Advancing Research on Mixtures:

New Perspectives and Approaches for Predicting Adverse Human Health Effects

Executive Summary

NIEHS Workshop

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Co-organizers:

Danielle Carlin, Ph.D., DABT Program Administrator, NIEHS DERT

Cynthia Rider, Ph.D., Toxicologist, NIEHS DNTP

Michael DeVito, Ph.D., Group Leader, Toxicology Branch, NIEHS DNTP

Claudia Thompson, Ph.D., Chief, Susceptibility and Population Health Branch, NIEHS DERT

Working Group Members:

Janice Allen, Ph.D.; Scott Auerbach, Ph.D.; David Balshaw, Ph.D.; Linda Birnbaum, Ph.D.; Chad Blystone, Ph.D.; Abee Boyles, Ph.D.; John Bucher, Ph.D.; Raj Chhabra, Ph.D.; Gwen Collman, Ph.D.; Caroline Dilworth, Ph.D.; Gregg Dinse, Ph.D.; June Dunnick, Ph.D.; Kim Gray, Ph.D.; Paul Foster, Ph.D.; Jerry Heindel, Ph.D.; Kembra Howdeshell, Ph.D.; Freya Kamel, Ph.D.; Grace Kissling, Ph.D.; Scott Masten, Ph.D.; Elizabeth Maull, Ph.D.; Sri Nadadur, Ph.D.; Keith Shockley, Ph.D.; Bill Suk, Ph.D.; Nigel Walker, Ph.D.; Mary Wolfe, Ph.D.

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EXECUTIVE SUMMARY

Overview: The NIEHS Workshop entitled "Advancing Research on Mixtures: New Perspectives and Approaches for Predicting Adverse Human Health Effects" was held on September 26-27, 2011 in Chapel Hill, NC (http://tools.niehs.nih.gov/conferences/dert/mixtures/). The purpose of the Workshop was to identify and focus on key issues that present challenges in mixtures research. A full summary report is available at http://www.niehs.nih.gov/about/visiting/events/pastmtg/2011/mixtures/index.cfm. The results from the Workshop will be used to inform the development of an NIEHS intramural and extramural mixtures research strategy and provide input to the scientific community for advancing mixtures research. The goals of this workshop were to:

- Identify and prioritize the knowledge gaps and challenges in mixtures research specific to each of the following disciplines: toxicology, epidemiology, exposure science, risk assessment, and statistics
- Obtain advice on integrating multidisciplinary capabilities to address critical topics in mixtures research
- Provide recommendations for research on key topics
- Inform the development of a long-term NIEHS mixtures research agenda
- Foster collaborations between extramural and NIEHS scientists

Day 1: The first day of the Workshop consisted of discipline-specific presentations from speakers in each of the areas listed above on the state-of-the-science and major challenges associated with mixtures research. These presentations were followed by breakout sessions consisting of discipline-specific groups tasked with developing and prioritizing a list of important knowledge gaps and research topics.

John Bucher, Ph.D., welcomed participants and provided a summary of previous NIEHS research on mixtures. Cynthia Rider, Ph.D. provided an overview of the Workshop format and goals. "Mixtures" (for the purposes of the Workshop) was defined broadly as any combination of chemicals that will contribute to our understanding of joint toxicity.

Glenn Ric, Sc.D., focused on the assessment of human health risk posed by exposures to environmental mixtures. He explained the various models available for conducting human health risk assessment of mixtures including component-based and whole mixture approaches. He used his work on disinfection by-products in drinking water as an example of a whole mixture case study.Rice discussed the toxic equivalency factor approach applied to dioxin-like chemicals as an example of a component-based risk assessment. He outlined the data needs of risk assessors including chemical analysis of complex mixtures, low-response region data, and high-throughput technology use and interpretation.

Paul Price, M.S., focused on the field of exposure assessment and specifically three issues in exposure science: lack of whole mixture data, lack of component data, and testing under the lamppost. He discussed the need for both monitoring and modeling in the characterization of real-world exposures. In terms of monitoring, Price discussed new sensor technologies that are being developed. Lastly, he described methods for prioritizing mixtures for study including the maximum cumulative ratio.

Earl Gray, Ph.D., discussed his work on chemicals that target androgen signaling in the developing male rat. He described the current paradigm that focuses on applying dose addition exclusively to chemicals

with the same mechanism of action. His work suggests that this is too narrow a definition and that dose addition should be applied to chemicals that target a common signaling pathway or tissue.

David Christiani, M.D., discussed his work on metal mixtures associated with neurodevelopment. Specifically, he mentioned his work within the Harvard University Superfund Research Center, where he is evaluating birth cohorts that will be followed into childhood in the U.S., Bangladesh, and Mexico City. He has found interactions among metals and other contaminants. Discussion following his presentation revolved around the different definitions of mixtures terminology in epidemiology and toxicology and the need for increased cross-disciplinary understanding.

Chris Gennings, Ph.D., focused on methods for predicting the toxicity of an "unknown" mixture based on the toxicity of a reference mixture. She discussed examples of testing for sufficient similarity of whole mixtures for both data rich and data poor scenarios. Methods for determining sufficient similarity were based on equivalence testing used by FDA to determine that generic drugs are similar to their name brand counterparts.

Following the Day 1 presentations, participants formed discipline-based breakout groups to discuss current challenges in mixtures research specific to their field. The epidemiology group highlighted the need for proper exposure characterization, translation between epidemiology and toxicology, and prioritizing mixtures for study. The risk assessment group focused on the need for more individual chemical data, development of methods to group compounds, and the potential use of high through-put screening (HTS) in mixtures research. The statistics group explored case studies that could benefit from statistical methods development including data mining, developing whole mixture reference libraries, and scenarios involving mediation of "unknown" mixtures. The biology/toxicology group discussed topics ranging from in vitro to in vivo linkages and understanding dosimetry to predictive models of mixture toxicity. Lastly, the exposure science group discussed the need to approach exposure characterization from both a chemical perspective and a disease perspective, consideration of temporal and spatial variables, inclusion of non-chemical stressors in exposure analyses, and diversity of populations.

Rider presented the findings from a Request for Information (RFI) conducted prior to the Workshop to gain insight into key mixtures challenges. Major themes included identification of interactions, mixture model development and validation, the need for better exposure characterization, temporal considerations, use of HTS and omics technologies in the study of mixtures, and inclusion of network/systems approaches. These themes were used in development of focus areas for Day 2 discussions.

Day 2: Presentations covered novel approaches for addressing mixtures challenges, such as cross-discipline experimental design considerations, Environment-Wide Association Studies (EWAS), and multi-pollutant epidemiological assessment tools. Following the presentations, multidisciplinary breakout sessions were held to address key topics, develop a priority matrix scheme consisting of timeframe and scientific impact, and propose suggested approaches to evaluate the most highly ranked research questions.

Linda Birnbaum, Ph.D., Director of the National Institute of Environmental Health Sciences (NIEHS) and the National Toxicology Program (NTP), provided an introductory presentation to begin Day 2 of the workshop. She expressed her enthusiasm for the study of complex exposures, which include both chemical and non-chemical stressors. Birnbaum also provided a brief history of NIEHS's success in the field of combined exposures by presenting examples of previously funded research, current efforts (e.g.,

DNTP), and future endeavors (e.g., development and utilization of comprehensive databases, the exposome, and the investigation of latent effects of combined exposures).

Paige Tolbert, Ph.D., discussed her work in developing strategies to advance multi-pollutant epidemiologic research in the context of the complex mixtures of air pollution at the Emory/Georgia Tech Southeastern Center for Air Pollution and Epidemiology. This Center has a multitude of ongoing studies on health effects in commuters, asthma development, and acute health outcomes in a number of cities across the United States. Tolbert discussed the various analytical and experimental approaches currently being used in these studies to determine which components of air pollution contribute to the health effects being studied.

Andreas Kortenkamp, Ph.D., discussed the dose addition model as an extremely powerful tool for approximating mixture effects. He provided two examples of applying toxicological methods to epidemiology studies. In one example, relative potency factors derived from toxicology studies could be used to weight component chemicals in deriving an aggregate exposure estimate. In the second example, a whole mixture approach could be used by testing the activity (e.g., estrogenicity) of human samples from epidemiology studies and then identifying the active mixture components through chemical analysis. Throughout his talk, Kortenkamp emphasized the need for collaboration among the various disciplines.

Chirag Patel, Ph.D., discussed his methods to conduct environment-wide association studies (EWAS), which utilize approaches adopted from "omics" research. Patel analyzes the expansive data available in the public domain to look for associations between environmental factors and health effects in order to develop testable hypotheses. He discussed an example of this approach in which he identified environmental exposures that may be contributing to endpoints associated with cardiovascular disease (e.g., cholesterol levels) and Type II diabetes.

Following the Day 2 presentations, participants congregated in multidisciplinary breakout groups to address the specific mixtures topics developed based on the RFI. The first group discussed "Modeling Mixture Toxicity: Constraints of Extrapolation." Participants discussed several issues including testing the interaction threshold, assessing the maximum cumulative ratio hypothesis, building a database to indentify interactions, exploring application of sufficient similarity approaches, and the need for statistical methods development. The second group was assigned the topic of "Exposure Assessment: Making Sense of Biomonitoring Data." They discussed goals including developing methods to generate and analyze high-density exposure data and databases for consolidating exposure information. The third group addressed "Epidemiology: Reconciling Edpidemiological and Toxicological Approaches to Mixtures" and covered translation of tools from toxicology to epidemiology and vice versa. The fourth group discussed "Chemical Interactions: Predicting the Unpredictable", focusing on improving *in vitro* assays, using sufficient similarity techniques, and integrating across data types to understand interactions. The fifth group was tasked with "Mixtures Across Time" and discussed incorporating temporally-relevant features (aging) into animal models and approaches for assessing exposure over time.

Rick Woychik, Ph.D. provided closing remarks and discussed the development of the NIEHS 2012–2017 Strategic Plan. Major themes of the Workshop highlighted by Woychik included: relationship of external to internal dose, application of innovative approaches to mixtures, a need for databases to house diverse mixtures-related data, use of a systems-based perspective in mixtures research, and evaluation of the total exposure scenario.