# Summary Minutes

# NTP Board of Scientific Counselors

# June 20, 2018

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# I. Frequently Used Abbreviations and Acronyms

| | |
|---|---|
| AAAI | American Academy of Allergy, Asthma and Immunology |
| ADME/TK | absorption, distribution, metabolism, and excretion/toxicokinetics |
| AOP | adverse outcome pathway |
| BD2K | Big Data 2 Knowledge |
| BMD | benchmark dose |
| BPA | bisphenol A |
| BPAF | bisphenol AF |
| BPS | bisphenol S |
| BSC | Board of Scientific Counselors |
| CDC | Centers for Disease Control and Prevention |
| CDER | Center for Drug Evaluation and Research (FDA) |
| CEBS | Chemical Effects in Biological Systems |
| CERHR | Center for the Evaluations of Risks to Human Reproduction |
| CLARITY-BPA | Consortium Linking Academic and Regulatory Insights on BPA Toxicity |
| CPSC | Consumer Product Safety Commission |
| CTB | Computer Technology Branch |
| DARPA | Defense Advanced Research Projects Agency |
| DERT | Division of Extramural Research and Training |
| DIR | Division of Intramural Research |
| DNT | developmental neurotoxicity |
| DNTP | Division of the National Toxicology Program |
| DPRA | direct peptide reactivity assay |
| ECHA | European Chemical Agency |
| EDSP | Endocrine Disruptor Screening Program |
| EFSA | European Food Safety Agency |
| EPA | U.S. Environmental Protection Agency |
| EPA IRIS | EPA Integrated Risk Information System |
| ER | estrogen receptor |
| FACT | Federal Accountability in Chemical Testing Act |
| FAIR | findable, accessible, interoperable, reusable |
| FDA | U.S. Food and Drug Administration |
| GLP | Good Laboratory Practice |
| GO | gene ontology |
| h-CLAT | human cell line activation test |
| HTT | high throughput transcriptomics |
| IARC | International Agency for Research on Cancer |
| ICs | NIH Institutes and Centers |
| ICATM | International Cooperation on Test Methods |
| ICCVAM | Interagency Coordinating Committee on the Validation of Alternative Methods |
| ICE | Integrated Chemical Environment |
| IgE | immunoglobulin E |
| ILS | Integrated Laboratory Systems, Inc. |

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|---|---|
| IPS | induced pluripotent stem cells |
| IVIVE | *in vitro* to *in vivo* extrapolation |
| LC/MS | liquid chromatography/mass spectrometry |
| LD50 | lethal dose, 50% |
| LOAEL | lowest observed adverse effect level |
| LoC | level of concern |
| MPS | microphysiological systems |
| NASA | National Aeronautics & Space Administration |
| NCATS | National Center for Advancing Translational Sciences |
| NCTR | National Center for Toxicological Research |
| NICEATM | NTP Interagency Committee for the Evaluation of Alternative Toxicological Methods |
| NIH | National Institutes of Health |
| NIOSH | National Institute of Occupational Safety and Health |
| NTP | National Toxicology Program |
| OCPL | Office of Communication and Public Liaison |
| OECD | Organisation for Economic Co-operation and Development |
| OESC | Office of Environmental Science Cyberinfrastructure |
| OHAT | Office of Health Assessment and Translation |
| OLRP | Office of Liaison, Policy, and Review |
| ORoC | Office of the Report on Carcinogens |
| OSC | Office of Scientific Computing |
| PCRM | Physicians Committee for Responsible Medicine |
| PFAS | per- and perfluorinated alkyl substances |
| PFCs | perfluorinated compounds |
| PFOA | perfluorooctanoic acid |
| PFOS | perfluorooctane sulfonate |
| PoD | point of departure |
| QSARs | quantitative structure-activity relationship models |
| RAPIDD | Rapid Acquisition of Pre/Post Incident Disaster Data protocol |
| REACH | Registration, Evaluation, Authorisation and Restriction of Chemicals |
| REACT | Rapid Evaluation and Assessment of Chemical Toxicity |
| RFAs | Requests for Applications |
| RFR | radiofrequency radiation |
| RoC | Report on Carcinogens |
| SACATM | Scientific Advisory Committee on Alternative Toxicological Methods |
| SBIR/STTR | Small Business Innovation Research/Small Business Technology Transfer |
| SOT | Society of Toxicology |
| SSS | Social and Scientific Systems, Inc. |
| TSCA | Toxic Substances Control Act |

# II. Attendees

**Members in Attendance:**

Cynthia Afshari, Amgen
Norman Barlow, Seattle Genetics
David Berube, North Carolina State University (*ad hoc*)
Weihsueh Chiu, Texas A&M University (*ad hoc*) (by phone)
Myrtle Davis, Bristol-Myers Squibb
Susan Felter, Procter & Gamble (*ad hoc*)
Kenneth McMartin, Louisiana State University Health Sciences Center (chair)
David Michaels, George Washington University (*ad hoc*)
Kenneth Ramos, Arizona Health Sciences Center
Anne Ryan, Pfizer (*ad hoc*)
Jennifer Sass, Natural Resources Defense Council
James Stevens, Paradox Found Consulting Services, LLC
Donald Stump, WIL Research
Susan Tilton, Oregon State University (*ad hoc*)
Katrina Waters, Pacific Northwest National Laboratory

**Other Federal Agency Staff:**
Goncalo Gamboa, FDA, BSC liaison
Kent Thomas, USEPA
Elizabeth Whelan, NIOSH, BSC liaison

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

Brian Berridge
Linda Birnbaum
Chad Blystone
John Bucher
Vesna Chappell
Sheba Churchill
David Crizer
Helen Cunny
Michael DeVito
Sue Fenton
Dori Germolec
Virginia Guidry
William Gwinn
Alison Harrill
Michelle Hooth
Gloria Jahnke
Nicole Kleinstreuer
Kelly Lenox
Ruth Lunn
Elizabeth Maull
Barry McIntyre
Suril Mehta
Alex Merrick
Esra Mutlu
Scott Redman
Julie Rice
Georgia Roberts
Veronica Robinson
Andrew Rooney
Kristen Ryan
Keith Shockley
Stephanie Smith-Roe
Matt Stout
Vicki Sutherland
Kyla Taylor
Molly Vallant
Suramya Waidyanatha
Nigel Walker
Vickie Walker
Amy Wang
Kristine Witt
Mary Wolfe
Rick Woychik
Michael Wyde

**Contract Staff:**
Dawn Fallacara, Battelle
Jenny Gorospe, Battelle
Steve McCaw, Image Associates

Jamie Richey, Battelle
Kelly Shipkowski, ICF
Barney Sparrow, Battelle
Anna Stamatogiannakis, ICF
Amy Zmarowski, Battelle

**Public:**
James Blake, RTI International
Mike Easterling, SSS
Reshan Fernando, RTI International
Ernie Hood, Bridport Services (rapporteur)
June Mader, GOFORWARD LLC
Joseph Manuppello, PCRM (by phone)
Olga Naidenko, Environmental Working Group (by phone)
Annie Sasco (by phone)
Marjo Smith, SSS

## III. Introductions and Welcome

The National Toxicology Program (NTP) Board of Scientific Counselors (BSC) convened June 20, 2018, 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. He noted that BSC members Mr. Daniel Kass and Dr. Paul Brandt-Rauf were unable to attend. Dr. Mary Wolfe, BSC Designated Federal Official, read the conflict of interest policy statement.

## IV. Report of the NIEHS/NTP Director

Dr. Linda Birnbaum, Director of NIEHS and NTP, briefed the BSC on developments at NTP and NIEHS since the December 2017 board meeting.

She began with a report regarding the federal budget and appropriations. She said that it was a good year, although the funding was not received until May 9. NIEHS saw an approximately $26M uptick in FY2017 over FY2016. In FY2018, the uptick was approximately $36M. NIH saw a $3B increase overall in FY2018. Much of the overall budget was earmarked for specific programs, which Dr. Birnbaum described in more detail. Information regarding appropriations for FY2019 is expected in the near future.

Dr. Birnbaum reported on a Congressional Briefing on Neurological Diseases Across the Lifespan, which took place March 8, where she was one of the three speakers. She

updated the board on the current leadership personnel at HHS, as well as the Reimagine HHS and Optimize NIH programs.

Dr. Birnbaum described progress in NIEHS/NTP disaster response research endeavors. The DR2 program started in 2013, and now has a new website that serves as a repository for surveys, questionnaires, protocols, and other validated data collection instruments and materials. There is also a new protocol called RAPIDD: Rapid Acquisition of Pre/Post Incident Disaster Data, which is a menu of standardized instruments that have been pre-reviewed by the NIEHS IRB for use. The protocol is designed to reduce the time to initiate data collection in a disaster situation. NIH researchers were involved within two weeks of Hurricane Harvey, and responded quickly to Hurricane Maria as well. The protocols are also intended to aid response to other time-sensitive EHS research opportunities such as wildfires and PFAS contamination.

Turning to scientific advances and recent publications, Dr. Birnbaum described DNTP papers on cell phone radiofrequency radiation (RFR) studies and the Tox21 S1500-plus gene sets.

She highlighted the new public NIEHS website, recent developments at *Environmental Health Perspectives*, and shared the news that in March she had received an award from the American Academy of Allergy, Asthma and Immunology (AAAAI).

## V. Report of the NTP Associate Director

Dr. Berridge delivered his first NTP Associate Director's report, calling it "a great honor."

He discussed several recent NTP staff changes, including new roles for Dr. John Bucher and Dr. Nigel Walker, new hires, and departures. He related several awards and recognitions of NTP personnel. He described recent efforts to reassess Tox21, including a new strategic plan for the next five years.

Dr. Berridge mentioned several recent events with NTP participation, including the 2018 SOT annual meeting, which traditionally has heavy involvement from NIEHS and NTP. He noted the new Strategic Roadmap for ICCVAM and NICEATM, which was published in January 2018.

He described several recent peer reviews of NTP reports, including antimony trioxide, cell phone radiofrequency radiation (RFR), CLARITY-BPA, and cancer chemotherapy agents.

Dr. Sass asked if it would be possible to conduct similar Tox21 toolkit training as took place at SOT for a public audience, perhaps via a webinar. Dr. Berridge said it was a

great suggestion. He cited the incredible number of resources developed by NTP, which empower the entire community.

Dr. Davis asked Dr. Berridge if there is an effort to engage with other institutes in areas where there are thematic overlaps in order to leverage partnerships to prioritize resources and strategies. Dr. Berridge said that leveraging partnerships would be a fundamental element of his strategic realignment. He cited a recent workshop on atherosclerosis co-sponsored with NHLBI and an upcoming workshop on chronic kidney disease co-sponsored with NIDDK as good examples of such partnership efforts.

## VI. Strategic Realignment: Translational Toxicology

Dr. Berridge continued with a presentation devoted to rolling out a new vision for translational toxicology; a strategic realignment of NTP's pursuits.

He began by describing what had attracted him to join the NTP, noting that one of the draws for him was the ability to tackle fundamental scientific challenges. He said that he absolutely agrees with NTP's mission and the program being a public health organization that uses scientific tools and expertise to pursue contemporary challenges in a space that affects everyone. He noted the trend in toxicology of evolving from an observational science to one that is more predictive and the importance of the many NTP partnerships, both within and outside of the program itself.

Dr. Berridge discussed the extensive internal review of the DNTP 2018 portfolio – the breadth and volume of the overall effort, with more than 500 projects in numerous different areas, as well as the extent of the organization's capabilities and expertise. He listed what he perceived to be NTP's core strengths, along with the significant challenges it faces.

He described what he sees as translational toxicology at NTP, which impacts policy, public health, and regulation. He aims for NTP to inform the present and innovate the future, through the use of innovative tools and strategies that are translatable, predictive, and timely. Among those tools are literature analysis, animal studies, *in vitro* systems, and *in silico*/computational analytics. He depicted a potential estrogen receptor (ER) pathway to breast cancer to illustrate the steps involved in translational toxicology. He showed the NTP Translational Toxicology Pipeline, with several steps along the way enabled by informed progression, and shared a graphic representation of the pipeline, in which each step leads to fit for purpose products designed to inform public health decisions. The solutions will include:

- Innovate at pace and for cause
- Leverage partnerships
- Leverage technology

- A portfolio that reflects contemporary needs and concerns
- Dynamic portfolio management
- Disciplined prioritization
- Judicious animal use
- Communication as a first intent
- Public engagement and education

Dr. Berridge noted that the four presentations following his would all serve as good examples of programs in the portfolio illustrating his ideas for translational toxicology.

Dr. Felter said she had noticed a recurring theme in Dr. Berridge's presentation about the importance of having contextual information when making problem-solving decisions. She asked what role he sees exposure science playing in the research programs that NTP takes on. Dr. Berridge said that the exposome has become a fundamental strategic area of focus, used to make relative risk decisions. Dr. Felter suggested integrating that type of information early in the process, before the design of experiments. Dr. Berridge felt that one basic tenet of toxicology, taking a study as far as it can go, should be rethought in order to become more contextual and relevant. He acknowledged that that would be a huge cultural shift in the field.

Dr. Ramos noted that in the diagrams Dr. Berridge had shown, he was making a case for fit for purpose toxicology. Dr. Ramos felt that the business idea of fit for purpose is applicable to scientific research, just with a different deliverable. He asked Dr. Berridge to describe his impression of what fit for purpose is for NTP. Dr. Berridge replied that it recognizes the dual mission of NTP, to inform the present and innovate the future. It has two phases; first trying to answer the problem articulated by the stakeholder. He described the need for more specific questions from stakeholders, which would help define the fit for purpose product. He added that the larger question is whether answering the problem advances the knowledge base by making the information more predictive and using mechanistic information to understand outcomes. Dr. Ramos said that he was struggling with the concept. A very specific deliverable to a stakeholder moves away from the domain of discovery, prediction, and advancement of the field, and becomes more of a concrete question with a single answer. He noted that this creates competing priorities that may end up diluting both missions and lead to a lack of focus.

Dr. Ramos's second question for clarification involved stakeholder communication and the renewed, intentional interest in ensuring that those communication lines are better defined or optimized. He noted that there had been no specifics and asked for more information on how it would be done. Dr. Berridge replied that the ideas he had

presented were still in draft form, and that the concepts were up for refinement based in part on feedback from the board.

Dr. Chiu said that Dr. Ramos's comments reflected the difference in the missions of NIEHS and NTP. Dr. Birnbaum noted that DIR is devoted to basic biomedical research, while DNTP and NTP as a whole are problem-solving programs. Dr. Berridge said the distinction between the NTP and NIEHS intramural programs is one of the issues he had to contemplate as he joined the organization. NTP is much more oriented toward the applied end of environmental science, and, he noted, that while there is a bit of bridging, NTP would never fit into a purely mechanistic, biomedical research setting.

Dr. Sass mentioned that there are issues with public and regulatory acceptance of alternative tests. She said that one of the barriers is that much of the data are being generated by outside contractors that hold portions of the data as proprietary. She added that it would be important to keep prenatal and early life susceptible windows of exposure in mind when focusing on acute or short-term outcomes. Dr. Berridge said that Dr. Sass's last point was a good one, and that it points to the complementary viewpoints active within NTP. Regarding transparency around assay systems, he said that the right balance must be found between proprietary rights and performance standards in order to establish confidence in alternative methods.

Dr. Stevens commented about the intellectual tug-of-war between basic and applied research, when in fact "one really informs the other." He asked how the NIEHS Strategic Plan would be leveraged within the NTP Strategic Plan. Dr. Birnbaum said that her vision for NIEHS has been "One NIEHS," with informed communication regarding areas that feed into each other. With the new NIEHS Strategic Plan, each division will create an implementation plan and work together to determine opportunities for interaction. She noted that in the last plan, nine cross-NIEHS programs were established, working to ensure that there is communication and interaction. Dr. Berridge said he was still learning about the interactions across divisions, and gaining a sense of programmatic, strategic partnerships. He said there is a natural complementarity between DIR and NTP, since many of the tools are common. Dr. Birnbaum added that external grantees should be counted in that equation as well.

Mr. Joseph Manuppello of the Physicians Committee for Responsible Medicine (PCRM) made an oral public comment via telephone. He said his group was concerned about the diagram for translational toxicology that Dr. Berridge had shown. He felt that it implied that *in vivo* animal tests are ultimately the only way to get answers that inform public health decisions. His group recommends a strategy for translational toxicology with multiple paths, reflecting that some decisions can be based exclusively on *in vitro* or *in silico* data, as well as human data from epidemiologic studies. Decisions about

whether to conduct *in vivo* tests should be made after results from *in vitro* or *in silico* tests are evaluated.

# VII. Examples from NTP's Portfolio

## A. NTP Synthetic Turf/Recycled Tire Crumb Rubber Research

Dr. Georgia Roberts from the DNTP Program Operations Branch summarized NTP research on synthetic turf/recycled tire crumb rubber.

She provided background information about the materials and the reasons for public health concerns, including potential for widespread and long-term exposure. The NTP research focus has been, "What conditions in an experimental setting have the potential to result in systemic exposure to crumb rubber constituents?" The research approach for evaluating crumb rubber has consisted of: 1) chemical characterization, 2) *in vitro* characterization, 3) feasibility to conduct *in vivo* studies, and 4) 14-day *in vivo* studies.

Chemical characterization showed that volatile and semi-volatile organic compounds comprise approximately 0.0007% by weight of the bulk material. *In vitro* studies showed that cytotoxicity was observed with human lung, skin, and small intestine cell lines, but was not observed in human liver cells. *In vivo* studies showed no signs of traditional toxicity and some evidence of systemic exposure. Gavage and bedding studies were successful, but feed studies were not practical due to the animals' avoidance of the test material.

Results from the studies were presented at the 2018 SOT Annual Meeting. Project outputs will be published in NTP Research Reports on each focus area, anticipated by the end of summer 2018.

Dr. McMartin asked for clarification regarding comparisons between the cell-conditioned medium, phosphate-buffered saline, and artificial lung fluid. He asked if the initial extractions were done in the three different fluids, while the actual incubations for the cytotoxicity tests were done in the same medium. Dr. Roberts confirmed that this was correct, and that they used time- and temperature-matched cell-specific medium to perform the dilutions.

Dr. Afshari asked if there was a difference in terms of the protein content of the media used. Dr. Roberts said that when the extraction was taking place, the serum was present, and then diluted with fresh media with serum; however, there were differences in protein content between cell lines based on the requirements for each cell line. Dr. Afshari asked for clarification of the elution methods used, and expressed concern about the chemical components sticking to plastic. Dr. Roberts acknowledged that this could have occurred but was not evaluated specifically. Dr. Afshari wondered if that

might account for the difference seen in cytotoxicity. She asked how similar the analytes were between the *in vitro* and *in vivo* experiments. Dr. Roberts said a side-by-side comparison had not been conducted, as the experiments were done by different labs using different equipment. Also, there was not 100% confidence in any of the tentatively identified compounds. Dr. Afshari said it would be important to articulate that when writing the report, especially in the context of trying to promote *in vitro* systems, as cytotoxicity was seen in the *in vitro* experiments.

Dr. Whelan asked about the acquisition of the bulk material, and whether it varied depending on the source. Dr. Roberts said that she was not sure about the variability between sources; she stated that this was being evaluated by other federal research efforts; The crumb rubber used for these experiments was provided to NTP by the California Office of Environmental Health Hazard Assessment (OEHHA). The material originated from two different manufacturers, which were combined to create the bulk lot.

Dr. Gamboa said the crumb rubber research program is a typical example of new studies dealing with "exotic" materials, and thought it showed the perils of trying to narrow each step of the process. He also found the project to be a good example of the importance of chemistry, and felt that Dr. Berridge's translational toxicology pipeline graphic should be restructured to include chemistry.

Dr. Sass was the first BSC discussant. She felt that the project was a good test case for the NTP strategic realignment, and said it would help tease out the utility and accuracy of non-animal tests moving forward. She said that the discovery portion of the studies was overly complicated for only looking at exposure; it was already known that there's toxicity with some constituents of crumb rubber and they've already been characterized. She noted that she was also struck by the differences in cytotoxicity between the different media, as well as the fact that it was not seen in human liver cells. She said she would have expected the opposite effect, with the serum proteins binding the components, making them less available, and therefore less toxic.

Dr. Roberts addressed the relevance of the *in vitro* results and the impression that they were being dismissed as unhelpful. She said that was not the intention; by altering the extraction environment, the thought was that a difference in outcome might be seen. It is a useful tool, she observed, but how it is approached, and the specific method used, is quite important. She added that it was helpful to hear Dr. Sass's impression that the studies were overly complicated, and felt that it may have been a communication issue.

Dr. Waters agreed that the chemistry aspect of the exposure was important. She asked why the untargeted LC/MS had been conducted at two different labs with different identification approaches, which meant they could not be compared and left the identifications tentative. Dr. Roberts said it was initially determined that a targeted

approach would not be appropriate, but said it would be considered if it could be done in a meaningful way. Dr. Waters noted that there are targeted metabolomics methods that can identify hundreds or even thousands of chemicals. Dr. Suramya Waidyanatha, DNTP Program Operations Branch, said that the data from the two labs can be compared, in particular the untargeted data. Dr. Waters observed that there can in fact be comparison between the tentatively identified compounds in the *in vitro* samples and plasma data. Dr. Roberts noted the caveat that there is not 100% confidence in either set, as all compounds are identified as “tentative".

Dr. Davis asked about the measurement of traditional toxicology endpoints, specifically hypersensitivity reactions and immune-mediated events as a result of the exposures. She said that as the skin is the first point of contact, it would be appropriate to explore those endpoints. Dr. Davis asked Dr. Roberts about the overall narrative and NTP’s recommendations regarding crumb rubber, as from a public perspective, there is conflicting information. Dr. Roberts replied that dermal contact was explored using keratinocytes in the *in vitro* studies, but there are challenges with *in vivo* dermal studies due to crumb rubber being a particle. She added that her personal opinion (not the NTP’s) is that she is not concerned about her relative playing on one of these fields.

Dr. Ramos said it was refreshing to see continuity in the program, with the idea taking shape over time. He asked if there were any human studies related to the research question being asked in *in vivo* and *in vitro* systems. Dr. Roberts said that several human studies are planned, including some human sampling and simulated activity monitoring. Dr. Ramos asked how the data so far can help inform the human studies, and what would come out of the NTP studies that would help with the design of an evaluation of human safety. Dr. Roberts said that the next big step is exposure monitoring, and that the NTP information on tentatively identified chemicals would be helpful at the stage of analyzing the samples. Dr. Ramos recommended that in crafting the research report, NTP should start with the question of how the data will help human exposure studies. He asked if any thought was given to spiking the samples with environmentally-relevant agents, such as pesticides, that might represent secondary exposures in the field. Dr. Roberts agreed that there are co-exposures, but said it was challenging to identify a consistent co-exposure. This is a difference between the NTP samples and the field samples, she observed. Dr. Ramos said that any final conclusions about the exposure need to be put in that context, as the stringency of chemical analysis methods may not be applicable to human exposure and analysis. Thus, the relevance of the exposure would be questionable. Dr. Ramos also asked about the rationale for the selection of the different cell lines used, and stressed that the choices should be made in a judicious fashion. Dr. Roberts said she would take that advice under consideration.

Dr. Stevens recalled that originally the primary public health concern was the cluster of leukemias among soccer goalies. He asked whether the project is a general characterization of crumb rubber toxicity or a set of studies to address the specific public health question. He felt that the exposure issue was "hamstringing most of what you've done." He asked how the public health question could be addressed in a risk assessment fashion, and whether the current data are approaching that direction, as the beginning of a long-term project. Dr. Roberts replied that it was more of a screening-level project that will hopefully help future work. Dr. Stevens said he did not see how that could be done, as the exposure in the NTP studies is not truly known. Dr. Berridge noted that the comments illustrated the complexity of the challenge. He said his impression is that the intent was to help determine feasibility, by getting some sense of the fundamental bioactivity of the material. He felt that doing so would allow the crafting of the more detailed questions. He said that it was designed to provide a foundation to explore those more fundamental questions. Dr. Roberts added that the effort was designed to be timely and provide information in concurrence with the other ongoing efforts. Dr. Stevens noted that "fit for purpose is the right data for the right question at the right time." He felt that the studies looked like "what we can do, rather than what should be done."

Dr. Davis observed that there is really no epidemiological evidence of exposure for any of the particular chemicals, beyond contact with crumb rubber, and perhaps association with leukemia and contact dermal sensitivity. However, there is no evidence of systemic human exposure to these chemicals. Dr. Roberts agreed with Dr. Davis's assessment. Dr. Nigel Walker, DNTP Toxicology Branch, added that it was known from the beginning that recapitulation of the real-world samples would not be feasible. It was a curtailed approach to examine bioactivity and explore some of the chemistry involved, and was by no means a full-blown hazard characterization. Dr. Davis said that what was lost in the translation of the data was that what was shown was whether or not there would be systemic exposure by multiple routes of administration. Dr. Roberts agreed with that summary.

Dr. Stevens said he fully endorsed how the limits of the study had been communicated by Dr. Walker.

Dr. Roberts thanked the board for their helpful comments.

Dr. Gamboa noted that the project was a hazard identification exercise, not risk assessment, which is often the expectation from NTP studies.

Dr. McMartin summarized the board's discussion. He felt the board was impressed with the work that had been done and its continuity. He noted that the board had many suggestions, with importance placed on how the information would be put together and

made relevant to the public. The *in vitro* dichotomy between the various cell culture tests should be explained, and the chemicals involved should be identified in the context of the targeted vs. untargeted approaches. Also, it would be important to determine how the data would be used to inform future human studies.

## B. Studies of Cell Phone Radiofrequency Radiation

### 1) Report on March 26-28, 2018 Peer Review of NTP Technical Reports

Dr. Chad Blystone, DNTP Toxicology Branch, reported to the board on the meeting that convened March 26-28, 2018, to peer review NTP Draft Technical Reports TR-595, which studied the effects of whole-body radiofrequency radiation (RFR) exposures in rats, and TR-596, RFR in mice.

He provided background information about NTP Technical Reports, the levels of evidence of carcinogenic activity against which the findings were evaluated, and the history of the NTP RFR research program. He described the design of the two studies, which involved a series of technical, logistical, and toxicity evaluations starting with the development, construction, and validation of the exposure chambers, followed by initial pilot studies in animals, 28-day toxicity studies, and ultimately the two-year study in both species.

The peer review began with an assessment of the exposure system by a panel of experts on RFR. A second panel comprised of experts in toxicology, pathology, or biostatistics reviewed the toxicity and carcinogenicity studies in the two species.

There was robust discussion by the panels on the exposure system and NTP's draft scientific interpretations. The panel recommended increasing the NTP's level of evidence calls regarding the heart in male and female rats, adrenal gland in male rats (GSM modulation only), and the brain in male rats at both modulations (GSM and CDMA modulations).

The panel's comments on the draft interpretations will be captured in the peer review report, and its recommendations will be carefully considered by NTP when finalizing the technical reports. Publication of the reports on the NTP website is expected in fall 2018.

Dr. Afshari asked Dr. Blystone about the slide he had shown detailing the calls, and why some of them were not highlighted. He explained that in those cases, the panel had agreed with the calls that had been made. The highlighting was to show where there had been a panel recommendation for a different level of evidence conclusion, he said.

Dr. Ramos asked Dr. Blystone to define the level of evidence calls. Dr. Blystone discussed the different levels, as he had shown earlier in his presentation, and described the process NTP uses to arrive at the conclusions. Dr. Ramos asked if there

was a sense of how the line between the calls was drawn. Dr. Blystone elaborated on the process and the various considerations, such as statistics and issues with the controls. Each one of the conclusions carries an explanation of the call, he added.

Dr. McMartin asked if the panel had given NTP a rationale for its recommendations to change some of the calls. Dr. Blystone confirmed that the panel did so, and those rationales are provided in the peer review report.

Dr. Berridge said that there is some subjectivity in where the line between levels is delineated, with reasonable people looking at the same data and coming up with slightly different interpretations. Dr. Blystone said that the conclusions are effectively hazard communication, and are in fact inherently subjective, engendering the need for peer review.

Dr. Barlow asked for more information about the process for generating the final reports. Dr. Blystone said that the final reports would include the updated conclusions. Dr. Wolfe mentioned that the peer review report would include the draft NTP recommendations, along with discussion of the panel's votes and the final recommendations. A short summary of the peer review report will appear in the Technical Reports, along with a link to the full document.

Dr. Annie Sasco, a cancer epidemiologist who formerly served at the INSERM (French NIH) and IARC-WHO, addressed the board with oral public comments via telephone. She summarized the evidence available in 2011 at the time of the IARC monograph meeting on RFR and cancer, and described what had been learned since that time. She said that the subsequent evidence provides argument for a future re-evaluation by IARC. She listed several suggested next steps for RFR-cancer research, with an "absolute need to keep on having independent agencies conducting valid, thorough evaluations."

Dr. Olga Naidenko of the Environmental Working Group (EWG), followed with her oral public comments via telephone. She expressed EWG's support for NIEHS and NTP research on this important topic, as it is providing invaluable information of immediate use by academic researchers or other federal government agencies, as well as the general public. She said that EWG feels that the NTP research likely did not capture where the dose-response curve is likely to be. She said that EWG believes that the lack of a dose-response does not imply that the data are not significant, but that the lowest dose tested was not in fact the LOAEL. They recommend that NTP scientists reanalyze the data, looking at the three exposure levels together.

Dr. Stump was the BSC liaison to the peer review. He said that the peer review was an incredible process, showing how complex and difficult the problem is. He speculated

that an RFR exposure study designed today would likely be different in many respects. He noted that the studies could not be replicated today, as the reverberation chambers took so long to build and were transported internationally. Thus, other ways must be found to conduct follow-up experiments and confirm the findings. Having only used one control group in the studies will add to the challenge. The bottom line, he noted, is that the studies were well-conducted, but, when the report is done, "what do we tell our kids?" In terms of how the studies were conducted, the peer review process, the scientific discussion at the meeting, and the overall approach were commendable. The challenge, he said, would be to determine how to move at the pace of the current technology, as the report will be vulnerable to criticism that it was conducted using outdated technology.

Dr. Ramos asked Dr. Blystone why the peer reviewers mainly upgraded the draft conclusions that had been provided by NTP. Dr. Blystone explained that the reviewers had more concerns about some of the findings in the heart and the brain. Dr. Ramos noted that the different outlooks in several instances call for a very deliberative way of writing the final report, so that the issues are clear, and more confusion is avoided. He thought the increases in incidences were still interesting and questioned how to best present the interpretations of the data to the public. He also questioned the use of the term "equivocal" in the calls, as its meaning may be unclear. He recommended that the term be defined carefully in the final report. Dr. Blystone pointed out that all of the calls are discussed in detail in the reports, with explanations. Dr. Michael Wyde, DNTP Toxicology Branch, added that some of the decisions were quite difficult, and the split votes by the peer reviewers were evidence of that.

Dr. Berube mentioned that many IRB committees are currently reviewing grant applications for study designs using cell phones as research tools. He noted that some of that research could be halted by the conclusions reached by NTP. He said the report must be done correctly, or research efforts could be affected.

Dr. Felter said that in NTP bioassays, the conclusions always clearly state that they are the conclusions of the findings "under the conditions of this study." She said the challenge will be that the public will want to know how the information relates to realistic exposures or potential future exposures. She recommended interagency collaboration on public messaging, particularly with the FDA and FCC. Dr. Wyde agreed and acknowledged that part of the difficulty with the studies is their relevance to humans, and "we're still not quite there yet with the data that we've produced." Bridging those gaps will be built into the RFR 2.0 program, he added.

Dr. Bucher commented that when the studies were designed nearly 20 years ago, there was a paucity of literature on exposure. In the intervening years, there have been many efforts to define exposures from different types of phone systems, so NTP is in a much

better position to articulate the information called for by the peer review panel, and is working to include it in the discussion of the technical reports. NTP will work closely with the FDA and FCC on communications.

Dr. Afshari said that it would be important to impart information about how to use cell phones safely.

Dr. Barlow noted that it would be important to contextualize the exposure regimen used in the studies, compared to actual typical cell phone use.

Dr. Michaels commented on the increasing usage of cell phones and changing technology, and recommended that the report describe those dynamics. Dr. Wyde said that today the issue is not the number of times the phone is used over the course of a day, but the conditions under which it is used, adding another level of complexity to the science – one that is unseen with chemicals.

Dr. Gamboa reiterated that risk assessment should not be expected with the reports, as they are intended to be hazard identification.

Dr. McMartin summarized the board's thoughts. The project was "an exceptional *tour de force*" and the peer review panel was exceptionally well done, he said. The board's concerns included careful definition of the calls, particularly the equivocal calls. The biggest issue was how the results would be communicated to make them understandable and usable by the public, in terms of possibly changing behaviors to prevent problems. The board emphasized care in the writing of the final report in terms of how certain aspects would be discussed.

**2) Follow-Up Studies on RFR**

Dr. Wyde described NTP's plans for follow-up cell phone RFR studies. They will be designed to address issues and criticisms raised during peer review of the NTP RFR studies in March 2018, including temperature measurement during periods of animal inactivity, evaluation of stress markers and behavior changes during exposures, and measurement of food consumption. Additional studies will have the potential to expand to newer, current technologies, as well as evolving technologies that will become the new standard in the telecommunications industry. The studies will probe potential mechanisms for RFR-induced effects, confirm RFR-induced DNA damage in the brains of rats and mice, and establish biomarkers of exposures to apply to studies of newer and emerging RFR-based technologies.

Dr. Wyde discussed the issues, specific questions raised, and proposed areas of research in:

- Stress and behavior
- Organ-specific evaluations
- Exposure factors
- The role of heat in RFR-induced effects

Dr. Felter asked whether the follow-up research would include the acoustic nerve. Dr. Wyde said there had been discussions about that, and that it is something NTP would be interested in. Dr. Felter encouraged inclusion of it in the discussions.

Dr. Afshari asked what cell type had been used in the comet assay. Dr. Wyde replied that cells had been isolated from the three regions of the brain and that the assay was performed with a heterogenous mixture of the cell types.

Dr. Ryan noted the outliers on the two graphs Dr. Wyde had shown, and asked him if they were the same animals between the cortex and the hippocampus. He replied that they were different animals, and that it was difficult to tell whether they were outliers or representative of a small number of animals responding.

Dr. Felter noted that Dr. Wyde had mentioned consideration of food consumption in the future studies. She said she recognized the challenge in including water consumption, but that it would be important to do so if possible. Dr. Wyde said that measuring the water consumption in the reverberation chambers is challenging because water bottles cannot be used, due to the RFR heating the water. Because of the design of the automated watering system, he added, it is nearly impossible to measure water consumption. In the initial NTP studies, food consumption measurement was not done because food was provided inside the cage in ramekins and could be spread in the bedding by the animals. There should be ways to get around that in the proposed studies.

Dr. Stevens asked if the intent to “confirm RFR-induced DNA damage in the brain of rats and mice” meant that the belief is that there was DNA damage, or was an effort to put the comet assay data in context. Dr. Wyde said the intent was to replicate the comet assay data to confirm DNA damage in the comet assay, but also to utilize other more-appropriate assays for DNA damage. Dr. Stevens suggested re-phrasing that particular bullet in the gaps’ discussion.

Dr. Stump was the first BSC discussant. He approved of the goals described by Dr. Wyde. He felt that the concept of a series of studies made sense, as did the idea of going with 10 animals per group. He said that it would be important to look at how long in the day exposures would be conducted, beyond simply time-on, time-off. He noted that behavioral assessments would be challenging, and recommended acquiring pre-test data. He encouraged prioritizing how to approach some of the questions, with so

many potential endpoints, and recommended focusing efforts to confirm that there are RFR-induced effects before looking at mode or mechanism of action.

Dr. Felter was the second BSC discussant. She agreed with the need to prioritize, concentrating on asking the most important fundamental question, which is the human relevance of the existing findings, whether they are attributed to a phenomenon that goes beyond tissue heating, or whether tissue heating is a critical component. It is a challenge not knowing what the actual heating was, particularly with the heart, which has a high-water content. She wondered about the impact of the ten minutes on, ten minutes off heating regiment, and whether there would be implications of continuing the exposures for 20, 30, or 60 minutes, for example. The question is associated with the potential temperature threshold for damage, and whether temporal length of exposure is relevant in terms of a health concern. She recommended again that the acoustic nerve be included as a target in the follow-up studies.

Dr. Wyde acknowledged the heating issue, and mentioned that the first studies in the project were dermal pilot studies to establish a threshold dose where heating would become an overriding issue. He agreed that human relevance is important.

Dr. Barlow noted that eventually the older technologies will go away, and he wondered which technologies would be used in future studies to maintain relevance for policy and regulatory decisions. Dr. Wyde agreed that 2G is being phased out, but noted that the benefit with 1-4G is that they are very similar in terms of frequencies and modulations. 3G and 4G will not be phased out for a long time. He said 5G does represent a jump into something totally different, but it is still in development and its specifications have not yet been clearly defined. Thus, it should be monitored, but cannot be studied at this point. Dr. Barlow asked whether it might be wise to investigate specific cell lines, such as glial cells, since it is suspected that that is where the damage occurs, or possibly employ a different assay that would yield a more robust answer. Dr. Wyde said both ideas would be considered.

Dr. Davis noted that there are other features of cell phones that have changed significantly in recent years, and wondered if there were other aspects that should be studied. She said she was not seeing a clear cause/effect relationship. She said it would be important to be able to give manufacturers information they could use in engineering devices that would be more protective. Dr. Wyde replied that the “G” defines a number of parameters, with many different protocols. He further explained the technical aspects of the G technologies. Dr. Bucher added that what is being studied is radiofrequency radiation, and not Gs, to establish whether there are biological effects that can be related to exposure to RFR. Conducting a series of short-term studies lasting weeks rather than years will allow NTP to conduct many studies in order to identify a set of biomarkers to evaluate different technologies.

Dr. Stevens mentioned that there is significant literature in the heat shock field about what happens when core temperature is raised. He said that it is certainly a great marker of a dose, and asked if using a heat shock response marker across tissues had been considered. He also asked if using early, short-term exposure had been considered. Dr. Bucher confirmed that all of those approaches had been considered and could be used.

Dr. Afshari said that the DNA damage and cytotoxicity seen provides a framework for further research regarding carcinogenicity. She noted that the brain is a very dynamic tissue for gene expression, and that looking at single cells may be better to find something realistic. Regarding the discussion on heat, she was unsure whether the experiments were conducted in a thermal-neutral zone for both mice and rats. She posited that stress and circadian rhythms may play a role and should constitute another variable in the design of the research.

Dr. Michaels asked whether the carriers or manufacturers had reached out to the study team with an interest in the research. Dr. Wyde said they had not heard from any carriers or manufacturers.

Dr. Gamboa said that core body temperature should be measured in subsequent studies, as well as increases in temperature in specific organs.

Dr. McMartin summarized the board's impressions. He said the board was positive on the follow-up studies, and approved of the goals, particularly trying to confirm a mechanism or mode of action. The board also felt that prioritizing the large list of potential tests would be important. Dr. McMartin said the board also wanted to ensure that the human relevance of the models would be considered, and that future studies would address heating concerns.

## C. Activities on Bisphenols

### 1) CLARITY-BPA Research Program: Peer Review of Core Study and Next Steps

Given extensive discussion on earlier topics and time constraints, it was announced that the report on the peer review of the draft NTP Research Report on the bisphenol A (BPA) core study would be truncated, and is anticipated to be brought back at the December BSC meeting after the integrated report is prepared and peer reviewed (see below).

Dr. Nigel Walker updated the board on NTP activities on bisphenols. He provided background information on BPA, which was the subject of an NTP Monograph in 2008. He described bisphenol analogues and derivatives, a variety of chemicals similar to

BPA with widespread exposure. He noted that bisphenols represent an example for multiple issues being addressed by NTP and NIEHS, including endocrine disruption and low dose considerations.

He briefly reported on the recent peer review of the CLARITY-BPA project, which was a consortium of NIEHS-funded academic researchers with federal scientists and regulators. He provided details of CLARITY-BPA, which generated a Core 2-Year Toxicology Study.

The peer review of the draft NTP Research Report on the core study was held April 26, 2018. Materials from the meeting have been posted on the NTP website. The Research Report is undergoing revision based on the comments received and the final report is anticipated to be completed by the end of August 2018. All grantee data sets should also be available by that time. Grantee publications are ongoing, and the next step will be integrated interpretation of all datasets and publications that will generate a report anticipated in 2019, which will be peer reviewed.

Dr. Sass discussed some of the public comments she had seen regarding the use of historical controls in the interpretation of some of the data from CLARITY-BPA. She said it seemed to be an important point. Dr. Walker noted that the issue had arisen with both the cell phone RFR and BPA studies. Dr. Sass added that the choice of statistical tests was another important issue that had emerged in her reading the pre-meeting materials.

Following Dr. Walker's presentation, it was noted that, due to time constraints, the last agenda topic regarding the BSC's Perspective for NTP Regarding Strategic Realignment would be postponed to a future webinar meeting. BSC and ad hoc members were informed that they would be contacted in the near future to discuss scheduling.

## 2) Evaluation of Bisphenol Analogues

Dr. Vicki Sutherland, DNTP Toxicology Branch, briefed the board on NTP activities related to evaluation of bisphenol analogues and derivatives. She outlined the testing program's assessment of bisphenol compounds, many of which are proposed to be in products to which the public is exposed (e.g., thermal paper, flame retardants, plastics, resins, and dental polymers). The initial plan provides for complete toxicological assessment of bisphenol A (BPA), bisphenol AF (BPAF), and bisphenol S (BPS) and utilizes data from these three chemicals to serve as reference information for comparison to other analogues. This reference information and any data available on other analogues will be integrated into a class assessment. This class assessment includes three primary workstreams: literature and *in vitro* evaluations (on all

analogues), *in vivo* studies (reference chemicals), and an integrative assessment using all of the data to develop a database and inform an iterative learning process. Dr. Sutherland described published and ongoing research from all three phases.

Once enough data is collected from each of the workstreams, the next step employs use of a sufficient similarity framework to determine if other bisphenol analogues are sufficiently similar to the three reference chemicals and can be toxicologically-characterized on that basis. The work will consist of two phases:

- Phase 1: Comparing reference analogues of interest within and across physiochemical/structural and biological activity data streams
- Phase 2: Integrating data and making an overall similarity to reference chemicals call for each analogue of interest

After phase 2, if the activity patterns seen in the analogue or analogues are similar to the reference compound, the conclusion may be that there is no need to comprehensively characterize the unknown analogue(s). On the other hand, if a different activity pattern is noted for an analogue and it is deemed to be non-similar, this may create the need to generate more data. Some of the comparisons have already been conducted, and results were described by Dr. Sutherland.

She delineated the work that is ongoing. For *in vivo* assessments of the reference chemicals, the BPA studies are almost complete; BPAF and BPS studies are in progress, as are ADME/TK studies. Plans for the future include:

- Finish collecting *in vivo* data for the initial reference chemicals
- Make an in-depth comparison of all data streams for the analogues
- Work to develop an integrative assessment of the bisphenol class
- Iterative process – what we learn will feed back into improving class evaluations

Dr. Tilton asked Dr. Sutherland about the phases as compared to the schematic she had presented, which appeared to be more linear, but with a non-sequential timeline. She replied that it is not a linear process as much as a phased approach with multiple phases ongoing at the same time, and that the research report she mentioned in the presentation has already been published, but there is still ongoing work. Dr. Tilton asked whether the *in vitro* and *in silico* data, along with the literature analysis, had helped to inform the reference chemicals that were used in the *in vivo* studies. Dr. Sutherland replied, “Yes and no.” The research report had helped make decisions for the BPS program, but the BPAF and BPA programs were already running before those data became available.

Dr. Barlow asked Dr. Sutherland to elaborate on the statement regarding a compound being well-absorbed but having low oral bioavailability. Dr. Sutherland asked Dr. Waidyanatha to respond, and she explained that a compound may be well-absorbed, but if it's quickly conjugated then not much will be freely available. Dr. Birnbaum added that the material is conjugated in the intestine before it gets anywhere else. Dr. Barlow asked if anything is known about the metabolism, and whether there is an active metabolite. Dr. Waidyanatha said none had been found, and that only conjugates were seen.

Dr. Barlow was the first BSC discussant. He said that he liked the approach, allowing for the ability to make decisions more quickly. He said he would like to have seen *in vivo* studies comparing the three reference chemicals, but understood the limitations involved. He noted that no one data point would ever predict everything, so it is important to look at all of the available data, to the point of sometimes needing *in vivo* studies as well. He pointed out that the approach taken for the program appeared similar to other NTP efforts, which Dr. Sutherland confirmed. He approved of that trend.

Dr. Tilton was the second BSC discussant. She approved of using the approach as a case study for the NTP strategic realignment. She was glad to hear that Dr. Sutherland was prepared and ready to conduct additional *in vivo* studies, if needed. Moving forward, the NTP can utilize data from earlier studies to inform *in vivo* studies with other compounds, potentially reducing animal use. She said it was a benefit that there are now human data for BPA and BPS.

Dr. Davis asked how the potency and plurality of molecular targets involved with the compounds were considered when trying to obtain a biological similarity profile, when the readout is not necessarily looking at potency for specific targets, leading to considerable variability. Dr. Sutherland said that some of the issue is still in flux, especially the potency aspect, but for biological similarity, approximately 43 different assays are involved, with yes/no scoring, looking at similarities and differences across the different analogues. Dr. Davis said that the branching in similarity scores was interesting, in that it could yield important mechanistic information.

Dr. Walker discussed the similar approaches being taken in other NTP programs, such as research in mixtures and botanicals. The challenge of biological similarity and potency arises in those areas as well, which calls for development of new tools. He noted that there currently aren't good statistical approaches for fully addressing the issues of potency when doing sufficient similarity work.

Dr. Ramos said that the ability to make decisions regarding further testing vs. no further testing would heavily depend on where the magnifying glass is put, reflecting the strength of the biomarker being used to make decisions. Given that, he asked if relative

weight had been assigned to the markers being used to make the classification. If not, he recommended doing so to reconcile *in vitro* and *in vivo* differences. Dr. Sutherland said that in her previous work in the pharmaceutical industry, there were such strategies used to limit the number of necessary *in vivo* assays. She noted that weighting of certain assays is under consideration, but exactly how to do so for each of the different classes had not yet been determined. Dr. Ramos suggested anchoring the weighting based on the available human data; i.e., reverse engineering from the human data.

Dr. Ramos asked about generational developmental effects, and how Dr. Sutherland would see the program evolving to capture those complex endpoints. Dr. Sutherland said that there are ongoing multi-generational studies for BPS and BPA.

Dr. Stevens observed that there seemed to be two separate problems to solve. First, prioritization of what should be tested further, and second, prediction of what would happen in humans. Dr. Sutherland said that although she had not presented the issues in that context, some of the comparative work is in progress. Dr. Walker noted that when the program was initiated, the thinking was how to leverage what is already known about BPA, so that study after study of the reference chemicals would not be required. He expressed the need to put the similar compounds into the existing BPA regulatory framework.

Dr. Afshari asked about PBPK modeling and potentially going across classes, specifically whether the concept of tissue resonance tied in with physical and chemical properties. Dr. Sutherland replied that that was not really her forte, but that modeling personnel at NCTR had been helpful in addressing that area.

Dr. Birnbaum said this is a really exciting movement in a new direction for how to deal with the thousands of chemicals in existence today. It is a way to improve short-term approaches to focus on identifying the “bad actors.” She added that the collaborations with both intramural and extramural scientists are important.

Dr. McMartin summarized that the board liked the approach very much and found it to be exciting.

### 3) REACT Program for Per- and Polyfluoroalkyl Substances

Dr. Michael DeVito from the NTP Laboratory informed the board about the Rapid Evaluation and Assessment of Chemical Toxicity (REACT) PFAS program. REACT is a general approach that NTP is developing to address environmental and public health challenges, focused on fit for purpose solutions and involving literature mining, computational, *in vitro,* and *in vivo* toxicological methods. REACT PFAS is a project focused on per- and polyfluorinated alkyl substances (PFAS). Dr. DeVito explained how the project is connected to the DNTP translational toxicology pipeline plan.

He provided background information on PFAS, a diverse group of more than 3,000 compounds used in a variety of commercial products. They are attracting significant regulatory interest by several agencies. NTP has been working on evaluating perfluorinated compounds (PFCs) since 2003, when they were nominated by the US EPA. Studies have focused on class assessment, including guideline toxicity studies on PFAS and PFOA. More than 100 PFAS are currently under evaluation, which creates challenges, comprising too many chemicals for traditional approaches, and necessitating a screening approach.

Questions facing the problem formulation and approach determination include:

- What types of biological activity and toxicological information can NTP develop in a responsive timeframe on these classes of chemicals?
    - How can this information be used to make public health decisions?
- What are the appropriate biological and computational tools to bring to the problem?
- How do we organize this information to provide useful products?
- How do we report this biological activity/toxicological information in a timely manner?

REACT PFAS is working on the EPA library of 75 chemicals, an NTP exploratory effort, and chemical-specific studies. QSAR modeling and high-throughput transcriptomics are being employed. Dr. DeVito provided details on each of the REACT PFAS initiatives. He noted that the PFAS assessment is based on read across. He described the current output of the projects. To date, the program has:

- Developed a data analysis pipeline in the CEBS database for *in vitro* data from the NTP Laboratory
- Developed a transcriptomic analysis and reporting pipeline
- Evaluated subsets of the PFAS library in several of the exploratory efforts
- Developed analytical methods (have methods for ~15 PFAS)
- Obtained the EPA library

Dr. Waters asked Dr. DeVito to comment on whether the screening and exploratory studies and the short-term *in vivo* studies would be run in parallel and help inform each other. Dr. DeVito said there would be iterative processes as *in vitro* assays are developed to represent appropriate biology to screen within the library, which would be included in the screening approaches. Understanding the screening approaches may inform how the chemicals are grouped, helping to decide which ones would go to a short-term assay for 5-day transcriptomic studies. Dr. Waters asked if the tissue had been selected for the short-term studies. Dr. DeVito said he favored studying the liver.

Dr. Ryan commented on the question of the translational toxicology pipeline being linear or non-linear, and suggested that it may be better to think of it as an adaptive study design. Dr. DeVito agreed that it is being thought of as adaptive or iterative. He felt that any one of the circles in the diagram is both a product and a point where a project halts.

Dr. Waters was the first BSC discussant. She said she liked how Dr. DeVito had tied in the REACT PFAS approach with the translational toxicology pipeline. She said she was concerned about the sense of linearity versus things happening in parallel. She wondered whether the liver would necessarily be the right place to start an evaluation, since the targeted pathways may be unknown. She suggested that information may emerge from the exploratory studies that would suggest a better cell type. Regarding the conflict between basic and applied science, she felt that EPA favors the transcriptome assay because it can be used as a surrogate of biological activity, particularly when thinking about biological activity and potency across many pathways, but it still will not yield information on mechanism within specific tissue types. She expressed concern that the focus in the collaboration with EPA is serving their needs more than NTP needs. Dr. DeVito said his comment about the liver had been in jest. He said that another concern is life stage. He noted that there is a philosophical debate going on within NTP, and that the side he endorses wants to predict dose, because regulatory decisions must be made. "How do we get to dose the quickest?" he asked. He speculated that transcription would get to dose quickest, possibly. He said that part of the effort is to test the models such as IVIVE. He said that there is an effort to predict *in vivo* biology from one cell type, and that that strategy does not work. He added that mechanism is important, but right now people are being exposed to PFAS, and decisions must be made.

Dr. Chiu was the second BSC discussant. He said that read-across is another type of prediction model, and that clustering a group of compounds together can show that information on one compound is predictive of information on another in that group. He noted that toxicokinetics and relative potency are examples of things that might be similar on a qualitative basis but different quantitatively, arriving at the issue of dose. He said the bioactivity, relative potency, toxicokinetics, and chemistry are all different dimensions, but all are not necessarily required to be similar in order to group them. Dr. DeVito said the issue had been discussed with statisticians in the *Gingko biloba* project, who said that nothing was similar because they did not produce the exact same response at the same dose. It was pointed out that there was an identical response, simply shifted by dose. Somehow, he said, it needs to be figured out how to compromise on what is similar, by defining what is "sufficient" and what is "similar." The advantage of the REACT PFAS project is NTP working with regulatory partners to help make those definitions, he noted. He said that all data from the project will be publicly

available, and encouraged others to analyze it for sufficient similarity, mechanisms, and pathways.

Dr. Chiu's second comment was more general, focusing on the adaptive toolbox involved with the program. He asked Dr. DeVito how he would see NTP's responsiveness to outside groups working to identify and fill specific data gaps in the future. Dr. DeVito said the product that would be most comforting to regulators would be the oral equivalent dose needed to produce the biological effects being seen *in vitro*, assuming that IVIVE works and that is has been adjusted specifically for individual classes of chemicals. It can then be applied to exposures, with stakeholder feedback being sought about how much effort to expend on which classes of chemicals. Dr. Chiu asked if that process is being envisioned as a template for future investigations. Dr. DeVito felt that the REACT program fits in well and sees it as becoming a more common approach to how NTP tackles problems. Dr. Chiu said that would engender an alternative chemical framework, as opposed to a one-chemical-at-a-time framework. Dr. DeVito agreed, and said that NTP is applying the REACT concept to its study of flame retardants. Dr. Chiu said that would pose different communication challenges in the future. Dr. DeVito said it allows NTP to provide data to regulators and the public so that they can make informed decisions.

Dr. Ramos said he had agreed with Dr. DeVito's presentation until he made his comment about "we're here to predict dose." He said that Dr. DeVito had discussed the importance of biology to the REACT program and did not understand why he had then made the statement about predicting dose. Dr. DeVito said that to learn whether certain chemicals cause diseases, such as Alzheimer's or Parkinson's, very specific animal models would be needed to arrive at useful predictions. He said it would be a long, protracted effort to connect the *in vitro* biology of models such as 3D organs-on-a-chip to reaching the apical endpoint in an animal model. Dr. Ramos said that perhaps the argument is one only of semantics, as Dr. DeVito shifts from dose, which is a number, to biological effects and outcomes. In that context, he asked Dr. DeVito how genetic diversity in response would be captured. Dr. DeVito acknowledged that understanding the biology would be interesting; however, given that EPA regulates based on dose, NTP's current focus is to provide EPA information so that it can determine safe exposure levels for the chemicals.

Bringing the discussion back to the strategic plan, Dr. Stevens said that he did not see a conflict. He noted that it is very important to be able to take a series of chemicals and say that all assays indicate they are biologically inert at a certain level of exposure. If the chemicals are biologically inert, the biological consequences of what they do are unlikely to be important for risk assessment. However, if they *are* biologically active, what are the consequences for human exposure? Mechanism then becomes important.

Dr. Stevens did not see the two as being in conflict, but it is a very important point for the strategic realignment plan. Arriving at some resolution between the two scenarios within the translational toxicology pipeline, or "bottle opener" model, would give a way to make prioritization decisions, working around the conflict of approaches.

Dr. McMartin noted that the issues being discussed would be good to bring up in the upcoming webinar on strategic realignment.

Dr. Birnbaum pointed out that, with regard to PFAS, there are several other regulatory agencies aside from EPA who are also interested, such as FDA, CPSC, and others.

Addressing Dr. DeVito, Dr. Stevens said that he liked the literature mining exercise on immunotoxicity, which then exploded out into a much broader screening approach. He asked if the intent had been to focus the assays on the literature-identified risk and then to take the whole class through the assays to see what the spectrum of response might be. Dr. DeVito said that EPA asked for the chemicals to be tested with BioSeek, for which they had information for over 1,000 chemicals, to be able to compare the tested chemicals with knowns from BioSeek. He elaborated on the immune assay that had been used.

Dr. Stevens said he felt that the biggest potential problem would be trying to predict immune responses *in vivo* from *in vitro*, when it is known that trying to predict immune responses in humans from whole animals has not been successful. Dr. DeVito agreed, but asked whether Dr. Stevens might suggest an alternative. Dr. DeVito said that it comes down to the ability to predict a concentration in blood that will impact cytokine expression, so that exposure is set below that level. He reiterated his hope that the information would lead to mechanisms, but was not optimistic that it would. Dr. Stevens noted that the immune system was seen as a target, but the assay decision was being made not really caring about mechanism. He felt that the cytokine assay was just one of the arrays of assays that had emerged from the literature review.

Dr. Gamboa said that regulators understand the characteristics of the different types of studies. He noted that in this case, a multi-step paradigm is being developed to ultimately arrive at a point of departure for the regulator to conduct risk assessment. He suggested that it would be useful for the process to incorporate some measure of uncertainty. Dr. DeVito said that the case study with seven PFAS would shed light on the uncertainty involved.

Dr. McMartin said that the sense of the board was that there had been a lively discussion which would be continued. Dr. Stevens said that regardless of conversation to the contrary, the board is very supportive of the program.

## VIII. Next Steps and Adjournment

Dr. Berridge thanked Dr. McMartin for his chairmanship of the meeting, as well as the NTP staff who had made presentations. He said that the experienced board members would appreciate the maturation of the challenges that NTP is taking on, while he hoped that the new board members had found the meeting insightful as to the complexity of the issues NTP tackles. He thanked the board for exceeding his expectations. He noted that the conversation will be ongoing in an effort to find a balance between near-term and future decisions. He said he appreciated Dr. DeVito's commitment to allowing people to make decisions in the near term. He said that one of the influential aspects for him as he has entered the community has been that unlike in pharma, the human experiment starts long before there is information about the chemicals, so NTP "can't just sit back and doodle around with these things, because people are at risk every single day."

Dr. McMartin adjourned the meeting at 5:15 pm, June 20, 2018.

Summary Minutes June 20, 2018
NTP Board of Scientific Counselors

[redacted]

Dr. Kenneth McMartin

Chair, NTP Board of Scientific Counselors

Date: 10-3-18