ADVANCING THE NEXT GENERATION (NEXGEN) OF RISK ASSESSMENT: THE PROTOTYPES WORKSHOP

EPA/600/R-11/100
September 2011
http://epa.gov/risk/nexgen

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NOVEMBER 1-3, 2010

RESEARCH TRIANGLE PARK, NORTH CAROLINA

National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC 20460

DISCLAIMER

This document summarizes the discussions presented at an experts' workshop held Nov 1-3, 2010, in Research Triangle Park, NC. The purpose of the workshop was to review conceptual approaches to prototype development. This document is not all inclusive or binding. Conclusions and recommendations to the U.S. EPA may not represent full consensus. The views expressed in this document are those of the Workshop Participants and do not necessarily reflect the views and policies of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This document was prepared initially by ICF Inc., an EPA contractor (Contract No EP-C-09-009 Work Plan, Budget, Work Assignment 1-37). This report captures the main points and highlights of the meeting. It is not a complete record of all detailed discussion, nor does it embellish, interpret, or enlarge upon matters that were incomplete or unclear. Statements represent the individual views of each participant.

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1. Background and Objectives of the Workshop

The U.S. Environmental Protection Agency (EPA), in collaboration with other federal and state agencies, is advancing the next generation of risk assessment through a project named "NexGen." The project aims to better incorporate recent advances in molecular and systems biology into risk assessment, thereby potentially making risk assessments faster, less expensive and/or more scientifically robust. This transition is expected to evolve over the next 10-20 years as new knowledge and approaches become available. NexGen partner organizations include the National Institute of Environmental Health Sciences (NIEHS), National Toxicology Program (NTP), Centers for Disease Control/Agency for Toxic Substances and Disease Registry, National Human Genome Research Institute, and the State of California's Environmental Protection Agency (CalEPA).

EPA convened a 3-day expert workshop on November 1–3, 2010, in Research Triangle Park, North Carolina to discuss a draft framework, early draft prototypes, research, and other project elements. The workshop sought individual input, rather than consensus, in meeting its discussion goals.

Days 1 and 2 of the workshop focused on deliberative drafts of data rich prototype health assessments (i.e., Tier 3 assessments). The goals for the first two days were to:

- Refine early-stage (i.e., early draft) case study health effects assessments of data-rich chemicals, referred to as "Prototypes."
- "Reverse engineer" from molecular system biology data to "known" public health risk estimates based on in vivo human and animal bioassay data and, ultimately, demonstrate proof of concept, elucidate value of information, and characterize decision rules with the final prototypes.
- Summarize options for expanded future work and research needs.

Day 3 of the workshop focused on approaches applicable to assessing the potential risks posed by chemicals with limited or no traditional data (i.e., Tier 2 assessments). The goals of the third day of the workshop were to:

- Identify and discuss a wider variety of new data, methods, and knowledge to help characterize data limited chemicals.
- Consider how this information may augment, extend, or replace traditional data in health assessment.
- Summarize options for expanded future work and research needs.

The workshop was attended by approximately 40 federal and non-federal experts and 80 EPA and NexGen partner organization staff.

2. Introduction

Dr. Ila Cote, EPA, provided a brief introduction to the NexGen project and the meeting. She noted that the NexGen Prototype assessments are not intended to change the current risk assessments for the specific chemicals evaluated, but rather will attempt to demonstrate proof of concept, characterize the value of information, and explore decision rules for appropriate use of molecular and systems biology data in general. For this initial effort, a narrowly defined set of diseases/disorders, causative chemicals, and mechanisms of action were used. Prototypes also rely on illustrative rather than comprehensive

data sets. A broad set of molecular systems biology disciplines and assays will be considered as data are available.

Day 1 continued with two plenary presentations of a proposed framework for NexGen risk assessment and an overview of NexGen risk assessment issues, each of which was followed by an associated question and answer session. Four breakout groups—one for each of the four draft Prototypes—deliberated for the remainder of Day 1 and the morning of Day 2 and reported back to the plenary on Day 2. A panel discussion on cross-cutting themes of the breakout groups concluded Day 2 of the workshop.

2.1. Towards a Framework for NexGen Risk Assessment

Dr. Daniel Krewski, of the University of Ottawa and Risk Sciences International, presented a draft framework for conducting NexGen risk assessments. This NexGen Risk Assessment Framework is comprised of the following three building blocks: *Toxicity Testing in the 21st Century* (NRC, 2007), McLaughlin Centre Framework for Population Health Risk Assessment (Krewski et al., 2007), and *Science and Decisions: Advancing Risk Assessment* (NRC, 2009)

Independently, each of these building blocks serve to advance the field of risk assessment. For example, the first building block, National Research Council's (NRC's) *Toxicity Testing in the 21st Century* report, provides a vision for the future of toxicity testing based on the identification and prevention of perturbations of toxicity pathways. The vision presented in this report focuses on predicting chemical properties and characteristics, where possible and appropriate, by using computational tools. The vision also emphasizes incorporating high throughput approaches using cells or cell lines, preferably of human origin, into toxicity testing. The risk assessment goal is to employ high throughput assays and computational methods in toxicology to efficiently identify potential toxic agents, and subsequently establish human exposure guidelines that will avoid pathway perturbations. Figure 1 illustrates the risk assessment process, where the four stages of risk assessment (as presented in the NRC's "Red Book," *Risk Assessment in the Federal Government: Managing the Process* (NRC, 1983)), overlap with the new scientific tools and technologies. This figure demonstrates that while this new NRC vision incorporates the traditional four stages of risk assessment, the technical activities conducted within each stage will change dramatically.

The second building block, the *McLaughlin Centre Framework for Population Health Risk Assessment*, addresses risk assessment on a population level. This Framework is based on the concept of integrating traditional human health risk assessment with population risk assessment, a comprehensive assessment of health risks in the general population based on biological, genetic, environmental, occupational, social, and behavioral determinants of health. By bringing together these two parallel fields and recognizing that there are a number of determinants of health outcomes, this Framework offers a more multidisciplinary and robust approach to the assessment and management of health risk issues, which is important for better assessing potential health risks to human populations.

The NRC's *Science and Decisions: Advancing Risk Assessment* report, informally known as "The Silver Book," is the third building block of the NexGen Risk Assessment Framework. This report provides guidance on new directions in risk assessment methodology, such as evaluating uncertainty and variability in risk in order to derive a distribution of risks that can be used as a more complete basis for risk management decision making. While the Silver Book includes the four core phases of risk assessment as presented in "The Red Book" (NRC, 1983), it places greater emphasis on the first phase of problem formulation and scoping. Specifically, the Silver Book promotes early and thorough planning that tailors the assessment's level and complexity to the demands of the problem and provides approaches for obtaining clearer estimates of population risk and advancing cumulative risk assessment.

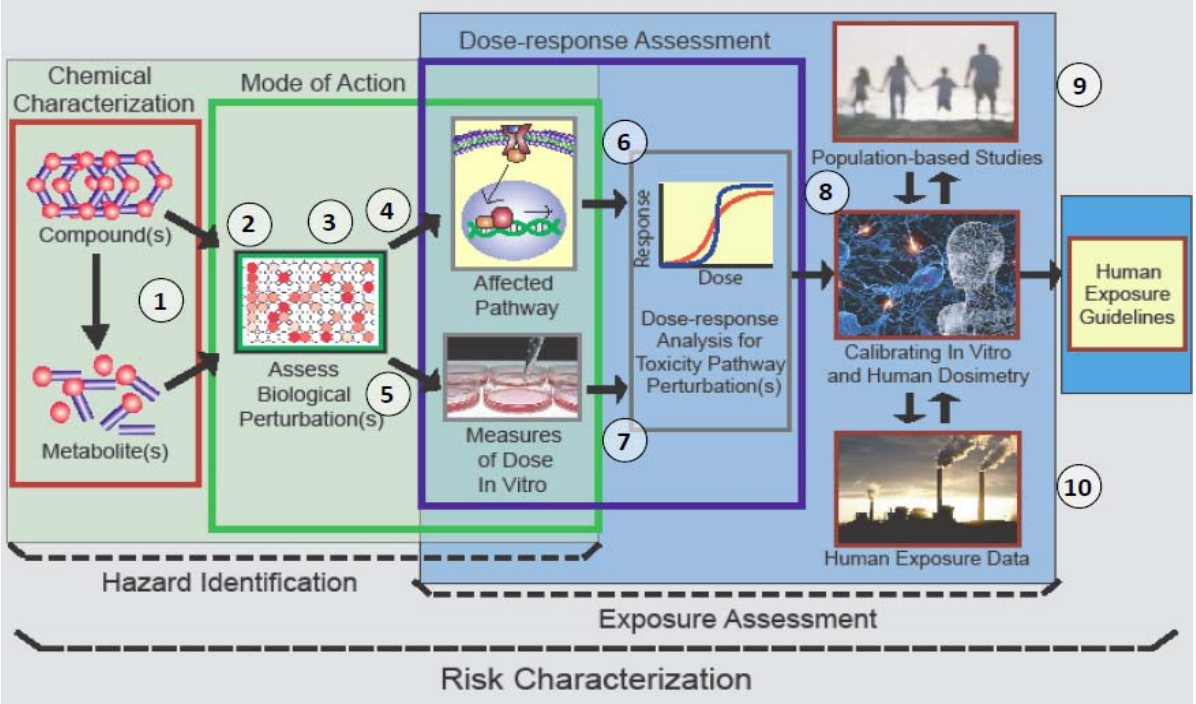

Figure 1. Scientific Tools and Technologies that can be used in Risk Assessment. (1) High throughput screens, (2) Stem cell biology, (3) Functional Genomics, (4) Bioinformatics, (5) Systems biology, (6) Computational systems biology,(7) Physiologically-based pharmacokinetic models, (8) Structure-activity relationships, (9) Biomarkers, (10) Molecular and genetic epidemiology.

Together, these three building blocks can help produce a NexGen framework that will shape the future of health risk science. However, it is important to keep in mind that although each of these buildingblocks will be useful in identifying future risk assessment principles, procedures, and practices, their integration into an overarching NexGen Risk Assessment Framework will continue to evolve as the technologies and the decision analysis approaches develop. As a result, the challenge in the near term is to begin the process of integrating these components into an overarching NexGen framework and developing acceptance of this broader view as risk assessment is transitioned from the current to the new paradigm in order to meet the demanding goals of human health risk assessment into the 21st century. An additional goal would be to build capacity for analyzing the data from the most recent scientific advances and incorporating them into risk assessment.

2.2. NexGen Risk Assessment Issues

The challenges and opportunities for NexGen risk assessment were addressed by Dr. Weihsueh Chiu of the Environmental Protection Agency's National Center for Environmental Assessment, Office of Research and Development. Dr. Chiu first identified the key challenges facing NexGen quantitative dose-response assessment as integration of new data and models, including high-throughput systems, in NexGen risk assessment; production of higher throughput assessments; definition of key terms like "adversity" from a quantitative perspective; prediction of metabolism and effects at environmental levels using in vitro assays; integration of assessments across different biological and temporal scales and determination of uncertainty and variability across these scales; assessment of cumulative interactions of multiple stressors; and achievement of diverse risk management goals.

Dr. Chiu outlined the previously proposed approaches to addressing these challenges, which included: (1) a procedurally simple approach similar to a point-of-departure/uncertainty factor approach (consistent with *Toxicity Testing in the 21st Century*), (2) a probabilistic dose-response assessment addressing incremental risk through evaluation of apical endpoints at the individual and population level, and (3) a biologically based dose-response modeling to predict apical endpoints. This would provide a quantitative link between precursor effects and adversity or risk, and a mechanistic basis for assessing cumulative endpoints. He noted, however, that there are some disadvantages to each approach individually, and existing proposals did not respond to all of the challenges for NexGen risk assessment. As a result, Dr. Chiu proposed a three-pronged approach to fill the remaining gaps. The first prong would involve a point-of-departure–based approach for screening and/or prioritization of chemicals, but augmented, as needed, by considerations for susceptibility and background conditions, as well as possibly probabilistic methods. The second prong would be an "off-the-shelf" adaptation of existing human biomarkers and prediction models, taking advantage of existing biomedical knowledge, in order to quantify different degrees effects across a population and integrate the effects of different stressors. The third prong would further extend this approach through the use of new (likely molecular) biomarkers and prediction models developed by integrating the next generation of biological data and understanding.

Dr. Chiu's three-pronged approach was well-received by the other participants. The need for further collaboration with the medical community and other entities was proposed as a source of additional research and an important component of developing needed expertise.

3. Data-rich Prototype Breakout Groups

Each Prototype breakout group considered the following general discussion questions:

1. Are the right questions being asked?
2. Have the most useful methods been identified?
3. What kinds of data are anticipated and how can results be used to (a) identify potential adverse health effects, (b) inform us about dose-response, (c) help link dose to exposures, and (d) improve our understanding of important issues such as sensitive subpopulations and mixtures exposures?
4. What are the weight of evidence criteria, key uncertainties, and areas of scientific disagreement that require particular consideration?

Breakout groups also discussed prototype-specific questions. Summaries of the breakout group discussions are provided in the sections that follow in the order shown in the accompanying text box.

Initial Draft Prototypes Discussed at the Workshop

- Lung Injury – Ozone
- Developmental Impairment – Thyroid Hormone Disruptors
- Cancer – Polycyclic Aromatic Hydrocarbons (PAH)
- Cancer – Benzene Prototype

3.1. Lung Injury – Ozone

The goal of this prototype is to evaluate the utility of molecular biology data in understanding health outcomes and the feasibility of developing a biologically based dose-response (BBDR) model using an integrated systems approach combining laboratory experiments and computational modeling for the

data-rich chemical ozone. This prototype is designed to help develop an in vitro model to predict in vivo effects for selected endpoints in toxicity pathways associated with ozone-induced lung injury and inflammation. Furthermore, this prototype may illustrate how BBDR modeling can be used to integrate diverse kinds of data at different scales of biological organization and how a toxicity pathway approach can be used to better understand the cellular and molecular events that underlie ozone-induced inflammation.

Dr. Robert Devlin, EPA's National Health and Environmental Effects Research Laboratory (NHEERL), led the Lung Injury – Ozone prototype breakout group with a presentation outlining the aims of the project, the proposed study design, and the basis for choosing ozone as a prototype chemical. Dr. Devlin noted that two projects are associated with this prototype, the first of which asks, "Can we expose cells in vitro and in vivo, run microarray analyses, and determine how well in vitro data can predict in vivo responses?" And the second project asks, "Can we model in vitro intracellular events that might explain why cells are making Interleukin-8 (IL-8)?"

The Lung Injury – Ozone breakout group discussion was loosely based around these questions and the nine more specific questions. The discussion points during the breakout group sessions were highly representative of the set of comments received prior to the workshop in response to these nine questions. The main topics of discussion were focused on clarification of the prototype terminology and approach, whether animal data are needed to supplement human data, whether toxicity pathway data are quantitative enough to use as model inputs, key considerations for incorporating population variability into models, whether other toxicants should be assessed concurrently with ozone to help validate models, whether multiple cell lines should be employed, whether the toxicity pathway approach is appropriate for this project, and whether there is value added from upstream real-time measurement of biomarker events.

The Lung Injury – Ozone breakout group concluded that in vitro modeling could potentially be used as a tool to rank the toxicity of various pollutants for risk assessment, and correlations could be sufficient to allow for prioritization of in-depth, chemical-specific analysis. Most participants were in support of supplementing the human data with animal data to allow for testing of more doses, time points, and measured variables. Some participants argued, however, that animal data was not needed for this phase of the project, but could be useful to fill data gaps for chemical-specific analyses beyond ozone. Ultimately, the group agreed that animal data was necessary for building a quantitative model because the human data alone was insufficient. One participant suggested that a comprehensive review of what is already known about ozone be conducted first to establish what kind of modeling is feasible given the animal, human, and in vitro data that already exist.

A few participants emphasized the importance of collecting data for other toxicants associated with an inflammatory response, and the group agreed that this was necessary for the acceptance of a predictive model within the scientific community. One participant specifically noted that the data used to generate a model cannot then be used to validate that model, thus generally necessitating incorporation of additional data from the same or other toxicants. The participants also generally agreed that if the budget could support only analysis of multiple toxicants or analysis of effects in multiple cell lines, priority should be given to the multi-toxicant approach. However, participants agreed that using multiple cell lines offered another valuable perspective, but that those cell lines would have to be well characterized. The group agreed that at this stage of the process, it was appropriate to use human primary cells.

The participants reached few conclusions on strategies to incorporate population variability into the in vitro models. While some participants argued that it was important to understand the "normal" state first by observing variability among healthy human cells, others argued that cells from humans with

susceptibility factors such as asthma should be used as the basic model. This discussion raised a number of important questions about whether toxicity pathways would be different for high responders (i.e., susceptible populations), whether the data are quantitative enough to distinguish between high and low responders, and whether sample sizes would be large enough to capture population variability. In the end, the question of whether genetic variability can be built into in vitro studies remained unanswered.

The Lung Injury – Ozone breakout group expressed some confusion over the meaning of toxicity pathways and the relevance of this approach to the ozone project. One participant remarked that multiple "toxicity pathways" are likely to lead to the same effects, and that trying to define a "toxicity pathway" in light of the many aspects of homeostasis is highly problematic. Another participant recommended that the term "toxicity pathway" be changed to "toxic signature," recognizing that there are examples of consistent signatures across different compounds, while another participant recommended using "stress pathways" instead because many of these pathways have already been identified and are distinct.

Finally, the group discussed the relevance of modeling upstream events in this prototype and strongly agreed that experimental data on the kinetics and dynamics for immediate, far upstream events (e.g., calcium changes, free radicals) was needed in addition to kinetic and dynamic data for midstream events (e.g., signal transduction pathways), and final downstream events (e.g., transcription factor activation). Participants further suggested that some of the highest-priority endpoints to analyze might be catalase activity, nuclear respiratory factor (Nrf), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), glucose metabolisms/solute carrier (SLC) transporters , and lipid mediator metabolism (cytochrome 4f15). One participant also suggested identifying five to ten variables in each known stress pathway to measure and map to microarray analyses.

3.2. Developmental Impairment – Thyroid Hormone Disruptors

Drs. Mary Gilbert and Kathleen Raffaele, EPA, opened the Developmental Impairment – Thyroid Hormone Disruptors prototype breakout group by discussing the relationship between endocrine disruption and the thyroid hormonal pathway. Disruption of thyroid hormone homeostasis has been linked to adverse neurological and developmental effects, including low intelligence and learning disabilities, making it a significant public health concern. The hypothalamic-pituitary-thyroid (HPT) axis, an important regulator of neurodevelopment, is well-studied and can provide insight into the toxicity pathways for thyroid hormone disruption. Distinct elements in these pathways are known to be disrupted by exposure to many environmental toxicants, including many chemicals that have been evaluated by EPA's IRIS Program. Available screening assays provide important data regarding specific elements of the toxicity pathways and many more are currently under development.

The ultimate goal of the breakout group was to develop approaches for predicting adverse impacts on brain development from exposure to environmental chemicals via interference with thyroid hormone homeostasis. The breakout group recognized, however, that developing such approaches would be difficult due to current data gaps. For example, an adverse outcome in the brain is not induced by direct interaction between the chemical and the brain, but is secondary due to changes in thyroid status. Additionally, the relationship between the magnitude of disruption and adverse outcome is not well characterized; timing and magnitude of disruption is critical for predicting an adverse impact on brain development. Furthermore, the mechanism by which thyroid hormone disruption interferes with brain development is not fully understood. In order to more accurately and efficiently screen chemicals for potential to disrupt thyroid hormone homeostasis, the group focused on the following three issues: (1) assay identification and refinement, (2) algorithm development for toxicity and hazard prediction, and (3) assay conduct, data analysis, and data reporting for risk assessment needs.

The participants addressed assay identification and refinement by stating that the ToxCast™ database, developed by EPA's National Center for Computational Toxicology, in collaboration with the National Institutes of Health National Chemical Genomics Center (NCGC) and NIEHS, needs to be expanded to include more assays that probe thyroid disruption toxicity pathways. They acknowledged that hepatic catabolism is well covered by available assays; however, there is minimal coverage of the other nodes in the HPT axis. While new assays are currently being developed, the breakout group identified opportunities for refining existing assays. The participants stressed the importance of comparing results from available assays to *in vivo* data from primary sources (e.g., ToxRef, EDSP, NTP), secondary sources (IRIS, ATSDR), and peer-reviewed literature. They also highlighted an existing opportunity to run known thyroid disrupting chemicals through available assays to better assess their predictive power and potentially elucidate other modes of action. For future assay development, the breakout group recommended incorporating quantitative capabilities to available assays to yield data for future use whenever feasible. They also suggested that assays be designed to cover more aspects of each pathway and assess across different nodes to assist in grouping of toxicants and reading across data.

Regarding algorithm development, the participants identified several keys to developing predictive algorithms including providing a probabilistic landscape of inputs, deriving a weight of evidence approach for integrating results from multiple assays, and utilizing new approaches for capturing higher level information (e.g., curve class descriptor and dose-response information for predictive modeling). Algorithm development is difficult because thyroid hormone disruptors potentially act on multiple molecular target sites and tissues, which is further complicated by the fact that extrathyroidal target tissues are less well understood than thyroidal target tissues and homeostatic pathways. Thus, the participants underscored the importance of addressing both direct and downstream impacts of perturbations to thyroid hormone homeostasis. For target sites such as hepatic catabolism and elimination of thyroid hormones, the participants demonstrated confidence in the ability to develop algorithms. For other nodes, however, more assay development is needed. They suggested that assay-specific and system-specific biological context be incorporated into the algorithms, and the results could then be ranked according to levels of confidence with the ultimate goal of optimizing assays for inclusion. Additionally, with sufficient concentrations and time points, it will be possible to model data and determine concentration-time-response curves.

In addition to reliable dose-response information, the participants agreed that interpretation, data analysis, and assay reliability are critical factors for use in risk assessment. Assays that take into account timing, sensitive life stages and population variability, and tissue-specific differences in response are desirable when assessing the numerous target sites potentially associated with thyroid hormone disruption. There also exists a need for concordance in responses between different types of tissues in order to achieve a systems integration approach; however, it is not clear what level of systems integration is necessary for using screening data for risk assessment. The breakout group concluded that the thyroid disrupting compounds will serve as a good initial case study. Despite clear data needs, the participants stressed that risk assessment is an iterative process and enough data exist currently to begin evaluating how approaches identified from the thyroid hormone disruption case study could inform regulatory decision making.

3.3. Cancer – Benzene

Dr. Martyn Smith, University of California–Berkeley, Dr. Bob Sonawane, EPA, and Dr. Kate Guyton, EPA, led the Cancer – Benzene prototype breakout group discussion. As discussed in the draft prototype, benzene exposure at high doses causes acute myeloid leukemia (AML) and myelodysplastic syndromes and has been associated with lymphoproliferative disorders including childhood lymphoblastic leukemia. Biological plausibility for a causal role of benzene (or its metabolites) in these diseases comes from its

genotoxic effects and toxicity to hematopoietic stem cells or progenitor cells, from which leukemias arise. The impact of this toxicity is manifested as lowered blood counts (hematotoxicity). However, the mechanism of action for benzene-induced leukemia is still unknown, making assessment of risk in the low-dose region uncertain. The draft prototype proposes a systems biology approach, encompassing toxicogenomic, epigenomic, and phenomic endpoints relevant to leukemia. The prototype proposes using a biomarker of early effect that is predictive of leukemia to examine dose-response relationship in low-dose region (e.g., hematotoxicity, chromosome changes and altered gene expression).

The Cancer – Benzene breakout group addressed four main questions: (1) what new data are available and can these new data and methods improve our understanding of risk in a meaningful way? (2) how can this new type of information best be incorporated into health assessments? (3) what new policies and procedures are needed? (4) what are the next steps to take to move forward with the goals set out in this prototype?

The breakout panel identified many sources of new data that can be used to further risk assessment procedures. Since the last EPA Integrated Risk Information System (IRIS) dose-response assessment was conducted for benzene in 2005, more than 60 new epidemiologic studies have been published and could be relevant for a NexGen assessment. A plethora of 'omics data is also now available, including work to identify the disease pathway initiated by benzene (or metabolites) exposure. Disease pathway data for benzene will establish a pattern and help identify effects from different chemicals that show a similar pattern. Hematoxicity and chromosome damage data, genetic factors (i.e., SNPs), toxicokinetic variability, life stage susceptibility (i.e., *in utero*), and birth defects data might be used to help understand the dose-response for benzene exposures. Recent studies have shown that pre-existing conditions, such as obesity and blood disorders, can increase an individual's susceptibility to benzene related diseases. Lastly, reproductive outcomes, such as reduced sperm count, are also showing potential as predictive endpoints for benzene-induced leukemia.

The breakout group discussed the best methods by which this new data can be used in the risk assessment paradigm. There was wide support to use the epidemiological data to verify the results from the new 'omics data, rather than incorporate the two data types together. Specifically, Dr. Smith suggested looking at the dose-response relationship of the new 'omics data to develop a point of departure. Some participants disagreed, stating that the epidemiological data and 'omics data will not align because the dosing regimens are varied and certain genes are expressed at low doses that are not at high doses and vice versa. If the 'omics' data can be used to support the interpretation of the epidemiologic studies, this will decrease the uncertainty of the assessment. Also, new data on preexisting conditions can be used to identify susceptible populations. Lastly, new mechanism data can possibly predict adverse co-exposures.

To incorporate this new data, new policies are needed, and procedures standardized, to ensure that the data can be compared across studies. New guidance and protocols are needed on the use of 'omics' data in a risk assessment. In addition to the guidance, training courses and communications are needed to support effective implementation and understanding of procedures among researchers and risk assessors.

The Cancer – Benzene breakout group recommended a number of research initiatives that could be pursued including: (1) developing a testing regimen that uses in vitro stem cells in a 3D niche; (2) exploring quantitative approaches (e.g., blood counts) for continuous health outcomes; (3) evaluating hematological data in a biomarker-based approach for parameters or states that predict leukemia risk; (4) conducting dose-response modeling of 'omics data and biomarker-based approaches to evaluate the predictability of comparing with the epidemiological data; (5) integrating single and multiple datasets (e.g., phenomics data, low exposure human studies, and disease-specific pathway

data) into a systems biology model that predicts risk from exposure to benzene; and (6) identifying data gaps and associated opportunities for model refinement.

3.4. Cancer – PAHs

Dr. Peter McClure and Ms. Heather Carlson-Lynch, Syracuse Research Corporation, led the breakout group discussion of their draft Cancer – PAHs prototype, which set out to evaluate whether 'omics data in combination with existing epidemiology, rodent bioassay data, and mechanistic data (1) can improve existing methods for evaluating human cancer risk of PAH mixtures, and (2) can lead to development of predictive tools for hazard identification and dose response for PAH mixtures. The initial objective of the project was to identify available 'omics data and examine how it could be used to inform human cancer risk assessment for PAHs.

Dr. McClure described the drivers for this case study. PAHs occur almost exclusively as complex mixtures, and in the environment, weathering alters mixture composition. Several complex PAH mixtures and/or occupations with PAH exposure have been shown to be carcinogenic in humans (e.g., coke oven emissions, diesel exhaust, tobacco smoke). Many individual PAHs and complex mixtures have been tested in animal bioassays and have been shown to be carcinogenic, but hundreds of known PAHs and most complex mixtures have not been tested. Given the universe of PAHs and potential mixtures of PAHs, testing all of them in carcinogenicity bioassays is not feasible.

Dr. McClure described the results of their literature review that examined studies of 'omics endpoints following exposure to benzo[a]pyrene and at least one other PAH. Their focus was on discriminating between carcinogenic and noncarcinogenic PAHs, high potency and low potency carcinogenic PAHs, and strongly genotoxic and less genotoxic PAHs and evaluating the carcinogenicity, genotoxicity, or potency of PAH mixtures relative to benzo[a]pyrene. The initial PAH Prototype analysis found that carcinogenicity in animal bioassays appears to correlate with modulation of genes in the p53 pathway. The results suggested that genes and gene products in the Mdm2-p53 network can serve as markers for DNA-damaging effect of PAH or PAH mixtures.

The Cancer – PAHs breakout group discussed the draft prototype analysis and concluded that while existing 'omics data are limited, they show promise for meeting the overall goal. They suggested that a directed research program will be required. In the short term, the group recommended identifying networks or pathways that serve as signatures for PAH-induced cancer. To support this, Dr. McClure presented the breakout group's short-term recommendations to the plenary:

- Take advantage of the literature for benzo[a]pyrene for further network or pathway inferences.
- Compare the benzo[a]pyrene 'omics signature with signatures for relevant cancers (skin, lung, bladder).
- Mine the literature and evaluate the weight of evidence for various pathways.
- Obtain and further analyze raw data from comparative studies of PAHs and mixtures.
- Evaluate consistency of 'omics data from in vitro and in vivo studies and across species.
- Assemble multiple data sets and consider possible meta-analysis.

The Cancer – PAHs breakout group also recommended assembling all information for 'omics and traditional bioassays to identify patterns associated with potency or carcinogenicity.

The group's longer-term recommendations were to design a specific research program strategy to relate 'omics data to carcinogenicity and/or cancer potency and obtain 'omics data in conjunction with apical endpoint data on a series of standardized complex mixtures and individual PAHs.

3.5. Panel Discussions and Cross-cutting Themes

Several questions developed prior to the workshop served as starting points for the panel discussions. The questions posed to each invited panelist are included in the text box to the right. In addition to providing answers to these questions, each panelist provided their insight on next steps for the NexGen initiative.

Day 2 Panel Discussion Questions

- Are we making progress in developing NexGen data and approaches that can be used in chemical assessments?
- What did we learn about using NexGen data and approaches to identify effects caused by chemicals?
- What did we learn about using NexGen data and approaches in quantitative assessments…
  - To evaluate relative potencies of similarly acting chemicals?
  - To account for susceptible populations in assessments?
  - To perform screening level risk assessments?

Dr. Krewski commented on the progress that has been made since 2007 in developing the context for toxicity testing in the 21st century, including advances in technologies, methodological approaches, and capacity. He indicated, however, that there is still a long path forward in terms of developing data to be used in a NexGen risk assessment context. He suggested that the NexGen concept as it currently stands, should be used as a concrete example of the direction in which risk assessment needs to proceed. Developing data to be used in a NexGen risk assessment will be iterative; therefore, it will be important to continually revisit the NexGen approach as new data emerges.

The second panelist, Dr. Bernie D. Goldstein, University of Pittsburgh, emphasized that a 20-year approach is realistic for this type of transformation in the field of risk assessment to be fully realized. He noted that the idea of starting with disease and working backwards is an enormous shift from the traditional approaches for toxicity testing and regulating chemicals; creating this shift will take time. He suggested that for future meetings, it would be beneficial to look at case studies that start with diseases (e.g., asthma, infertility) and work backwards. This would help in developing a better molecular understanding of disease. He also stressed that it is important to effectively communicate the reasons for improving approaches to risk assessment. Regardless of the number of tests and assays available, there will always be uncertainties associated with chemical risk assessment, which may result in chemicals with toxic effects entering into commerce. For this reason, more effort should be directed towards approaches and tools that can effectively manage the uncertainty aspects of risk assessment and fortify the decision making process.

Dr. Ken Ramos from the University of Louisville noted that this workshop is an effective step in the right direction and is taking place at the right time. The debate is no longer whether risk assessment can be advanced, but how risk assessment should be advanced. Going forward, he recommended asking more targeted research and application questions to enable the development of tangible products in a shorter timeframe. As new knowledge builds, risk assessors need new approaches for embracing and capitalizing on that knowledge, while still remaining scientifically grounded in a process. He cautioned that there is no need to reinvent the wheel; rather, it is important to look at existing resources and

applications (e.g., the NRC reports *Toxicity Testing in the 21st Century* and *Science and Decisions: Advancing Risk Assessment*) and ensure that they are being fully utilized.

Dr. Gary Ginsberg of the Connecticut Department of Public Health described some current limitations associated with transitioning from the traditional animal based toxicity testing to a new generation where toxicity testing data is derived from higher throughput systems. There are limitations in any system in terms of uncovering how they function and respond across nodes within a cell, across cells and tissues, and in regards to timing (e.g., periodicity, development). He noted that there is always the question of whether we are testing the right things at the right time within a system to effectively predict human biology; this question is even more relevant for in vitro systems as there is the potential for issues with simulating dosimetry in culture versus in vivo systems, as well as metabolic differences, and determining whether the cell types are biologically relevant (i.e., do they simulate human systems). There is also a limitation in determining population risk using in vitro systems. He concluded by sharing his thoughts on some immediate uses for NexGen data, which include conducting hazard identification (e.g., chemical screening against well anchored prototypes), understanding mechanistic pathways and responses (e.g., screening not only for toxicant fingerprints but also for various points along pathway), screening for chemical-chemical interactions, developing biomarkers, and conducting contextual dose-response modeling. A potential longer term use for NexGen data would be to conduct quantitative risk assessments.

Dr. Martyn Smith of the University of California-Berkley, highlighted the importance of starting to conceptualize how the emerging research will fit into the risk assessment process. He noted that a critical aspect of advancing the NexGen concept and advancing risk assessment is building funding capacity and providing opportunities for emerging scientists to further develop risk assessment approaches and concepts. For example, resources are needed to develop robust assays; before moving to high throughput testing, it is important to ensure that the assays that have already been developed are relevant for low throughput testing.

Dr. Frederic Bois, Institut National de l'Environnement Industriel et des Risques (INERIS), spoke about the deluge of data resulting from the European Union's REACH (Registration, Evaluation, Authorisation and Restriction of Chemical substances) regulation and how people are struggling with approaches to expedite the registration process. He noted that one of the lessons learned from this registration process is that since this is a multidisciplinary effort and, since people have different ways of interpreting the same terms, there is a need to establish a common vocabulary that allows everyone to communicate with each other. In terms of NexGen, he recommended developing guidance and a range of agreed upon approaches that will enable interaction between prototypes. However, he cautioned that care should be taken when extrapolating from prototypes to other chemicals; this will require thinking about the process and prioritization of the process so that the outcomes from these prototypes are more generically able to be applied. It is important to develop a continuum of chemical information that allows for risk based decision making within a variety of contexts.

Overall, the panel felt that it is important to determine which methods could be currently utilized in risk assessment, and to set priorities and target future research needs. Based on the four prototype discussions and presentations described in Section 2.4, the panel and workshop participants identified several cross-cutting research needs, which are presented in the following text box.

Days 1 & 2 Cross-Cutting Themes

- Develop specific plans for what molecular and systems biology approaches could be utilized in the near term
- Start using approaches as they currently exist and recognize that these approaches are iterative and will continue to develop in stages
    - Feedback into the development process as we learn new information
    - Refine initial data and approaches as needed
- Benchmark case studies against:
    - Suite of tools that are currently available
    - Risk assessment methodology criteria
    - Public health population outcomes and other human data, as available
    - NexGen framework
- Elucidate networks or related pathways that serve as signatures, i.e., we have identified many key events/nodes in toxicity pathways for four prototypes, but generally need to more broadly elucidate critical pathway(s)
    - Mine the literature and molecular biology databases, and evaluate the weight of evidence for various pathways
    - Use data-rich chemicals to extrapolate mechanisms and responses for data-poor chemicals when feasible
- Develop approaches that incorporate population variability and susceptibility that are biologically relevant (e.g., occurring at environmentally relevant concentrations)
- Obtain data on a series of standardized complex mixtures and develop approaches for analysis
- Further explore dose-response information to develop more informative dose-response curves
- Integrate and compare data sets (e.g., epidemiology, exposure, biomarker) in a systems biology approach to develop integrated models of human risk
- Evaluate the consistency of data obtained from in vitro and in vivo approaches across species
- Consider variabilities to the extent feasible, including assays, interspecies, intraspecies (i.e., male/female/lifestage/physiological condition) exposure scenarios, and progression of disease processes
- Identify: data gaps, opportunities for methods, model/assay refinement, and the needs for additional reseach, articulate options from future applications of molecular and systems biology to risk assessment

4. Day 3 – Approaches for Chemicals with Less Data (Tier 2 Assessments)

Dr. Stan Barone, EPA, opened Day 3 of the workshop. The focus of which was whether high-throughput screening assays can help solve the problem of the paucity of data for chemicals in the environment. The goal is to design new approaches that will allow us to:

- Screen and rank thousands of chemicals for further evaluation rapidly and relatively cheaply.
- Identify potential adverse effects and relative potencies for specific effects for hundreds of chemicals.
- Derive points of departure for many chemicals with limited data with the additional application of reverse dosimetry.
- Provide information on mixtures interactions.
- Provide EPA program offices with a way to address the many chemicals for which there are no or inadequate data, e.g., Office of Air and Radiation's National Air Toxic Assessment, urban air sheds, and residual risk, Office of Water unregulated contaminants, Superfund chemicals, and emergency urgent response (e.g., World Trade Center, Katrina, Gulf Oil spill).

Dr. Barone described how this collaborative effort involve the EPA Office of Research and Development labs and centers, NIEHS, NTP, NCGC, and the Centers for Disease Control/Agency for Toxic Substance and Disease Registry. The specific programs include, but are not limited to, ToxCast Phase I and II, Tox21, and NexGen. Dr. Barone presented risk assessment questions that were provided to the Day 3 speakers and considered by the panelists later in the day.

Day 3 Risk Assessment Questions

Hazard Identification

- How can key information be rapidly and effectively summarized in an automated fashion?
- What toxicity pathways are affected by the chemical(s) in question?
- What are the implications of pathway alteration for specific adverse effects?
- Can specific weight of evidence criteria for high throughput (HT)/high content (HC) assay data be articulated that would indicate a known, likely or suggestive relationship between chemical exposure and adverse effect?

Dose-Response

- How can relative potencies, and/or dose-response be estimated?
- Can upstream events that predict well characterized public health risks, based on traditional data, be identified?
- How can recent scientific advances help describe adaptation, additivity to disease background, & implications for low-response rates?
- How can recent scientific advances help describe probability of harm and uncertainty?

Both Hazard Identification and Dose-Response

- How can recent scientific advances help describe human variability and susceptible subpopulations?
- How can recent scientific advances help describe the impacts of exposures to mixtures?

4.1. Day 3 Presentations

Dr. Derek Knight, Approaches from the European Union: REACH

Dr. Derek Knight from the European Chemicals Agency (ECHA) presented an overview of ECHA's REACH, Registration, Evaluation and Authorisation of CHemicals Regulation.

Registration of substances is central to REACH and the evaluation, authorization, and restrictions processes for chemicals rely on the registration data collected. There is a system of targeted registration deadlines so that the highest priority chemicals will be registered first. Thus, the first phase of registration includes high production and high volume chemicals (HPV), substances that are carcinogenic, mutagenic and toxic to reproduction, and medium tonnage substances that are classified as toxic to aquatic organisms and that may cause long-term adverse effects. ECHA expected that approximately 4,500 substances would be registered by the November 30, 2010 deadline for Phase I chemicals, but since multiple companies might submit dossiers for registration of a particular chemical, the total number of dossiers could reach 30,000. EHCA estimates that companies will register an additional couple of thousand substances in Phase II and several thousand more in Phase III. For registration, ECHA requires standard data including chemical information, information on the use of the chemical throughout its life cycle and potential exposures, and an assessment of the hazardous properties of the substance at each stage of the life cycle. In addition, for chemicals produced in excess of 10 tonnes per year, the registrant must submit a chemical safety report including a risk assessement for hazardous substances. Registration for high and medium tonnage substances must include proposals to fill data gaps for higher-tier toxicological and environmental studies, such as long term mammalian toxicology studies as well as soil or sediment studies; however, ECHA emphasizes that conducting new studies, particularly animal studies, should be a last resort. Instead, registrants should consider using "non-standard data" including existing data, weight of evidence, QSAR, in vitro methods, and chemical groupings and read-across approaches, which may provide adequate information and hence be acceptable. While registrants are encouraged to consider all of these options, they must provide robust scientific arguments to justify the use of non-standard data. In September 2010, ECHA conducted an experts' workshop to discuss with experts the challenges and uncertainties related to using non-test data in a regulatory context; this includes both the scientific uncertainty of using the data and the uncertainty associated with applying that data in a risk management context.

ECHA expects that Phase I registration data will be available on a public Web site in early to mid 2011, and then it will be possible to use this information for scientific purposes such as developing new QSAR.

Dr. Karen Leach, Approaches for Safety Assessment to Pharmaceuticals

Dr. Karen Leach from the Compound Safety Prediction group, which is part of the Medicinal Chemistry Division of Pfizer Incorporated discussed the methods and applications of compound safety predictions in the drug development process at Pfizer. In drug development, toxicity accounts for approximately 60% of drug attrition; that is, 60% of potential drugs are abandoned because of toxic effects discovered during either preclinical or clinical Phase 1, 2 or 3 testing. When these potential toxic effects are discovered earlier in the development process, the overall cost of developing a drug can decrease since money and time are no longer invested in drugs that are later found to be toxic. Previously, drug development focused first on pharmacology then on pharmacokinetics and lastly on safety. This sequential approach is being abandoned in favor of assessing all three simultaneously primarily through predictive assays that are based on pathway knowledge, computational analysis, and in silico models.

Dr. Leach's department at Pfizer develops predictive screening tests that can help inform the drug design process. Adverse safety events resulting from compound treatment can be the result of the primary pharmacology, the chemical structure of the compound, its reactive metabolites, the

physicochemical properties of the compound, and the off-target or secondary pharmacology effects. Using a combination of in vitro assays (e.g., genetic toxicity assays such as Ames test, cell viability assays, mitochondrial toxicity assays), researchers will be able to make more informed predictions of in vivo outcomes. Recently, scientists at Pfizer physical-chemical property associations and created a map of physical properties such as molecular weight and polar surface area for central nervous system drugs that are currently on the market. By comparing the physical-chemical properties of a potential drug to those of existing drugs, one can produce a probability-type estimate about chemical safety, rather than a simple binary, yes or no prediction of chemical safety. Dr. Leach emphasized that the challenges in developing predictive assays provides an opportunity for scientists from industry, academia, and regulatory groups to collaborate when investigating toxicity mechanisms, identifying acceptable biomarkers and high throughput screening applications, and determining how this data can be combined to support decisions.

Dr. Michael DeVito, Tox21 Targeted Testing

Dr. Michael DeVito, NIEHS's NTP, presented an overview of Tox21, a collaborative partnership between the NTP, NGCG, EPA, and U.S. Food and Drug Administration (FDA). The goal of Tox21 is to pool resources and expertise towards developing predictive toxicity models and high throughput screening assays based on mechanisms of chemically-induced biological activity to prioritize chemicals for more extensive toxicological evaluation, and to develop toxicity data that can be used to support risk management decisions. Dr. DeVito presented a brief overview of a targeted testing study that evaluated a predictive model for non-genotoxic liver carcinogens. The study was designed to answer questions such as "Do human assays predict rodent in vivo results?", "What is the impact of metabolism?", and "Is a hit in vitro really a hit in vivo?" Tier 1 testing will begin with 30 chemicals known to either cause liver tumors in Sprague-Dawley rats or not cause liver tumors; the animals will be dosed once daily for two years, and in vitro assay signatures will be compared to biomarkers measured in the animals.

Dr. DeVito also presented an example of the challenges of quantitatively extrapolating from in vitro data to predict in vivo responses. Using a toxicokinetic model, Dr. DeVito and his team determined how well in vitro data on the metabolism and distribution of deltamethrin derived in cells predict pharmacokinetic data for deltamethrin derived from in vivo studies. The team also had in vivo data on changes in motor activity in rats. The results included accurate predictions of in vivo blood concentrations, but in vivo brain tissue concentrations were not accurately predicted. Dr. DeVito presented several caveats regarding interpretation of these data. For example, further study is needed on the relationship between in vitro cell exposures and chemical concentrations and interactions in the media, and how best to model this relationship. Similar approaches will be evaluated in the Liver Targeted Testing study; the details of that study were presented later in Day 3 by Dr. Richard Judson.

In conclusion, the ongoing Tox21 efforts are providing insight into the capabilities and uncertainties of extrapolating HTS data to predict in vivo biological responses.

Dr. Christopher Portier, Genetic and Genomic Risk Assessment for Identifying Hazards

Dr. Christopher Portier, Centers for Disease Control, National Center for Environmental Health/Agency for Toxic Substances and Disease Registry, discussed the importance of linking genomics to pathway perturbations to guide prioritization of chemicals for high-throughput screening. He stressed the need for using a systems biology approach, in which data from human clinical laboratory, epidemiology, animal model, tissue culture, cell culture, and molecular biology studies are used in conjunction to predict human health risk. It is also important to take into account how humans interact with their environment as well as other human characteristics (e.g., nutrition, socioeconomic status). Once human disease pathways are identified, genomic signatures of chemicals can be linked to these pathways to

predict risk from chemical exposure. Determining the gene target for pathways often linked to human diseases can guide chemical prioritization for high-throughput screening. The NIH Genetic Association Database and the Comparative Toxciogenomics Database contain gene-centered data, but Dr. Portier emphasized the growing need for genome-wide association studies.

Audience participants pointed out that a method must be developed to screen out and prioritize which genes are really linked to disease. Dr. Portier responded that the development of such a method is beyond the scope of current research but must be addressed in the future. An audience participant highlighted the opportunity to use genome sequencing previously conducted for certain cancers as a tool to derive pathways. Another audience participant suggested using multiple strains rather than a single strain to identify pathways. Dr. Portier agreed, arguing that once the pathways in the mouse are identified, this information can be used to extrapolate to humans.

Dr. Alexander Tropsha, Combined Application of Chemical and Molecular Biology Information

The presentation by Dr. Alexander Tropsha, University of North Carolina-Chapel Hill, focused on the combined application of cheminformatics and high-throughput screening data to improve chemical safety assessment. Dr. Tropsha expressed the need to begin building multiple quantitative structure-activity relationship (QSAR) models as a virtual screen database of chemicals that allows for prioritization of chemicals for high-throughput screening based on toxicity. QSAR modeling uses statistical techniques to relate a characterized chemical structure to biological data. Dr. Tropsha pointed out that this process is only effective when chemical structure knowledge and biological response data are error free, prompting the need for thorough curation of existing chemical and biological data. High-throughput screening in vitro data or chemical descriptors alone do not predict in vivo results as well as the combination of those data. Dr. Tropsha emphasized that in vitro data, especially concentration-response high-throughput screening profiles, can improve the results of the QSAR modeling of in vivo endpoints. Concentration-response biological high-throughput screening descriptors further enhance the accuracy of the models. Dr. Tropsha concluded that any developments in combining cheminformatics and high-throughput screening data should be made publically available.

Dr. David Reif, A Toxicological Priority Index (ToxPi) for Prioritizing Chemicals based on the ToxCast Data

Dr. David Reif, EPA, presented an overview of Toxicological Priority Index (ToxPi), a flexible prioritization support software tool that incorporates ToxCast bioactivity profiles, inferred toxicity pathways, dose estimates, and chemical structural descriptors. Instead of developing an absolute threshold, ToxPi consists of a numerical index that is more flexible for different prioritization tasks and can better accommodate new data, chemicals, and data adjustments. The data can be sorted according to pathway, and combined with additional information such as genetic susceptibility factors and chemical-specific factors. Dr. Reif described efforts to examine confidence in ranking, alternative chemical sets, and treatment of missing data to better communicate uncertainties and increase transparency in decision making.

Dr. Richard Judson, A Framework for High-Throughput Risk Assessment

Dr. Richard Judson, EPA, discussed the benefits of using high throughput risk assessment (HTRA) to inform decisions regarding health protective exposure levels for chemicals. HTRA aims to use in vitro data to estimate the dose at which a given pathway is perturbed in vivo, and it may potentially be used to evaluate hundreds to thousands of chemicals with little to no in vivo data. Presently, he proposed focusing on Tier 1 chemicals with molecular pathways and targets where existing data suggest a link

between perturbation and signs of adversity. These results would then be used to prioritize chemicals for inclusion in Tier 2 and Tier 3 assessments.

He presented five key ideas that comprise the HTRA approach, which include defining biological pathways whose alteration can lead to adverse outcomes, developing in vitro assays that measure chemical activity in biological pathways, determining the in vitro concentration required to alter a pathway (i.e., the biological pathways altering concentration [BPAC]), estimating the oral dose required to reach the BPAC (i.e., the biological pathway altering dose [BPAD]), and incorporating variability and uncertainty. The BPAD required to reach the BPAC is determined using the Reverse Toxicokinetics (RTK) approach. RTK yields a concentration at steady state based on human plasma protein binding data that accounts for population variability. Uncertainty is incorporated by taking the 95% bound on the lower 99% tail of the BPAD, resulting in a more protective lower bound. As assays are developed to measure chemical activity in biological pathways, he emphasized the power of this approach to quantitatively predict in vivo human responses from different target sites and pathways.

Audience participants suggested additional potential uses for the HTRA approach. For example, one participant pointed out that a tremendous amount of pharmacodynamic data exist, presenting an opportunity to interface this approach with in vivo data. Another observer suggested taking a relative potency approach for cases where you have a lot of uncertainty (e.g., Tier 2). The presenter explained that there is not enough data to build models in these instances, but such an approach would be qualitatively informative. A third person stressed the need to take into account gender differences, but the presenter asserted that such details are beyond the scope of the approach at the present time, and that the outlined approach is only the first step in the process of developing HTRA.

Dr. Russell Thomas, Can Genomics Be Used to Derive a Meaningful Points-of-Departure for Cancer and Noncancer Risk Assessment?

The final presentation by Dr. Russell Thomas from the Hamner Institutes for Health Sciences focused on incorporating genomics into Tier 2 risk assessments. He reviewed how the field of genomics has matured over the past decade and indicated that numerous studies have demonstrated the sensitivity and reproducibility of current gene expression microarray technology. Genomics has the potential to be useful in risk assessment because it can provide quantitative information on the dose at which cellular processes are affected and the underlying biology of dose-dependent transitions. It also has the potential to increase efficiency, reduce animal numbers, and cut costs associated with chemical risk assessment.

For example, to determine how relatively short-term genomic changes correlate with apical toxicity endpoints as a function of dose, he exposed whole animals to chemicals and measured transcriptional changes in selected target tissues using microarrays. Each gene was then fit with a statistical model, a benchmark dose was calculated, genes were grouped by cellular function (e.g., proliferation, apoptosis), and a dose at which cellular function was perturbed was estimated. He then identified points of departure and used these values to estimate provisional references doses (RfDs) or cancer slope factors.

To further demonstrate the promise of this approach, he presented the results from 90-day exposure studies using five chemicals that had evidence of tumor development when tested by NTP. For the most sensitive gene ontology (GO) category, he reported slightly more sensitive results, suggesting that there is good correlation between transcriptomic dose response alterations and both noncancer- and cancer-related apical endpoints. While this approach is promising, more work needs to be conducted in the interpretation and use of genomic data for mode-of-action risk assessment. As a result, future work includes evaluating the use of genomics in Tier 2 risk assessment over a three-year period in conjunction with EPA's Office of Research and Development.

4.2. Panel and Open Discussion

A panel convened on Day 3 which included the speakers and was moderated by Drs. Stan Barone and David Dix, EPA. The panel considered each others' presentations and the risk assessment questions followed by an open discussion among all of the workshop participants. Discussion points are summarized in the following paragraphs.

It was recognized that the focus of Tier 2 assessments to date has been on toxicity assessment and exposure is not addressed. A lot is unknown about how the chemical gets to the target tissues and gets into the cell and modulates the cell activity. Pharmacokinetics could be added as a factor for the screening approach. Tier 1 ADME will be in silico and in vitro.

There was agreement that single predictive values are inadequate and that we need to look deeper to understand the shape of the dose-response curve. It is important to understand where the exposure is on the dose-response curve. It was asked if there were plans to compare dose-response curves between in vivo and in vitro. Dr. Judson remarked that indeed they are starting this. They are comparing oral equivalents of bioactivity with estimated human exposure, which provides context to get at bioactivity. Three different approaches were suggested for semi-quantitiative estimates. One was to take the median transcriptional BMDL and develop an ordinal scale of severity.

It was asked whether we have progressed for being able to screen for neurotoxicity, immunotoxitiy, reproductive toxicity, and endocrine disruption. Dr. Leach replied that there is work going on in these areas, especially immunotoxicity and reproductive toxicity. The least work is being done on neurotoxicity. Cardiotoxicity is also challenging. Liver and cardiotoxicity are major challenges for the pharmaceutical industry. There is a group in EPA ORD looking at cardiotoxins.

There was discussion about where the different studies fit on the continuum between Tier 1, Tier 2, and Tier 3 assessments and how much information is needed at each Tier. Dr. Cote clarified that Tier 1 is exclusively high throughput testing based. It was noted that decision-makers and stakeholders need information quickly and in a format that they can use. How can the information be integrated into the decision-making process earlier? The need for stakeholder engagements (e.g., with States and EPA Regions) was acknowledged.

Following the open discussion, Dr. Dix summarized key points from the discussions. He projected Figure 2 which was refined by the workshop participants. It is a preliminary proposal for what delineates Tier 1 from Tier 2.

Key points from the Day 3 discussion are provided in the text boxes on page 19 and 20.

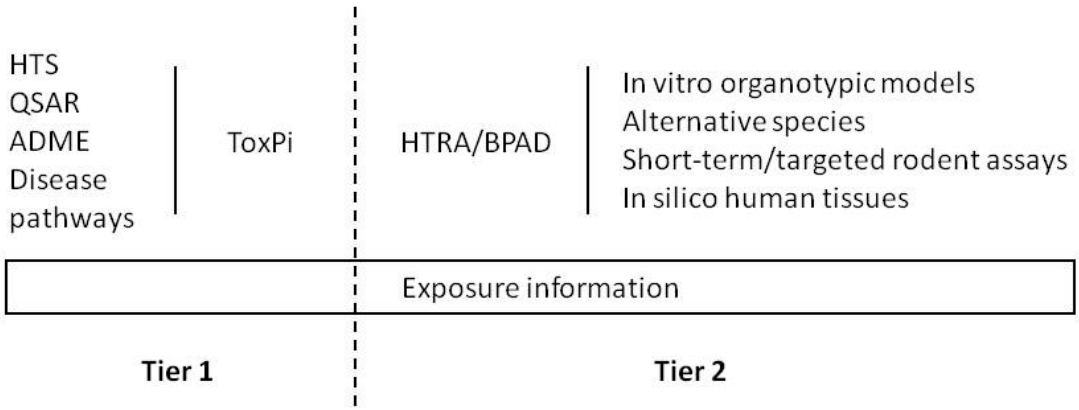

Figure 2. The NexGen Tier 1 and 2 continuum with a vertical dashed line distinguishing between data elements and approaches relevant to these tiers. Tier 1 data elements of High-throughput Screening (HTS), Quantitative Structure Activity Relationships (QSAR), Absorption-Distribution-Metabolism-Elimination (ADME), and disease pathways inform a Toxicological Prioritization index (ToxPi) for ranking chemicals for Tier 2 evaluation. Tier 2 High-Throughput Risk Assessments (HTRA) yielding Biological Pathway Altering Doses (BPAD)would benefit from addition data from in vitro organotypic models, alternative non-mammalian species, short-term and targeted rodent assays, and in silico human tissue models. Exposure information would be important for both Tier 1 and 2 assessments.

Day 3 Key Points

- With respect to in vitro to in vivo correlation, what are we benchmarking high-throughput screening data against (i.e., human disease interactomes or in vivo data from experimental animal data)?
- Grouping criteria/metrics for chemical mixtures are needed.
- There is uncertainty in the biological data that are in-hand. There may be even more uncertainty in how the information is subsequently employed in decision making/risk management.
- There is a need to refine a tiered framework for Tier 2 assessments.
- Optimization analysis (i.e., inclusion of assays that address, in part, both kinetics and dynamics) should be considered.
- Decision makers are faced with a variety of situations to be addressed, e.g. ranking chemicals for futher research to regulatory decision making. NexGen-type approaches/data will vary depending on the type of situation to be addressed.

(Continued on next page)

Day 3 Key Points (Continued)

- Variabilities should be considered to the extent feasible, including assays, interspecies, intraspecies (i.e., male/female/lifestage/physiological condition) exposure scenarios, and progression of disease processes.
- We have been focused primarily on hazard identification and dose-response assessment. Exposure assessment is a critical piece and needs consideration.
- There is a need to investigate approaches to correlating biological perturbations with the incidence/severity of apical events both as a function of dose-response and duration of exposure (e.g., an extension of Dr. Thomas' approach). For example, transcriptomic changes at a BMR of X correlates with apical phenotype/condition Y.
- How might screening/prioritization feed into a more risk assessment centric outcome (e.g., quantitative dose-response data, identification of the point-of-departure)?

5. References

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Appendix A. Final Agenda: Advancing the Next Generation (NexGen) of Risk Assessment: The Prototypes Workshop

Day 1 – November 1, 2010 – EPA Campus at Research Triangle Park
Data Rich Prototypes (Tier 3 Assessments)

Morning Plenary Session – EPA Conference Room C111 A/B

8:00-8:50	**EPA Security Check-in and Registration** – Directions provided in General Travel and Workshop Information document.
9:00-9:20	**Welcome and Introduction** – Origin of effort, goals and objectives, structure for this workshop – Dr. Ila Cote, U.S. EPA
9:20-10:30	**Framework for Prototype Development** – Presentation (30 minutes); Q&A and facilitated discussion (30 minutes) – Dr. Daniel Krewski, University of Ottawa/Risk Sciences International
10:30-10:45	**Break** (EPA's Lakeside Café will be open for beverage purchases)
10:45-11:15	**NexGen Risk Assessment Issues** – Presentation (20 minutes) and Q&A (10 minutes) – Dr. Weihsueh Chiu, U.S. EPA
11:15-11:45	**General Charge to Breakout Groups** – Presentation (20 min) and Q&A (10 min) – Dr. Ila Cote, U.S. EPA (1) Lung Injury – Ozone (2) Developmental Impairment – Thyroid Hormone Disruptors (3a) Cancer – Benzene (3b) Cancer – Polycyclic Aromatic Hydrocarbons (PAHs)
11:45-1:00	**Lunch** (EPA's Lakeside Café, on your own)

Afternoon Breakout Sessions

1:00-3:15	**Breakout Sessions** ▪ Prototype overview presentations by Prototype Team Leads ▪ Discussion	**Breakout Conference Rooms** (1) Lung Injury – Ozone: Room C111A (2) Developmental Impairment – Thyroid Hormone Disruptors: Room C111B (3a) Cancer – Benzene: Room C112 (3b) Cancer – PAHs: Room C113
3:15-3:30	**Break** (Lakeside Café will be open for beverage purchases)	
3:30-5:00	**Breakout Sessions** – Continued discussion	

Day 2 – November 2, 2010 – EPA Campus at Research Triangle Park
Data Rich Prototypes (continued)

Morning Breakout Sessions	
7:30-8:30	**EPA Security Check-in**
8:30-10:00	**Breakout Sessions –** (Same conference rooms as Day 1) ▪ Conclude discussion ▪ Summarize
10:00-10:30	**Break** (Lakeside Café will be open for beverage purchases)
Day 2 Plenary Session – EPA Conference Room C111 A/B	
10:30-12:00	**Breakout Group Report by Prototype Chairs** (each 30 minute presentation & 15 minute Q&A) (1) Lung Injury – Ozone (2) Developmental Impairment – Thyroid Hormone Disruptors
12:00-1:00	**Lunch** (Lakeside Café, on your own)
1:00-2:30	**Breakout Group Reports by Prototype Chairs** (continued) (3a) Cancer – Benzene (3b) Cancer – PAHs
2:30-3:15	**Panel Discussion –** Moderated by Dr. Lauren Zeise, California EPA Dr. Gary Ginsberg—Connecticut Department of Public Health Dr. Bernard Goldstein—University of Pittsburg Dr. Daniel Krewski—University of Ottawa Dr. Ken Ramos—University of Louisville Dr. Martyn Smith—University California—Berkeley
3:15-3:45	**Break** (Lakeside Café will be open for beverage purchases)
3:45-4:30	**Panel Discussion** (continued) – Discussion and Q&A
4:30-5:15	**Refinement of Cross-Cutting Key Points** – Moderated by Dr. Rob DeWoskin, U.S. EPA
5:15-5:30	**Next Steps, Discussion of 3rd Day, and Close** – Dr. Ila Cote, U.S. EPA

Day 3 – November 3, 2010 – Hilton Raleigh-Durham Airport at Research Triangle Park, Grand Ballroom

Approaches for Chemical with Less Data (Tier 2 Assessments)

Dr. Stan Barone and Dr. David Dix, U.S. EPA – Co-chairs

7:30-8:30	**Workshop registration at the Hilton** (outside of Grand Ballroom)
8:30-8:45	**Introduction – What is Tier 2 and what questions are we trying to address?** – Dr. Stan Barone, U.S. EPA ▪ In a risk assessment context, how is Tier 2 different from Tier 3? What is being learned from the Tier 3 prototypes about proof of concept, value of information, and decision rule that can inform Tier 2? ▪ Can the Tier 2 approach be applied to 100 to 1000s of chemicals per year? ▪ Is the Tier 2 approach acceptable for selected regulatory and policy decisions even though it provides less WOE than Tier 3?

8:45-10:15	**Speakers** – 20 minute presentations, 10 minute Q&A
	Example Questions Posed to Each Speaker—Moderated by Dr. Stan Barone, U.S. EPA
	▪ What kind of information does this approach provide about potential adverse effects when combined with in vivo data and in the absence of in vivo data?
	▪ How could these data inform potency estimates or dose-response relationships?
	▪ How qualitatively or quantitatively predictive is the data of in vivo human responses the information generated from this approach?
	▪ How could this information be use in a weight of evidence scheme?
	▪ Can this approach inform us about:
	  ◦ Variability and susceptibility in the human population?
	  ◦ Mixtures interactions?
	▪ What are the strengths and weaknesses of this approach for assessing risks in the human population?
	▪ Are there other approaches that you are aware of that might provide similar or improved information on these topics?

Day 3 – November 3, 2010 (continued)

8:45-10:15	**Speakers** – ▪ Approaches from the European Union: REACH – Dr. Derek Knight, European Chemicals Agency ▪ Approaches for Safety Assessment to Pharmaceuticals – Dr. Karen Leach, Pfizer ▪ Tox21 Approaches –Dr. Michael DeVito, National Institute of Environmental Health Sciences
10:15-10:30	**Break**
10:30-12:00	**Speakers** (continued) ▪ Genetic and Genomic Risk Assessment for Identifying Hazards—Dr. Christopher Portier, CDC-NCEH/ATSDR ▪ Combined Application of Chemical and Molecular Biology Information – Dr. Alexander Tropsha, University of North Carolina-Chapel Hill ▪ A Toxicological Priority Index (ToxPi) for Prioritizing Chemicals based on the ToxCast Data – Dr. David Reif, U.S. EPA ▪ First Steps Towards High Throughput Risk Assessment (HTRA) – Dr. Richard Judson, U.S. EPA
12:00-1:15	**Lunch** (Hotel restaurant on your own; buffet with beverage available for $11+tax)
1:15-1:45	**Speakers** (continued) ▪ Can Genomic Data be Used to Derive Meaningful Points-of-Departure for Cancer and Noncancer Risk Assessment? – Dr. Rusty Thomas, The Hamner Institutes for Health Sciences
1:45-3:00	**Panel and Open Discussion**
3:00-3:10	**Development of Key Meeting Observations** – Facilitated by Dr. Jason Lambert and Dr. Ila Cote, U.S. EPA
3:10-3:30	**Break**
3:30-5:00	**Development of Key Meeting Observations** (continued) ▪ Integrated toolbox approaches targeted for further development in health assessment applications ▪ Weight of evidence issues ▪ Needed next steps
5:00-5:15	**Next Steps and Close** – Dr. Stan Barone and Dr. David Dix, U.S. EPA

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