Advancing Research on Mixtures: New Perspectives and Approaches for Predicting Adverse Human Health Effects

Details

Organized by the National Institute of Environmental Health Sciences (NIEHS) Divisions of Extramural Research and Training (DERT), the National Toxicology Program (DNTP), and Intramural Research (DIR)

September 26 – 27, 2011

Sheraton Chapel Hill, 1 Europa Dr., Chapel Hill, NC 27517

Workshop Goals

The goal of the NIEHS Mixtures Workshop is to identify and focus on key issues that present challenges in mixtures research (for this workshop, "mixtures" includes combined independent exposures). The NIEHS will use the results from the workshop to inform the development of an intramural and extramural mixtures research strategy. This workshop will also provide input to the scientific community for advancing mixtures research.

Specifically, this workshop will:

- 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 (see topics below)
- 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

Workshop Product

Discussions from the workshop will be captured in a manuscript in the peer-reviewed literature. This manuscript will also provide suggestions for future directions in mixtures research for both NIEHS scientists and the broader research community. Additionally, advice from the experts will be used to enhance ongoing NIEHS mixtures research efforts.

Agenda at a glance

Day 1 – September 26		
Time	**Topic**	**Speaker**
8:30-8:45 EDT	Welcome	John Bucher
8:45-9:00	Introduction to Workshop	Cynthia Rider
9:00-9:25	Chemical Mixtures: What Dose-Response Analysts Really Want	Glenn Rice
9:25-9:50	What are the Combinations of Chemicals to which We are Exposed, How Can We Identify the Relevant Combinations, and Why is this Important?	Paul Price
9:50-10:15	A Biological/Toxicological Perspective on Mixtures Research	L. Earl Gray
10:15-10:30	Break	
10:30-10:55	Epidemiologic Approaches to the Study of Mixed Exposures	David Christiani
10:55-11:20	Empirical Whole Mixture Approach for Risk Evaluation: Combining Exposure and Mixtures Toxicity Data	Chris Gennings
11:20-11:35	Charge for Day 1 Breakout	Danielle Carlin
11:35-12:30	Day 1 Breakout: Discipline-based Groups Identify Critical Research Issues	
12:30-1:30	Lunch (box lunch available for purchase)	
1:30-3:15	Day 1 Breakout (continued)	
3:15-3:30	Break	
3:30-5:30	Report Back/Discussion	Mike DeVito (chairperson)

Day 2 – September 27		
Time	**Event**	**Speaker**
8:30-8:45 EDT	Welcome	Linda Birnbaum
8:45-9:05	Developing Strategies to Advance Multi-Pollutant Epidemiologic Research	Paige Tolbert
9:05-9:25	Mixture Effects and Epidemiology – The Need for Unbiased Approaches to Exposure Assessment	Andreas Kortenkamp
9:25-9:45	Environment-Wide Association Studies: Creating Data-Driven Hypotheses Regarding Environmental Correlates to Common Disease on a Population Scale	Chirag Patel
9:45-10:00	Break	
10:00-10:15	Charge for Day 2 Breakout	Claudia Thompson
10:15-12:00	Day 2 Breakout: Multidisciplinary Groups Address Key Topics	
12:00-1:00	Lunch (box lunch available for purchase)	
1:00-2:00	Day 2 Breakout (continued)	
2:00-2:15	Break	
2:15-4:00	Report Back/Discussion	Claudia Thompson (chairperson)
4:00-4:15	Closing Remarks	Richard Woychik

Supporting Material for Day 1 Breakout Session

Background

In March 2011, NIEHS published a Request for Information (RFI) titled: Needs and Approaches for Assessing the Human Health Impacts of Exposure to Mixtures (http://ntp.niehs.nih.gov/go/rfimix). The RFI was developed to identify critical knowledge gaps in mixtures research from a broad cross-section of perspectives. Day 1 activities will be aimed at further developing and refining a comprehensive list of challenges and research needs toward understanding the health effects of chemical mixtures.

The Day 1 plenary talks will provide the audience with an overview of current approaches and major challenges in mixtures research from the perspective of each discipline (risk assessment, exposure science, biology/toxicology, epidemiology, and statistics). Following the plenary session, participants will be organized into discipline-based groups. At this information-gathering stage, discipline-specific issues will be discussed with representatives from academia, government, industry, and non-profit organizations that are familiar with the particular challenges facing mixtures research in their field. Further, the participants will be asked to move beyond listing challenges to discussing prioritization of those challenges. Effort should be focused on ranking challenges in terms of the impact that addressing the issue will have on advancing the field. Written comments from observers will be collected at the end of the day and will be incorporated into the final workshop report.

Questions from the RFI to be addressed by each of the discipline-based breakout groups:

1. What are the underlying scientific knowledge gaps for assessing the effects of mixtures on human health?
2. What are the scientific issues encountered in performing risk assessments of mixtures that can be addressed by new research?
3. What types of scientific data (e.g., mechanistic, epidemiological, etc.) are needed to address these underlying knowledge gaps?
4. What are the new technologies and innovative approaches that could be leveraged to address these underlying knowledge gaps?

Note: Priority should be given to addressing questions 1 and 2, as subsequent questions support the initial queries.

Goals

- Develop a list of key challenges in mixtures research and cumulative risk assessment specific to each discipline
- Prioritize issues based on impact
- Discuss potential approaches, tools, and technologies that offer promise for addressing key challenges

Groups

Participant group assignments are shown below and appear in table format on page 34.
Observer group assignments are located in the observer directory (page 22).

Epidemiology Discussion Group Participants: Rosemary Castorina (University of California, Berkley), David Christiani (Harvard School of Public Health), Julie Daniels (University of North Carolina, Chapel Hill), Lyndsey Darrow (Emory University), Caroline Dilworth (NIEHS DERT), Kimberly Gray (NIEHS DERT), Russ Hauser (Harvard School of Public Health), **Todd Jusko (Rapporteur; NIEHS DIR)**, Irva Hertz-Piccioto

(University of California, Davis), Kembra Howdeshell (NIEHS DNTP), Freya Kamel (NIEHS DIR), Susan Korrick (Harvard Medical School), Scott Masten (NIEHS DNTP), Paige Tolbert (Emory University), Tom Webster (Boston University), **Beth Whelan (Chairperson; NIOSH)**

Risk Assessment Discussion Group Participants: Michael Babich (CPSC), George Bollweg (EPA Region 5), Mike DeVito (NIEHS DNTP), **Bethany Hannas (Rapporteur; EPA NHEERL)**, Andrew Hotchkiss (EPA NCEA), Ronald Lorentzen (FDA), Anna Lowit (EPA OPP), Jennifer McPartland (Environmental Defense Fund), **Moiz Mumtaz (Chairperson; ATSDR)**, Glenn Rice (EPA NCEA), Andrew Salmon (California EPA), Bill Suk (NIEHS DERT), Linda Teuschler (EPA NCEA), Claudia Thompson (NIEHS DERT), Nigel Walker (NIEHS DNTP), Tracey Woodruff (University of California, San Francisco)

Statistics Discussion Group Participants: John Bailer (Miami University), Abee Boyles (NIEHS DNTP), Gregg Dinse (NIEHS DIR), Chris Gennings (Virginia Commonwealth University), **Paul Feder (Chairperson; Battelle)**, Dale Hattis (Clark University), Richard Hertzberg (Biomathematics Consulting), Amy Herring (University of North Carolina, Chapel Hill), Grace Kissling (NIEHS DIR), Elizabeth Maull (NIEHS DNTP), Shyamal Peddada (NIEHS DIR), Cynthia Rider (NIEHS DNTP), Keith Shockley (NIEHS DIR), Woody Setzer (EPA NCCT), **Sheetal Thakur (Rapporteur; NIEHS DNTP)**

Biology/Toxicology Discussion Group Participants: Mamta Behl (Rapporteur; NIEHS DNTP), Chad Blystone (NIEHS DNTP), Christopher Borgert (Applied Pharmacology and Toxicology, Inc.), Danielle Carlin (NIEHS DERT), Deborah Cory-Slechta (University of Rochester), Stephania Cormier (Louisiana State University), Kevin Crofton (EPA NHEERL), Paul Foster (NIEHS DNTP), Julia Gohlke (University of Alabama, Birmingham), L. Earl Gray (EPA NHEERL), Jerry Heindel (NIEHS DERT), Marike Kolossa-Gehring (Umweltbundesamt/ Federal Environment Agency), Andreas Kortenkamp (University of London), Susan Schantz (University of Illinois), Jane Ellen Simmons (EPA NHEERL), **Raymond Yang (Chairperson; Colorado State University)**

Exposure Science Discussion Group Participants: Scott Auerbach (NIEHS DNTP), David Balshaw (NIEHS DERT), Dana Barr (Emory University), Herb Buxton (USGS), **Antonia Calafat (Chairperson; CDC)**, Brian Curwin (NIOSH), June Dunnick (NIEHS DNTP), Richard Kwok (NIEHS, DIR), David Lawrence (Wadsworth Center), Joanna Matheson (CPSC), **Minerva Mercado-Feliciano (Rapporteur; NIEHS DNTP)**, Sri Nadadur (NIEHS DERT), Chirag Patel (Stanford University), Paul Price (Dow Chemical Company), James Rusling (University of Connecticut), Rogelio Tornero-Velez (EPA NERL)

Supporting Material for Day 2 Breakout Session

Background

The plenary and breakout sessions on Day 2 will move the discussion from an information-gathering phase to a problem-solving phase. The Day 2 plenary talks will present select innovative approaches for the study of mixtures. These talks are meant to stimulate thought on novel, cross-discipline approaches for addressing mixtures problems in advance of the Day 2 breakout group session.

Following the plenary session, participants will be organized into multidisciplinary breakout groups. Each breakout group will be given one topic. The key topics chosen for the Day 2 breakout groups were developed based on information from the RFI, discussions with regulatory agency partners and mixtures experts, and NIEHS working group deliberations. The key topics reflect major themes in mixtures, where research is needed to advance the field.

Goals

The overarching goals of the Day 2 breakout session are to identify the most important issues in each of the key topic areas, and to discuss the most appropriate research approaches for addressing those issues. Information from this breakout session could potentially be used to develop DNTP projects or inform the direction of future mixture-related DERT programs. Written comments from observers will be collected at the end of the day and will be incorporated into the final workshop report.

Specific goals for the Day 2 Breakout Session include:

- Matching important mixtures-related scientific questions with suggested approaches
- Determining the timeframe and impact of these question/approach combinations
- Identifying the types of multi-disciplinary collaborations that could address the identified question/approaches

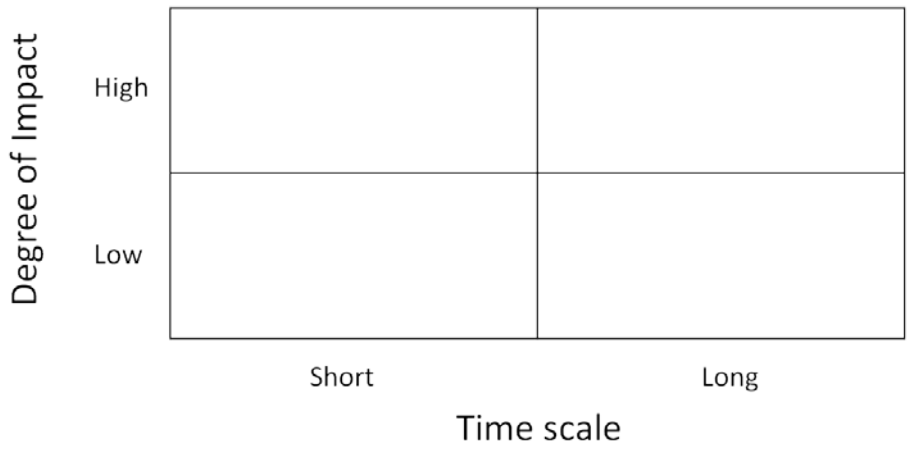
Instructions

Each multi-disciplinary breakout group will address one of the topics (see topic descriptions below). A brief description of each topic (along with accompanying scenarios) has been provided to all participants. Breakout groups should work through the following **General Questions** for each topic:

1. What key questions need to be addressed to move forward on this topic?
2. What scientific approaches could be used to address these questions?
3. What are the scientific or technology barriers and anticipated limitations to implementation of these approaches?
4. How does each key question/proposed approach rank according to time and impact? Use the priority matrix provided (see instructions below).

Priority matrix:

The priority matrix is designed to categorize projects in terms of their impact and timeline – this will help to match capabilities to projects. Rapporteurs for each breakout group will capture the prioritization of questions/proposed approaches on a priority matrix that will be visible to the group (note: rapporteurs will draw the priority matrix on a flip chart).



Priority matrix instructions:

- For each question/suggested scientific approach combination proposed by the group, indicate where on the chart it belongs

- "Short" time is < 3 years, "long" time is > 3 years; "low" impact will only incrementally advance a limited area of mixtures research, "high" impact will advance the field significantly across disciplines
- More than one combination can go in each square
- All squares do not need to be filled

Notes about Scenarios: The scenarios have been provided to offer potential direction for discussion. They can be used at the discretion of the chairperson in consultation with the breakout group. If conversation does not flow freely or if more direction is wanted, one or more of the scenarios can be addressed by the group. They are meant to provide examples of the kinds of problems that are applicable to the main topics. However, the group is not required to address any of the scenarios and is free to offer alternative scenarios for discussion. *All scenarios described below are fictional.*

Groups

Participant group assignments are shown below and appear in table format on page 35.
Observer group assignments are located in the observer directory (page 22).

Group 1 Topic – Modeling Mixture Toxicity: Constraints of Extrapolation

Group 1 Participants: John Bailer (Miami University), Lyndsey Darrow (Emory University), Mike DeVito (NIEHS NTP), Gregg Dinse (NIEHS DIR), Chris Gennings (Virginia Commonwealth University), L. Earl Gray (EPA NHEERL), **Bethany Hannas (Rapporteur; EPA NHEERL)**, **Richard Hertzberg (Chairperson; Biomathematics Consulting)**, Marike Kolossa-Gehring (Umweltbundesamt/ Federal Environment Agency), Anna Lowit (EPA OPP), Paul Price (Dow Chemical Company), Glenn Rice (EPA NCEA), Cynthia Rider (NIEHS NTP), James Rusling (University of Connecticut), Bill Suk (NIEHS DERT), Raymond Yang (Colorado State University)

Group 1 Topic Description: Two basic approaches for predicting the toxicity of mixtures that are promising for widespread application are: models that use single chemical dose-response data to calculate mixture toxicity (i.e. dose addition, response addition, and integrated addition) and models that compare the toxicity of a known mixture to estimate the toxicity of a related mixture (sufficient similarity). Dose Addition (DA), Response Addition (RA), and Integrated Addition (IA) are mathematical models that have been used to assess the toxicity of mixtures when the mixtures in question are relatively simple and individual dose-response data of the constituents is available. Whereas, approaches based on Sufficient Similarity (SS) have been suggested as a path forward in cases dealing with complex mixtures that are often ill-defined and when constituent dose-response data is not available. In this breakout session, participants will focus on questions that challenge the application of these approaches and discuss how they should be incorporated into biologically-based models (e.g. PBPK), or other relevant predictive models of mixture toxicity. General questions, example scenarios, and specific questions are provided below to help guide discussion.

General Questions:

1. What key questions need to be addressed to move forward on this topic?
2. What scientific approaches could be used to address these questions?
3. What are the scientific or technology barriers and anticipated limitations to implementation of these approaches?
4. How does each key question/proposed approach rank according to time? Use the priority matrix provided (see instructions above).

Group 1 Example Scenarios:

Scenario 1 – Modeling complex, low dose mixtures
Leachate from a particularly contaminated site has reached groundwater that supplies a small population with their drinking water. Chemical analysis of the groundwater identified over 100 chemicals that target a common signaling pathway. Although many of the chemicals have different primary targets, all of these chemicals have the capacity to disrupt the signaling pathway of interest. All chemicals are present well below the levels that would elicit significant toxicity individually. Is it possible to test the hypothesis that the 100 chemicals will contribute to dose additive toxicity and if so, what is the best approach to test this hypothesis?

Scenario 2 – Sufficient similarity of complex mixtures
The Gulf Oil Spill and subsequent remediation efforts (e.g. application of chemical dispersants) resulted in a complex and dynamic mixture. What is the best way to move forward on using a sufficient similarity approach to estimate the toxicity of this type of mixture with the example of polycyclic aromatic hydrocarbons (PAHs)? Consider the following specific issues: 1) what characteristics of the complex mixtures should be used to determine sufficient similarity of the unknown mixtures to the reference mixture(s) (e.g. concentration of the most potent component, ratios of top 10 components); 2) how many defined mixtures do you need as "references" to have confidence in the sufficient similarity of the unknown mixture; 3) what is the best approach for designing reference mixtures (e.g. design reference mixtures based on human exposures versus statistical approaches for designing mixtures that differ in systematically in components and ratios of components)?

Scenario 3 – Target tissue approach to applying mixture models
A population of factory workers is experiencing unusually high incidence rates of liver-related diseases and you suspect that some combination of exposures is responsible. You identify several chemical exposures that are known to target the liver, but through different biochemical pathways. What experimental support would you need to determine whether these chemicals are contributing to liver toxicity in an independent (i.e. response additive), dose additive, or interactive (synergistic or antagonistic) manner?

Group 2 Topic – Exposure Assessment: Making Sense of the Data

Group 2 Participants: David Balshaw (NIEHS DERT), Rosemary Castorina (University of California, Berkley), Brian Curwin (NIOSH), Julie Daniels (University of North Carolina, Chapel Hill), Paul Foster (NIEHS DNTP), **Julia Gohlke (Chairperson; University of Alabama, Birmingham)**, Kimberly Gray (NIEHS DERT), Grace Kissling (NIEHS DIR), Susan Korrick (Harvard Medical School), Richard Kwok (NIEHS DIR), Scott Masten (NIEHS DNTP), **Minerva Mercado-Feliciano (Rapporteur; NIEHS DNTP)**, Moiz Mumtaz (ATSDR), Chirag Patel (Stanford University), Woody Setzer (EPA NCCT), Rogelio Tornero-Velez (EPA NERL)

Group 2 Topic Description: The ability to identify and characterize exposures is rapidly advancing as new tools for characterizing exposure are developed and methods for chemical analysis improve and become more sensitive. Vast stores of data on potential co-exposures are being collected from programs such as the Exposure Biology Program, NHANES, and the evolving exposome project. However, translating exposure and health status data into a meaningful basis for designing relevant toxicological and epidemiological studies has been less clear-cut. How can we use these data to understand the contribution of environmental exposures to human disease? In this group, participants will focus on developing suggested paths forward in this area. General questions, example scenarios, and specific questions are provided below to help guide discussion.

General questions:

1. What key questions need to be addressed to move forward on this topic?
2. What scientific approaches could be used to address these questions?
3. What are the scientific or technology barriers and anticipated limitations to implementation of these approaches?
4. How does each key question/proposed approach rank according to time? Use the priority matrix provided (see instructions above).

Scenario 1 – Looking for unknowns

Humans are constantly exposed to mixtures of chemicals. Through biomonitoring programs, we are beginning to get picture of the chemicals to which people are most frequently exposed. However, the chemicals included in screening panels are often chosen because of their known effects. Conversely, sensor arrays and functional screens can provide information on exposures to multiple chemicals with less specificity. How can we best use exposure metrics to identify the chemicals that are likely to cause a human health problem without prior knowledge of what these chemicals might be?

Scenario 2 – Highly exposed populations

Exposures to chemical and nonchemical stressors tend to concentrate in certain populations. For example, a heavy concentration of manufacturing and highway pollution concentrated in geographical regions with a high percentage of people of lower socioeconomic status and decreased access to healthcare. How can we identify patterns of exposure in the most highly exposed populations, including both the most frequently occurring co-exposures and the maximum total exposures? Would epidemiological and toxicological studies focused on these populations provide a meaningful "worst case" scenario?

Scenario 3 – Getting a more complete exposure picture

Following the lessons of DDT and other persistent organic pollutants, current-use chemicals tend to be short-lived and therefore, it is difficult to accurately assess exposure in a population. Determination of accurate exposure profiles are further complicated by consideration of multiple short-lived chemicals with differing metabolic profiles. However, due to cost and sample limitations, it is often difficult to

move beyond “snapshot-in-time” exposure assessments. What are some practical approaches for getting more accurate assessments of multiple, short-lived chemical exposures? Consider both chronic or historic exposures and exposures from multiple routes.

Group 3 Topic – Reconciling Epidemiological and Toxicological Approaches to Mixtures

Group 3 Participants: Mike Babich (CPSC), Antonia Calafat (CDC), Caroline Dilworth (NIEHS DERT), Dale Hattis (Clark University), Irva Hertz-Piccioto (University of California, Davis), Kembra Howdeshell (NIEHS DNTP), **Todd Jusko (Rapporteur; NIEHS DIR)**, Freya Kamel (NIEHS DIR), Andreas Kortenkamp (University of London), Jennifer McPartland (Environmental Defense Fund), Sri Nadadur (NIEHS DERT), Susan Schantz (University of Illinois, Urbana-Champaign), Paige Tolbert (Emory University), Nigel Walker (NIEHS DNTP), **Tom Webster (Chairperson; Boston University)**, Beth Whelan (NIOSH)

Group 3 Topic Description: Epidemiology intrinsically involves consideration of complex exposure scenarios that include both chemical and non-chemical stressors. Against this backdrop, the probability of an association between select chemicals or groups of chemicals and disease outcomes is evaluated (i.e. chaos → order). The toxicological approach to mixtures is the direct opposite, wherein chemical mixtures are designed and carefully controlled to provide information on the joint action of concurrent exposures to multiple chemicals and the information gathered is then extrapolated outward to real-world exposure scenarios (i.e. order → chaos). In this group, participants will focus on research strategies that incorporate lessons from both disciplines to increase our understanding of the role of environmentally-relevant mixtures in human disease.

General questions:

1. What key questions need to be addressed to move forward on this topic?
2. What scientific approaches could be used to address these questions?
3. What are the scientific or technology barriers and anticipated limitations to implementation of these approaches?
4. How does each key question/proposed approach rank according to time? Use the priority matrix provided (see instructions above).

Scenario 1 – Dose addition in epidemiology

A mixtures study to assess the toxicity of 18 common workplace chemicals that target the same tissue was conducted in an animal model. The findings indicated that these chemicals acted in a dose additive manner to contribute to the toxicity of the mixture. An epidemiology study has found a positive association between one out of the 18 measured chemicals, which were all present in the population at relatively low concentrations, and development of disease in the target tissue. Are there preferred methods for grouping the 18 chemicals in the epidemiology study to find an association between the disease and the total mixture exposure?

Scenario 2 – Biomarkers approach

Methods have been developed for using a biomonitoring approach to measure the total burden of mixtures of chemicals that share a common target (e.g. using biomarkers of exposure, effect, or response). What are the advantages and disadvantages of this approach? How can we better integrate these biomarkers across epidemiological, toxicological, and clinical sciences?

Scenario 3 – Statistics and epidemiology

Decreased power to determine significant associations is often cited as a major problem in assessing joint effects or effect modification of multiple chemicals in epidemiological studies. Are there study designs or statistical methods that could increase the power for multiple chemical assessments? Another major problem in epidemiological studies where co-exposure is common is an inability to separate out effects of individual chemicals. Are there approaches (e.g. in vivo studies, novel statistical

approaches, etc.) that could be used to discern the contributions of the individual components to the observed outcome?

Group 4 Topic – Chemical Interactions: Predicting the Unpredictable

Group 4 Participants: Scott Auerbach (NIEHS DNTP), **Mamta Behl (Rapporteur; NIEHS DNTP)**, Christopher Borgert (Pharmacology and Toxicology, Inc.), Abee Boyles (NIEHS DNTP), David Christiani (Harvard School of Public Health), **Deborah Cory-Slechta (Chairperson; University of Rochester)**, Kevin Crofton (EPA NHEERL), Paul Feder (Battelle), Amy Herring (University of North Carolina, Chapel Hill), Andrew Hotchkiss (EPA NCEA), Elizabeth Maull (NIEHS DNTP), David Lawrence (Wadsworth Center), Ronald Lorentzen (FDA), Keith Shockley (NIEHS DIR), Claudia Thompson (NIEHS DERT)

Group 4 Topic Description: One key roadblock to understanding the effects of complex mixtures has been the insurmountable task of evaluating the toxicity of what is seemingly an infinite number of potential chemical combinations. The evolving fields of computational chemistry and systems biology coupled with high-throughput/high-dimensional screening tools offer the potential to characterize chemical interactions with high efficiency. However, in order to move this area of research forward a number of issues must be addressed, the most important of which is: what constitutes a biologically meaningful chemical interaction (i.e. an interaction that translates to an in vivo context) in an in vitro or in silico system and what approach(es)/tool(s) (e.g. SAR, genomics, cell biology, etc.) are likely to be most effective at addressing the latter question? In this breakout session, participants will address the types of in vitro assays that can potentially be used to predict in vivo chemical interactions and discuss how they can best be applied to provide useful information. General questions, example scenarios, and specific questions are provided below to help guide discussion.

General questions:

1. What key questions need to be addressed to move forward on this topic?
2. What scientific approaches could be used to address these questions?
3. What are the scientific or technology barriers and anticipated limitations to implementation of these approaches?
4. How does each key question/proposed approach rank according to time? Use the priority matrix provided (see instructions above).

Scenario 1 – Understanding interactions

Although interactions are perceived as a significant challenge in the effort to predict the toxicity of mixtures, they are relatively infrequent and often subtle. Before embarking on efforts to identify interactions, it is important to agree on what constitutes a significant interaction. For example, what kind of increase above the "no interaction" case represents a significant synergistic interaction (i.e. is a statistically significant deviation enough or does it require a level that is biologically significant)? For the sake of identifying interactions, is dose addition an appropriate default for the "no interaction" scenario, since it provides a more conservative estimate of additive toxicity in the overwhelming majority of cases?

Scenario 2 – Translation of interactions from in vitro to in vivo

Assuming that a high throughput method is developed that is able to systematically identify chemical interactions, how can we move from in vitro identification of interactions to in vivo characterization of these interactions? What kinds of targeted testing approaches should be employed to provide data that will help develop fundamental principles of chemical interactions?

Scenario 3 – Gene-environment interactions

Chemical-chemical interactions are the focus of the majority of work in the interaction arena. However, it is an accepted fact that both genetics and environmental (e.g. chemical, nutrition, life-stage, lifestyle)

factors can affect the same pathways and can play a role in the etiology of certain diseases. What are some research strategies and considerations for identifying important gene-environment interactions?

Group 5 Topic – Mixtures across Time

Group 5 Participants: Dana Barr (Chairperson; Emory University), Chad Blystone (NIEHS DNTP), George Bollweg (EPA Region 5), Herb Buxton (USGS), Danielle Carlin (NIEHS DERT), Stephania Cormier (Louisiana State University), June Dunnick (NIEHS DNTP), Russ Hauser (Harvard School of Public Health), Jerry Heindel (NIEHS DERT), Shyamal Peddada (NIEHS DIR), Joanna Matheson (CPSC), Andrew Salmon (California EPA), Jane Ellen Simmons (EPA NHEERL), Linda Teuschler (EPA NCEA), **Sheetal Thakur (Rapporteur; NIEHS DNTP)**, Tracey Woodruff (University of California, San Francisco)

Group 5 Topic Description: Mixtures research has often focused on concurrent exposures to multiple chemicals. However, constituent chemicals have different toxicokinetic and toxicodynamic properties, so that even concurrent exposures do not overlap entirely. An additional layer of complexity occurs with real-world exposures, as chemicals can be present at different life stages with their unique sensitivities (e.g. developmental versus adult exposures). In this breakout session, participants will address the types of studies required to elucidate principles of mixture exposures across time. General questions, example scenarios, and specific questions are provided below to help guide discussion.

General questions:

1. What key questions need to be addressed to move forward on this topic?
2. What scientific approaches could be used to address these questions?
3. What are the scientific or technology barriers and anticipated limitations to implementation of these approaches?
4. How does each key question/proposed approach rank according to time? Use the priority matrix provided (see instructions above).

Scenario 1 – Assumptions of averaging

A person is exposed to different magnitudes of various chemicals during different stages of their lives. However, regulatory agencies typically average exposures across a lifetime. What are the uncertainties associated with this practice? Are there specific chemical classes, life-stages, or health outcomes where averaging exposures is inappropriate? What are suggested practical alternative approaches for defining lifetime exposure?

Scenario 2 – Biomarkers for measuring aggregate exposures

Biomarkers are available to measure various exposures (e.g. micronucleus or comet assays for exposure to mutagenic chemicals). These biomarkers offer promise for assessing exposures across time. What should be considered in developing biomarkers for measuring exposures across time? Is it possible to tease apart the effects of chemical mixtures from non-chemical stressors (e.g. disease, psychosocial stress) using biomarkers? Will biomarkers be useful in elucidating principles related to exposures across time?

Scenario 3 – Sensitive life stages

Endocrine active compounds can have organizational and activational effects depending on the timing of exposure. What experimental approaches would be best to elucidate the combined effects of exposure to endocrine active chemicals during development, puberty, and adulthood? Besides endocrine disrupting chemicals, what other types of targets will provide useful scenarios to address this issue (e.g. initiators and promoters in cancer, developmental versus adult neurotoxicity) and which cases offer the most public health impact (i.e. available experimental model, relevance to human exposures/disease)?

Recommended Reading List

General References

Kortenkamp, A., T. Backhaus, and M. Faust. 2009. State of the Art Report on Mixture Toxicity: European Commission - Environment. http://ec.europa.eu/environment/chemicals/pdf/report_Mixture%20toxicity.pdf

Lökke, H. 2010. Novel methods for integrated risk assessment of cumulative stressors - Results from the NoMiracle project. *Science of the Total Environment* 408 (18):3719-3724.

Sexton, K., and D. Hattis. 2007. Assessing cumulative health risks from exposure to environmental mixtures - Three fundamental questions. *Environmental Health Perspectives* 115 (5):825-832.

U.S. Environmental Protection Agency. 2000. Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=20533

Topic 1: Mixture Modeling

Krishnan, K., S. Haddad, M. Beliveau, and R. Tardif. 2002. Physiological modeling and extrapolation of pharmacokinetic interactions from binary to more complex chemical mixtures. *Environmental Health Perspectives* 110:989-994.

Teuschler, L. K. 2007. Deciding which chemical mixtures risk assessment methods work best for what mixtures. *Toxicology and Applied Pharmacology* 223 (2):139-147.

U.S. Environmental Protection Agency. 2009. Determinants of Whether or Not Mixtures of Disinfection By-Products Are Similar. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=205944#Download

Topic 2: Exposure Assessment

NIOSH Mixed Exposures Research Agenda - A Report by the NORA Mixed Exposures Team. 2004. http://www.cdc.gov/niosh/docs/2005-106/2005-106d.html

Preau, J. L., L. Y. Wong, M. J. Silva, L. L. Needham, and A. M. Calafat. 2010. Variability over 1 Week in the Urinary Concentrations of Metabolites of Diethyl Phthalate and Di(2-Ethylhexyl) Phthalate among Eight Adults: An Observational Study. *Environmental Health Perspectives* 118 (12):1748-1754.

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Day 1 Breakout Session – Discipline-based groups

Epidemiology	Risk Assessment	Statistics	Biology/Toxicology	Exposure Science
Rosemary Castorina	Mike Babich	John Bailer	Chad Blystone	Scott Auerbach
David Christiani	George Bollweg	Abee Boyles	Christopher Borgert	David Balshaw
Julie Daniels	Mike DeVito	Gregg Dinse	Danielle Carlin	Dana Barr
Lyndsey Darrow	Andrew Hotchkiss	**Paul Feder (chair)**	Stephania Cormier	Herb Buxton
Caroline Dilworth	Ron Lorentzen	Chris Gennings	Deborah Cory-Slechta	**Antonia Calafat (chair)**
Kim Gray	Anna Lowit	Dale Hattis	Kevin Crofton	Brian Curwin
Russ Hauser	Jennifer McPartland	Amy Herring	Paul Foster	June Dunnick
Irva Hertz-Piccioto	**Moiz Mumtaz (chair)**	Richard Hertzberg	Julia Gohlke	Richard Kwok
Kembra Howdeshell	Glenn Rice	Grace Kissling	Earl Gray	David Lawrence
Freya Kamel	Andrew Salmon	Liz Maull	Jerry Heindel	Joanna Matheson
Susan Korrick	Bill Suk	Shyamal Peddada	Marike Kolossa-Gehring	Sri Nadadur
Scott Masten	Linda Teuschler	Cynthia Rider	Andreas Kortenkamp	Chirag Patel
Paige Tolbert	Claudia Thompson	Woody Setzer	Susan Schantz	Paul Price
Tom Webster	Nigel Walker	Keith Shockley	Jane Ellen Simmons	Jim Rusling
Beth Whelan (chair)	Tracey Woodruff		**Raymond Yang (chair)**	Rogelio Tornero-Velez

Day 2 Breakout Session – Focus on Topics

Topic 1: Modeling	Topic 2: Exposure	Topic 3: Epi and Tox	Topic 4: Interactions	Topic 5: Temporal Mixtures
John Bailer	David Balshaw	Mike Babich	Scott Auerbach	**Dana Barr (chair)**
Lyndsey Darrow	Rosemary Castorina	Antonia Calafat	Christopher Borgert	Chad Blystone
Mike DeVito	Brian Curwin	Caroline Dilworth	Abee Boyles	George Bollweg
Gregg Dinse	Julie Daniels	Dale Hattis	David Christiani	Herb Buxton
Chris Gennings	Paul Foster	Irva Hertz-Piccioto	**Deborah Cory-Slechta (chair)**	Danielle Carlin
Earl Gray	**Julia Gohlke (chair)**	Kembra Howdeshell	Kevin Crofton	Stephania Cormier
Richard Hertzberg (chair)	Kim Gray	Freya Kamel	Paul Feder	June Dunnick
Marike Kolossa-Gehring	Grace Kissling	Andreas Kortenkamp	Amy Herring	Russ Hauser
Anna Lowit	Susan Korrick	Jennifer McPartland	Andrew Hotchkiss	Jerry Heindel
Paul Price	Richard Kwok	Sri Nadadur	David Lawrence	Joanna Matheson
Glenn Rice	Scott Masten	Susan Schantz	Ronald Lorentzen	Shyamal Peddada
Cynthia Rider	Moiz Mumtaz	Paige Tolbert	Liz Maull	Andrew Salmon
Jim Rusling	Chirag Patel	Nigel Walker	Keith Shockley	Jane Ellen Simmons
Bill Suk	Woody Setzer	**Tom Webster (chair)**	Claudia Thompson	Linda Teuschler
Ray Yang	Rogelio Tornero-Velez	Beth Whelan		Tracey Woodruff

Notes

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