She felt that NTP is the right entity to be asked to undertake the issue, but the state of the science and the ambiguity of the problem limit the readiness to implement and scope out a specific program. She suggested communicating back to the nominating party to point out how NTP could effectively approach the issue moving forward.

Dr. Masten said that the discussants’ comments reflected several of the internal discussions that had taken place.

Dr. Birnbaum asked the BSC to consider whether the proposal is at the stage to go forward, or whether it would benefit from having a workshop. Dr. Davis wondered if there might be an opportunity to scope the project differently, and suggested that a workshop might be a means by which that could occur.

Dr. Brandt-Rauf noted the similarity to the crumb rubber program, and said he could envision someone asking why that program was pursued, but this one was not. He asked what the response would be to such a question. Dr. Birnbaum said that one is a specific class of products, but the other is an almost infinite variety. She described some of the other specific aspects of the crumb rubber program. Dr. Nigel Walker wondered if a future could be envisaged when thousands of products at a time could be analyzed simultaneously, as is now the case with chemicals. He said the nomination under consideration could be a starting point for the long-range process of developing those capabilities. Dr. Brandt-Rauf agreed that a workshop would be a good first step.

Dr. Kass found the concept of a workshop to be interesting. He recommended inclusion of regulators and risk assessors.

Dr. Sass said it was important to begin to tackle the issue, because it represents how people are exposed in the real world. She added that the end goal is not risk assessment, but about hazard and an informed public. Dr. Birnbaum noted that NTP does not conduct risk assessment, but provides information that may be useful to risk assessors.

Dr. McMartin summarized the sense of the BSC. He said it was apparent that the issue is a huge concern for the public, but the board was being quite cautious about recommending that NTP move forward with the project as of now.

## VI. Report of the NTP Associate Director

Dr. Bucher, Associate Director of NTP, informed the BSC about developments at NTP since the last BSC meeting. He welcomed new DNTP staff and trainees, and said goodbye to several DNTP staff members who recently departed the division.

With a new NIEHS strategic plan being formulated, Dr. Bucher described the DNTP response to the 2012-2017 Strategic Plan. He listed NTP programs in terms of individual programs and initiatives that responded to several of the Strategic Goals contained in the plan. For example, for Strategic Goal #1, Fundamental (and applied) research, he described Toxicology and Carcinogenesis Technical Reports, and Tox21. He listed responses to Strategic Goals 2 and 3. For Strategic Goal #4, Combined environmental exposures and disease, he described NTP projects to inform risk assessment of mixtures, including component-based and whole mixture programs, with the 2014 Elk River spill analysis as an example. He also delineated NTP responses to Goals 7, 9, 10, and 11.

Dr. Afshari asked whether the NTP fellows program would be protected given diminishing funds, to help continue a sustainable workforce. Dr. Bucher replied that NTP is doing all it can to keep the level of training constant. He said it had been suggested that NTP partner with some academic institutions to bring in their trainees for short periods, if the current levels of trainee numbers could not be sustained. Dr. Birnbaum added that budget constraints have reduced trainees in DIR from 250 to about 200. She said she sees it as “an extremely high priority.” She praised Dr. Bucher’s presentation of how the NTP has been “living” the Strategic Plan.

Dr. Bucher introduced Beth Bowden from the Program Operations Branch, who briefed the BSC on the redesign of the NTP website. The redesign has taken an incremental approach, with new content going live as it is updated. Ms. Bowden showed several examples of redesigned web pages, including a new home page and a new search tool.

### BSC Discussion

Dr. Kass asked about the issue of orphan documents. Ms. Bowden said that orphan documents are not an issue, but the website is very deep, requiring many clicks to get to a particular document. Part of the reorganization of the website is flattening that user experience.

Dr. Brandt-Rauf asked about NTP's use of social media to drive people to the website. Ms. Bowden replied that NTP currently relies on NIEHS Office of Communications and Public Liaison for social media activity. Dr. Nigel Walker noted that NTP does have its own hashtag.

## VII. Transgenerational Inheritance of Health Effects: A State of the Science Evaluation

Ms. Vickie Walker updated the BSC on the state of the science in research on transgenerational inheritance of health effects.

She defined a transgenerational effect, and noted that it has not been consistently defined in the literature, resulting in a challenging literature base.

She reported on the state-of-the-science review, which was developed using the OHAT approach for conducting literature-based health assessments. The objective was to systematically collect and map transgenerational studies by evidence stream, health effects, and exposures. The review also looked at a subset of studies to assess the risk of bias.

The review identified 49 human and 232 animal studies with a transgenerational study design. Data extraction files are publicly available in the HAWC web-based tool.

Ms. Walker noted that many studies reported transgenerational effects, but datasets are limited for reaching conclusions on consistency of the findings given the heterogeneity of the data. There were few bodies of evidence or groups of studies for which the same exposure and health effects were studied. Evidence mapping showed "pockets" of transgenerational evidence, suggesting potential directions for future research.

The review suggests that rather than pursuing additional studies on a wide range of exposures, the field would benefit from targeted research addressing inconsistencies discovered in the review, such as improving study design and minimizing bias. This would help to establish bodies of evidence needed to critically assess transgenerational effects.

Dr. Brandt-Rauf asked Ms. Walker why foreign language studies were eliminated from consideration, and whether the same two reviewers had reviewed all 63,000 studies

initially looked at. Ms. Walker said it would have been costly to have the foreign language studies translated. She added that there were 8 reviewers for the initial screening. Two reviewers took part in the full text review of 1,125 studies. She noted that there was a piloting phase at the outset of the process, where everyone evaluated the same 200 studies as a group.

### Oral Public Comment

Dr. Naidenko from the EWG spoke on the systematic review. She said her group was excited about the combination of themes explored in the BSC meeting. She spoke positively about the developmental and reproductive toxicology that the NTP is developing. She said that the “golden outcome” for her group was the effort to reduce false negatives. She asked that NTP make the nomination information on transgenerational health effects available.

### BSC Discussion

Dr. Brandt-Rauf, first discussant, said that transgenerational health effects is a very important topic. He felt that Ms. Walker had been rigorous and transparent about the review process. The process was broad, thorough, comprehensive, and well-documented, he observed, as well as being appropriately critical of the evidence. He said that the review had yielded a trackable product. He hoped that the process would achieve a higher level of public awareness about the issue, and is confident that the results would engender a high degree of trust and credibility. He reiterated the importance of social media in adding to public awareness.

Dr. Stump, second discussant, commended the very rigorous assessment of the literature. He said he was not surprised that the vast majority of the studies screened did not comply. He felt that the process followed had made good sense. He said he has often been skeptical of transgenerational claims, and commended the review process to ensure that the assessments had been conducted properly. He approved of the approach to assessing statistical significance, which he noted was often faulty in such studies. Route of administration and dose are also important, he averred. He agreed that the review had yielded a trackable product. He felt that it was a good first step, and that now the information would be helpful to researchers interested in conducting these types of studies. He asked Ms. Walker if future searches would be easier.

She said that a future search strategy would probably focus on a particular chemical or a particular outcome. She added that it would be very helpful if the keywords in the area were indexed on PubMed. Dr. Bucher added that NTP is working on automated tools using artificial learning activities to aid searches. Dr. Stump said that would be

helpful to all in the field who sometimes struggle with literature searches. Ms. Walker said the tools were used in this review for priority ranking, and worked well.

Dr. Kass asked whether the underlying data would be available. Ms. Walker said they would be available in HAWC, along with Excel sheets to allow filtering. Dr. Kass said he was concerned about publication bias, and noted it would be interesting to look for studies with negative results.

Dr. Ramos asked for more information on quality assessment of the studies used in the review, and how it enters into filtering. Ms. Walker said that risk of bias had not been conducted on all of the studies, and that key factors were considered in the write-ups of the individual papers. Dr. Ramos asked about the process used to define what quality assessment criteria would be used. He asked whether, for example, dose would be considered. Ms. Walker replied that this evaluation did not go quite that far; that dose was considered but was difficult to assess across individual studies. Dr. Ramos felt that it would become critical in the final reporting. Dr. Ramos asked if the review had included a review of the 281 papers, selected for a particular agent, and then using that data, conducting the more comprehensive database expansion exercise – and if so, what had been found? Ms. Walker clarified that no conclusions had been reached in the review document since the goal had been to identify the areas of evidence.

Dr. Davis asked about whether natural language processing had been used. Ms. Walker said the methods were in development when the project was underway, but that they are now being actively used.

Dr. Brandt-Rauf asked why the Dutch famine studies were not included. Ms. Walker said the effects had not been reported in a sufficient generation to be included.

Dr. Birnbaum noted that there are many multi-generational studies, but few truly transgenerational. She said that NIEHS and NTP have funded a considerable number of studies in the area. She described existing cohorts that continue to be followed, including the availability of biospecimens in some cases. Overall, she observed, “what’s really important and what the analysis has shown is that there has been lots of talk about transgenerational exposure, and the data is just not that strong if you look at it carefully.”

## VIII. A Roadmap for the Implementation of New Approaches to Safety Testing

NICEATM Director Dr. Warren Casey updated the BSC on progress in implementing new approaches in safety testing.

He provided background information and a history of the effort, noting that despite technological advances, toxicity safety testing and regulatory practices remain close to where they were several years ago. So, he said, a new roadmap is required to focus the efforts to move beyond animal testing. The roadmap will:

- Help federal agencies identify consensus goals and coordinate key activities required to achieve them.
- Provide a framework to support the planning and coordination of technology development.
- Facilitate communication and collaboration within and between government agencies, stakeholders, and international partners.

Dr. Casey related details of a February, 2017 face-to-face interagency meeting to start the process of establishing mission, vision, goals, and objectives for the roadmap. Participants attended from 85 agencies and stakeholders.

A draft of the new roadmap is to be published in August, 2017. SACATM holds its annual meeting in September. The final draft is scheduled to be published in December.

Dr. Kass asked Dr. Casey for an example of an early success. Dr. Casey cited the example of acute toxicity testing, with EPA slated to do away with the acute “six-pack” test by 2020.

#### Oral Public Comment

Megan Amos spoke on behalf of the Physician’s Committee for Responsible Medicine (PCRM). She said that NICEATM has done an excellent job coordinating the ICCVAM federal agencies to obtain consensus on a vision, mission, and objectives to promote implementation of the new approaches to toxicity testing. “We support the vision, mission, and objectives, and look forward to opportunities to assist ICCVAM in implementation,” she said. She spoke positively about the roadmap themes of enhanced communication and improved training. She expressed PCRM’s appreciation of the BSC’s work advising NTP in its efforts to protect human and environmental health.

#### BSC Discussion

Dr. Afshari, first discussant, commented on the inclusion of “chemicals and medical products” in the Roadmap vision statement. She felt that it potentially would exclude several areas, such as foods and tobacco products. She noted that the process is not just about the three R’s, but also allows an opportunity “to do things better.” She said that the new technologies can not only work in normal, healthy cells, but the cells



themselves can also be manipulated to mimic disease states. She felt that that was a huge opportunity, and should be worked into the vision statement. She noted that there would need to be a process for prioritizing according to the needs of different stakeholders. She recommended examination of the gaps in the current science, with the potential path forward being offered by some of the alternative methods. She suggested taking the opportunity to talk to others who have worked in similar partnerships around validation. She suggested a case study workshop with the regulated and the regulators together.

Dr. Davis, second discussant, complimented Dr. Casey on his engagement with the many stakeholders and others in creating the roadmap. She asked whether he felt that each of the objectives should have equal weight or should be prioritized, understanding that he was working to be comprehensive in the initial document. She felt that many of the objectives had dependencies outside the scope of ICCVAM, and that the implementation section should take those outside factors into account, allowing prioritization. She noted that the document had moved away from referring to ICCVAM delivering approaches, which is not ICCVAM's role. She agreed that communication is vitally important, since it is important for stakeholders to understand the advances in the field. She felt that the document presented by Dr. Casey is a great start and would highlight ICCVAM's leadership role in advancing the effort.

Dr. Casey pointed out that the words in the document had been laboriously arrived at. He said FDA had specifically asked for inclusion of the phrase "medical products." He emphasized that the document is still a draft. He agreed that there had been amazing progress, but one of the most commonly used animal safety tests dated from 1928. He said there is "all this amazing technology, but it's not changing some aspects of how we do toxicology."

Dr. McMartin summarized the sense of the BSC. He felt that the board found the revised roadmap to be a good move forward, with good progress through enlisting all of the stakeholders. There were some specific suggestions made for the roadmap, and more generally, the board felt that communication was the key.

## IX. Adjournment

Dr. Afshari commented that she felt the format of the meeting's agenda had worked quite well.

Dr. Bucher felt that the meeting had been successful in delving deeply into the area of mixtures. He appreciated the board's response to the wealth of material that had been included, and thanked them for their comments. He thanked the members for their hard work, which makes the NTP a better program.

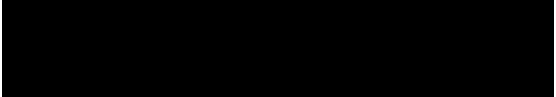
Concluding the meeting, Dr. Birnbaum asked for additional comments about the meeting. She called for a round of applause for Dr. Bucher, assuming that his replacement would be on board by the next BSC meeting in December. The board responded enthusiastically.

Dr. McMartin thanked everyone who had provided comments during the meeting.

Dr. Bucher thanked Dr. McMartin for chairing the meeting, the staff for their presentations, and Dr. Birnbaum for “the privilege of serving as the Associate Director.”

The meeting was adjourned at 4:30 pm, June 29, 2017.

Summary Minutes June 29, 2017  
NTP Board of Scientific Counselors



Dr. Kenneth McMartin

Chair, NTP Board of Scientific Counselors

Date: 9-18-17