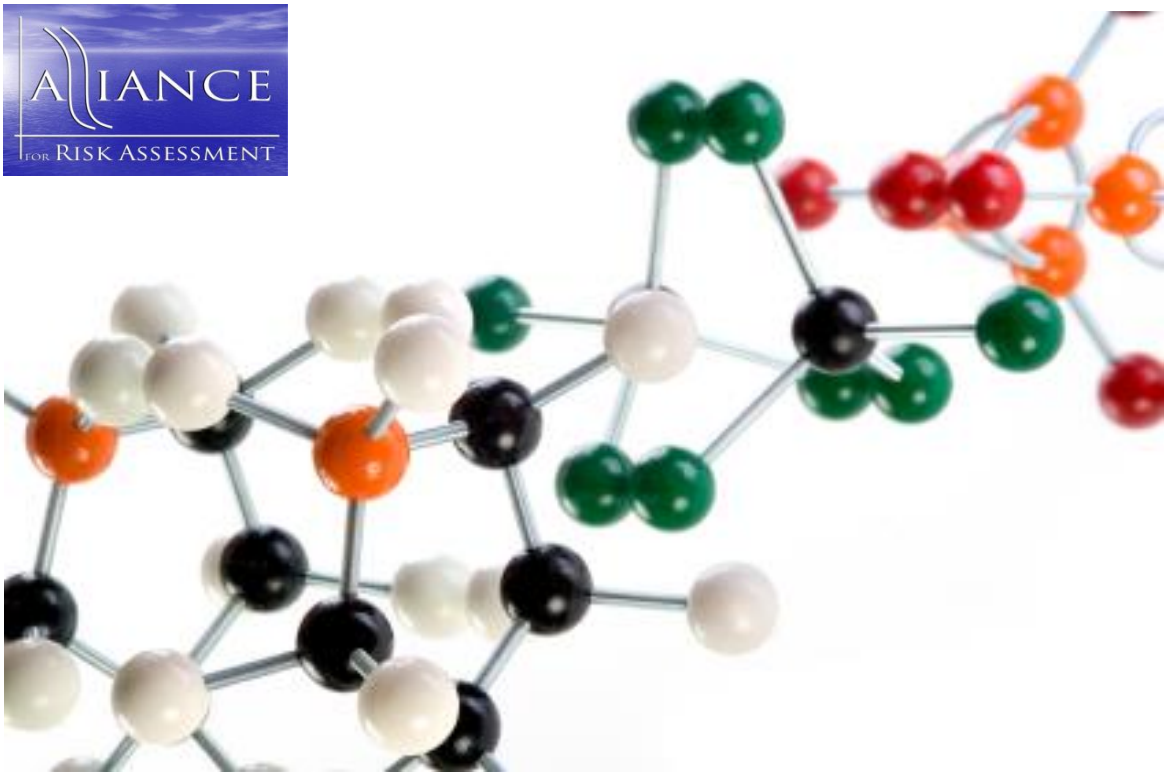

Beyond Science and Decisions: From Problem Formulation to Dose-Response
Report from Workshop IX

Workshop Held:
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Cincinnati, OH
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FINAL Report Prepared By:
Toxicology Excellence for Risk Assessment (TERA)
Lead Rapporteur and Report Author: Lynne Haber (Lynne.Haber@uc.edu)

Supporting Rapporteur: John Reichard

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Abbreviations

ADME	Absorption, distribution, metabolism, and excretion
AFB1	Aflatoxin B1
AHF	Altered hepatic foci
AHR	Aryl hydrocarbon receptor
AOP	Adverse outcome pathway
AOP-KB	AOP Knowledgebase
ARA	Alliance for Risk Assessment
AS3MT	Arsenic methyl transferase
cpAOP	Computationally-predicted AOP
CTD	Comparative Toxicogenomics Database
DMA	Dimethyl arsenic
DRAC	Dose-Response Advisory Committee
DSL	Domestic substances list
FR	Flame retardant
HCC	Hepatocellular carcinoma
HHRA	Human health risk assessment
HTS	High-throughput screening
iAs	Inorganic arsenic
IPCS	International Programme on Chemical Safety
KE	Key event
KEGG	Kyoto Encyclopedia of Genes and Genomes
KER	Key event relationship
MIE	Molecular initiating event
MMA	Monomethyl arsenic
MOA	Mode of action
MOA/HR	Mode of action/human relevance
OECD	Organisation for Economic Cooperation and Development
QSAR	Quantitative structure activity relationship
TG-GATE	Toxicogenomics Project-Genomics Assisted Toxicity Evaluation System
WHO	World Health Organization
WOE	Weight of evidence

Introduction

Workshop Scope and Objectives

The workshop series, Beyond Science and Decisions: From Problem Formulation to Dose-Response continues and expands upon the discussion initiated by the National Academy of Science report: *Science and Decisions: Advancement of Risk Assessment* (NRC, 2009). The workshops utilize a multi-stakeholder format to support the development of a practical and solution-oriented compendium of risk assessment methods. Conducted under the aegis of the Alliance for Risk Assessment (*ARA*), the workshop series explores both currently available and evolving methodologies, through the development and application of case studies. The workshop series is based on the fundamental premise that the appropriate methodologies for dose-response assessment need to be based on objectives specific to the intended application; this will include varying levels of analysis.

The workshop series continues to advance the framework of Meek et al. (2013) on problem formulation and dose-response analysis (beta version available at http://chemicalriskassessment.org).

The purpose of this workshop report is to document and communicate the workshop results to the workshop participants and interested others. The report contains summaries of the Science Panel discussions with the authors of invited presentations, as well as the Science Panel review of case studies presented at the workshop. The draft Workshop report was reviewed by the panel and presenters, and their comments have been incorporated into the final report.

Members of the Science Panel provide input on the utility of the case study methods to address specific problem formulations, and identify areas for additional development of the case study and/or method. **Inclusion of a method or case study in the framework as an illustration of a useful technique does not imply panel acceptance of the chemical-specific outcome.** In the context of the current workshop agenda, the panel is providing input and advice, not peer review. For the AOP discussions, the panel is not drawing conclusions on chemical assessments per se but providing input as to how the data help to elucidate the key events of a given AOP.

Workshop IX Organization

The workshop was organized by the Dose-Response Advisory Committee (DRAC) on behalf of the 60 workshop sponsors. The DRAC determined the agenda (see Appendix 2) in consultation with the Science Panel. The sponsors of the workshop series are listed at http://allianceforrisk.org/sponsors/. The workshop included both invited presentation providing background context related to the case study topics, and case studies being reviewed by the Science Panel. The case studies included three related to adverse outcome pathways (AOPs) and a framework for evaluating flame retardants based on hazard and exposure). The workshop was open to the public for both in-person participation and participation via webcast. Public

comments were invited at selected times during the workshop. The list of workshop participants is included in Appendix 3 of this report.

The following were invited presentations at the meeting. Summaries of the panel discussions following the presentations are provided in this report.

- Daniel Villeneuve, U.S. EPA. *An Introduction to Adverse Outcome Pathways (AOPs)*
- Stephen Edwards, U.S. EPA. *Adverse Outcome Pathways – Tailoring Development to Support Use*
- Bette Meek, McLaughlin Centre for Population Health Risk Assessment, University of Ottawa. *Distinguishing Modes of Action and their Analysis from Development and Evaluation of Adverse Outcome Pathways (AOPs)*
- Joel Tickner, University of Massachusetts Lowell. *An Introduction to Alternatives Assessment and Its Application in the Science and Policy of Safer Chemicals*

Much of the workshop was dedicated to review of case studies. Each review began with a presentation by the case study author(s) on key elements, followed by a panel discussion. The purpose of the panel discussion was to identify areas for additional development of case studies and/or refinement of methods. The following case studies were presented:

- Harvey Clewell, The Hamner Institutes for Health Sciences. *Vicinal Dithiol Binding Cancer Adverse Outcome Pathway*
- Robinan Gentry, Ramboll ENVIRON. *Vicinal Dithiol Binding Non-Cancer Adverse Outcome Pathway*
- Smadar Admon, ICL Industrial Products. *A systematic assessment methodology for flame retardants (FRs) based on hazard and exposure- the FR framework*
- Lynn H. Pottenger, TERC, The Dow Chemical Company; Martha M. Moore, Ramboll ENVIRON. *AOP for a Mutagenic MOA for Hepatocellular Carcinoma*

All presentations are available at http://allianceforrisk.org/workshop-ix-case-studies-and-presentations/. The abstracts for all invited talks were provided by the speakers, and the speakers have had the opportunity to review the summary of the discussions after their presentations.

Science Panel

The science panel for workshop IX included a mix of standing Science Panel members and *ad hoc* members chosen for their expertise related to specific case studies. Panel biographies are provided in Appendix 1, as well as at http://allianceforrisk.org/science-panel/. The Science Panel for Workshop IX consisted of the following, including standing panel members and five *ad hoc* members:

- *Barbara Beck, Gradient (ad hoc member for arsenic review)*
- *Michael L. Dourson, Toxicology Excellence for Risk Assessment (co-chair)*
- *Stephen W. Edwards, U.S. EPA NHEERL (ad hoc member for AOP review)*
- *Annie M. Jarabek, U.S. EPA, NCEA (co-chair)*
- *Mike Jayjock, Jayjock Associates (ad hoc for framework, via phone)*

- *R. Jeffrey Lewis, ExxonMobil Biomedical Sciences, Inc.*
- *Bette Meek, McLaughlin Centre for Population Health Risk Assessment, University of Ottawa*
- *Gregory Paoli, Risk Sciences International*[1] *(pre-meeting comments)*
- *Joel Tickner, University of Massachusetts, Lowell (ad hoc member for framework, via phone)*
- *Daniel Villeneuve, U.S. EPA NHEERL (ad hoc member for AOPs, via phone and written comments)*

Standing Panel, Unable to Attend:
- Richard Beauchamp, Texas Dept State Health Services
- James S. Bus, Exponent

Panel Discussions of Presentations

An Introduction to Adverse Outcome Pathways, Dr. Daniel Villeneuve

ABSTRACT:

High throughput and *in silico* methods are providing the regulatory toxicology community with capacity to rapidly and cost effectively generate data concerning a chemical's ability to initiate one or more biological perturbations that may culminate in an adverse ecological or human health outcome. Translation of those data into scientifically-defensible predictions of outcome that help support regulatory decision-making depends on the ability to efficiently access and assemble the wealth of accumulated toxicological evidence and biological understanding distributed throughout the scientific community. We propose that this challenge can be met through the assembly and description of adverse outcome pathway (AOPs) in a common knowledgebase. Adverse outcome pathways are frameworks for organizing knowledge in a manner that supports the extrapolation of mechanistic data, often measured at low levels of biological organization, into regulatory outcomes of concern, typically observed at higher levels of biological organization. A set of key principles and conventions for AOP development have been defined. Computational approaches can be leveraged to support the process of AOP discovery, quantitative prediction of dose-response time course behaviors and transitions between key events, and derivation and analysis of complex networks of AOPs. This presentation will provide an introduction to the AOP framework, key principles of AOP development, and highlight the potential applications of the AOP framework for predictive risk assessment and regulatory decision-making. *The contents of this abstract neither constitute nor necessarily represent official US EPA views and policies*.

[1] Member of the NAS *Science & Decisions* panel

DISCUSSION:

In response to a panelist question, Dr. Villeneuve stated that an AOP describes the likely impacts of any chemical that perturbs the molecular initiating event (MIE) with sufficient potency and duration; AOPs provide biological motifs of failure. Key event relationships (KERs) are the functional unit of inference/extrapolation, and can be used to aid in a quantitative understanding of the biology. AOP networks can be assembled to better capture the complexity of biological systems, such as how MIEs feed into the same series of key events (KEs) or a single MIE can trigger multiple adverse outcomes.

The panel discussed with the speaker some areas where the AOP concept is continuing to develop. The AOP community has noted that it would be useful to leverage knowledge of human pathogenesis in the understanding of adverse outcomes, although formal interactions have not yet occurred. A panelist noted that EPA's Human Health Risk Assessment (HHRA) program has a task called "disease based data integration" to help with this integration, looking at the disease and key events leading to it (the "top-down" approach) as well as a "bottom up" approach starting with the MIE. Another panelist noted that an understanding of human disease processes has been considered critical to evaluation of mode of action (MOA). The similarity intersection between AOPs and systems biology was also noted. For example, if a chemical triggers multiple MIEs, systems biology can aid in determining whether the resulting pathways intersect and how the resultant network will affect the ultimate outcome.

With regard to addressing potential alternative MIEs for an AOP, Dr. Villeneuve stated that the AOP framework is an attempt to organize the available knowledge in a systematic fashion. AOP networks can capture the variety of outcomes from a single MIE, depending on the dose, species, etc. The assessor can then ask what outcome is most likely based on the specific scenario of interest.

Adverse Outcome Pathways – Tailoring Development to Support Use, Dr. Stephen Edwards

ABSTRACT:

Adverse Outcome Pathways (AOPs) represent an ideal framework for connecting high-throughput screening (HTS) data and other toxicity testing results to adverse outcomes of regulatory importance. The AOP Knowledgebase (AOP-KB) captures AOP information to facilitate the development, evaluation, and use of new AOPs. The AOP-KB is designed to structure information to facilitate computational modeling efforts while also capturing free-text descriptions to provide additional information important for regulatory decision-making. Fully describing an AOP can be labor intensive and requires a broad range of expertise, so the AOP-KB is specifically designed to encourage crowd-sourcing and expert review of the AOP development effort. In particular, the key events (KE) within the AOP are shared across all AOPs in the system, so that no one has to repeat information that has been previously entered and so all information for a key event is captured in a single location. The AOP-KB consists of four main components: AOP-Wiki, Effectopedia, AOP-Xplorer, and Intermediate Effects DB.

This talk will describe the current capabilities of the AOP-KB with an emphasis on the AOP-Wiki component, which is currently the primary location for AOPs developed under the OECD AOP Development Programme. To generate more AOPs within the KB, we have developed data mining approaches to expedite the inclusion of computationally-predicted AOPs (cpAOPs) that include biological pathways containing ToxCast assay targets. A variety of input data sources have been used including large-scale toxicogenomics data such as the Toxicogenomics Project-Genomics Assisted Toxicity Evaluation System (TG-GATEs) and public annotation databases such as the Comparative Toxicogenomics Database (CTD). By combining AOPs with exposure and absorption, distribution, metabolism, and excretion (ADME) predictions developed in a similar manner, we can recapitulate the mode of action for a given chemical from reusable components, allowing more extensive use of AOPs in hazard characterization and risk assessment.
The views expressed in this abstract are those of the author and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

DISCUSSION:

In response to a audience question about how dose-response is handled in the pathway context, Dr. Edwards noted that there are two approaches. In the first situation, the biology is constant with dose, but the magnitude or sign of the response changes with the degree of perturbation of the MIE. In this case, the AOP is not affected, but the quantitative description captures the change in sign or magnitude of the following KE. In contrast, if the biology changes (different effects) as dose changes, then this is described as two different AOPs, both of which can be perturbed by the chemical

Distinguishing Modes of Action and their Analysis from Development and Evaluation of Adverse Outcome Pathways, Dr. Bette Meek

ABSTRACT:

A recent update of the World Health Organization/International Programme on Chemical Safety (WHO/IPCS) mode of action/human relevance (MOA/HR) framework, reflects evolving experience in its application and incorporates recent developments in toxicity testing and predictive modeling at different levels of biological organization. The modified framework is incorporated within an iterative roadmap, encouraging continuous refinement of problem formulation and mode of action based (integrated) testing and assessment strategies.

The framework can be used where the outcome of chemical exposure is known, or in hypothesizing potential effects resulting from exposure, based on information on putative key events in established modes of action from appropriate *in vitro* or *in silico* systems and other evidence. The update includes illustration of MOA analysis in various case examples such as prioritizing substances for further testing, the development of more efficient testing strategies and addressing read-across for priority setting and combined exposures.

Weight of evidence considerations for hypothesized MOAs have been developed additionally in the update and more recently evolved as a basis to contribute to the revision of guidance and electronic tools for an international knowledge base on AOPs being developed for an initiative of the Organization for Economic Cooperation and Development (OECD). The implications of this evolution are discussed with focus on similarities and distinctions between the assessment and evaluation of AOPs and mode of action analysis.

DISCUSSION:

Panel members discussed how chemical metabolism relates to MOA and AOPs, with the concept that AOPs are chemical agnostic biological pathways. A panel member noted that EPA had included ADME (toxicokinetics, expressed as absorption, distribution, metabolism and excretion) as part of mechanistic considerations (U.S. EPA, 1994) and in the first definition of MOA in 1998 (Wiltse and Dellarco, 1998), but it was not emphasized as part of the MOA in the 2005 cancer guidelines (U.S. EPA, 2005) except to consider species differences. It was clarified that while the 2005 cancer guidelines did not include toxicokinetics as an integral component of MOA, this was an important component of its qualitative and quantitative extension to human relevance/species concordance analysis in international frameworks (e.g., Meek et al., 2003; Seed et al., 2005; Boobis et al., 2006; Boobis et al., 2008). Another panelist noted that metabolic key events for similar chemicals are being considered as part of the development of integrated testing strategies for AOPs. Dr. Meek clarified that each of the component applications of AOPs (e.g., integrated testing strategies or MOA analysis for risk assessment for specific chemicals) would have application-specific add-ons to the AOP. (See Figure 1.) This talk was focused on MOA vs. AOP, but other applications would require additional add-ons not specified in the figure. For example, consideration of toxicokinetics plays a critical role in the evaluation of species concordance and dose-response, and so toxicokinetics is an important part of international frameworks for MOA analysis. Another panelist noted that the AOP knowledgebase is developing the structure for including ADME and chemical-specific aspects, as a complement to the AOP.

An Introduction to Alternatives Assessment and Its Application in the Science and Policy of Safer Chemicals, Dr. Joel Tickner

ABSTRACT:

There are increasing regulatory and market place drivers for the substitution of chemicals of "concern". There is also increasing recognition that existing chemical assessment methods are resource intensive and may not be easily applied to decisions regarding alternative chemical selection. Also, there are many examples where uninformed chemical substitutions may lead to unintended health consequences. Alternatives assessment is a process for comparing chemical and design options to a chemical of concern. The goal of alternatives assessment is informed substitution, the thoughtful transition towards safer chemistry on the basis of the best available information. The past decade has seen a significant growth in alternatives assessment frameworks, tools, and approaches. The recent National Research Council Framework to Guide Selection of Chemical Alternatives (NRC, 2014), much like the NRC Red Book on risk

assessment, builds on these efforts by outlining a multi-step framework and basic guidance to provide direction to this growing field. This presentation provides an overview of the foundations of the field of alternatives assessment, outlines current efforts and identifies research needs moving forward. Alternatives assessment is a robust and growing science policy field. It is important that evolving methods be adaptable to different decision-contexts and responsive to the decision-time frames required in alternatives assessment, to avoid paralysis by analysis. There are important research needs to build alternatives assessment including: addressing tools for addressing uncertainty and integrating data from different sources (in silico, high throughput), tools for rapid exposure characterization, to identify potential trade-offs; and tools for multi-attribute comparison.

DISCUSSION:

A panel member noted the importance of communication between groups prioritizing chemicals for assessment and those doing alternatives assessment, and that functional categories can aid in evaluating exposure. Dr. Tickner agreed with both points, noting that he has a paper in Environmental Science and Technology (Tickner et al., 2014) that looked at functional substitutions using function rather than the chemical as a starting point. A panel member noted the importance of specifying decision criteria. In addition, iterative refinement of the approach is important, based on increasing experience. One typically finds that additional professional judgment is needed, and additional analyses based on the experience obtained can help to identify and codify that professional judgment; transparency is important. In response to a question about whether alternatives assessment frameworks include cost-benefit considerations, Dr. Tickner noted that the alternatives assessments conducted under the California Safer Consumer Products program (see http://www.dtsc.ca.gov/scp/index.cfm) has the most extensive economic valuation, including both external and internal costs. The National Research Council panel on a "Framework to Guide Selection of Chemical Alternatives" noted that the U.S. EPA has an active program in sustainability assessment, and that alternatives assessment could be an important contribution to a sustainability assessment. Other considerations include the impact of the manufacturing process, function and cleanup, as well as unintended consequences in the production train. Dr. Tickner agreed that lifecycle assessment tends not to evaluate production or occupational exposure. Other areas that could be considered include degradation products and the synthetic history of the materials, but the current frameworks have not yet identified an approach for including all of these aspects. A tool (called p2OASyS, available at http://www.turi.org/Our_Work/Research/Alternatives_Assessment/Chemical_Hazard_Comparison_Tools/P2OASys_Tool_to_Compare_Materials) does exist that looks at worker tradeoffs, including ergonomic tradeoffs. These additional issues need to be considered even if they cannot be quantified.

A panel member noted the importance of recognizing data gaps (e.g., ecological effects) and providing incentives to fill the gaps. Dr. Tickner stated that there are many approaches for addressing data gaps. Some frameworks use uncertainty factors, others use the entirety of the data, including *in silico* modeling, some assume potential impacts in absence of data, and still others do not address gaps. In the realm of practical application, some companies prefer to use a known chemical with known risks rather than a substitute with data gaps. One of the goals of the

new, emerging community of practice (and potential professional society) on alternatives assessment is to help build a research agenda, including consideration of data gaps. A panel member suggested that retrospective analyses would be useful in considering the impact of data gaps. Such analyses would consider what the outcomes were in the presence of different data gaps and what influenced the outcomes.

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A panel member noted the importance of distinguishing different types of risk assessments, that some may be data-intensive and require a lot of resources, while faster approaches are used in screening and in industry in the development of new products. (See www.chemicalriskassessment.org for different problem formulations.) Dr. Tickner stated that alternatives assessment is not a subset of risk assessment, but is about risk management and comparing alternative options to meet a particular chemical function. For example, a manufacturer may not want to put a carcinogen in a product that will touch a consumer's body, even if the risk is low, due to issues of public perception and opinion.

Case Study Discussions

Four new case studies were presented. Panel input was sought on the utility of the methods to address specific problem formulations, and on areas for additional development. Inclusion of a method or case study in the framework as an illustration of a useful technique does not imply panel acceptance of the chemical-specific outcome. All case study presentations are available at http://allianceforrisk.org/workshop-ix-case-studies-and-presentations/.

Table 1. Workshop VIII-Summary of Case Study Discussions

New Case Studies	
Case Study: Vicinal Dithiol Binding Cancer Adverse Outcome Pathway	**Authored by: Harvey Clewell, Robinan Gentry, Jan Yager, Petra Begemann, and Tracy Greene**
The Adverse Outcome Pathway (AOP) method provides a means to organize existing and developing toxicological understanding into a format that can facilitate application of mechanistic information to risk-based decisions. This case study is intended to present an adverse outcome pathway (AOP) for carcinogenesis resulting from vicinal dithiol protein binding by systematically organizing key toxicological and molecular data. This AOP addresses the key events (KE) and the key event relationships (KERs) for this pathway. The panel thought that the overall case study was well done and they supported carrying the method forward into the ARA dose response framework, but recommended a number of revisions before including it on the framework. As discussed in the rest of this summary, a key comment was that the case study reviewed by the panel was really a description of an MOA, not an AOP, and the AOP (i.e., the chemical agnostic biological pathway) needs to be separated from the chemical-specific aspects, especially with respect to metabolism. Panel members also	

noted that a number of useful details were in the presentation but not in the case study documentation and need to be captured. For example, co-mutagenicity is mentioned in the presentation but would need to be more explicitly documented as part of a description of the inorganic arsenic MOA (not AOP). Rather than focusing on revising the case study text, the panel proposed that the authors should enter the AOP into the web-based AOP-Wiki website (http://aopwiki.org/), and then the ARA framework can include a link to the Wiki. The Wiki structure provides guidance on the needed elements to support the identified KEs and KERs. The panel also recommended that the authors follow the guidance in the OECD Users' Handbook (OECD, 2014).

A key enhancement recommended by the panel was to strengthen the text related to weight of evidence (WOE) and the degree of confidence in the AOP, as described in the Users' Handbook. The panel noted that data on the general biology could be used to enhance the WOE evaluation. For example, the hallmarks of cancer (Hanahan and Weinberg, 2000, 2011) could be used to enhance the data and WOE for downstream KEs. The authors noted that the WOE discussion could be strengthened for the AOP by including additional evaluation of cell signaling network relationships and generic cell responses. This toxicodynamic information supports greater network understanding as part of the confidence/WOE analysis and plausibility of the AOP.

The panel noted that AOP, MOA, and biomarkers are all conceptually related but distinct components of the exposure-dose-response construct and acknowledged a great deal of confusion about the differences among these concepts. The AOP framework is defined as a chemically-agnostic system for organizing information on toxicodynamics. Since AOPs are not chemical-specific, multiple chemicals can potentially elicit the same MIE. The AOP framework is modular, consisting of KEs, which are observable pathway nodes reflecting changes of the biological state. KEs are linked together by key event relationships (KERs) (edges, support for which is described in the context of the biological plausibility and empirical support. In light of these definitions, the case study reviewed by the panel was really a description of a MOA, not an AOP, and much of the discussion reflected the steps needed to tease out and document the AOP (i.e., the chemical agnostic biological pathway).

The panel discussed extensively how dose-response is addressed within the AOP framework. Rather than thinking about response in relation to the applied (or internal) dose of a chemical, it is important to think about relationships (i.e., KERs) in terms of response-response relationships (i.e., the amount of change in one KE needed to cause a specific degree of change in the downstream KE). This is consistent with the idea that the AOP is chemically-agnostic. By describing quantitation in terms of the response-response relationship, the actual chemical dose is removed from the description.

However, it was recognized by the panel that dosimetry and ADME are an important part of MOA analysis in chemical-specific evaluation. Rather than using the term "pre-MIE," it was recommended that the authors consider the events occurring in that node [inorganic arsenic metabolism to MMA(III) and DMA(III)] to be key events, though part of arsenic dosimetry, and thus part of the MOA, rather than part of the AOP. ADME factors govern the availability of a chemical to interact with one or more target molecules and therefore the magnitude of the MIE.

Implications of dosimetry are captured when the AOP is incorporated into the framework of a MOA analysis, as well as other comparative WOE evaluations. While the ADME components are not considered part of the AOP, the AOP knowledgebase will contain this information, and tools are under development that will better structure the ADME-related information in a manner analogous to the AOP and biological effects.

It was recognized that dosimetry has two different impacts on AOPs. The first is the magnitude of the MIE perturbation: the greater the magnitude of the MIE, the greater the outcome response, within the bounds of the biological dynamic range. The second is that as the dose increases, chemicals interact with an increasing number of targets. This target promiscuity can initiate a greater number of AOPs, and consequently result in additional adverse effects. For this reason, the dose-response of a specific chemical can be described by more than one AOP, for example where one AOP may predominate at one dose and another may predominate at higher doses. However, panelists noted that the *chemical's MOA* is described by more than one AOP under these conditions, and each AOP stands as a chemically-agnostic description. Presumably, in such circumstances, current risk assessment procedures would result in the selection of the most sensitive AOP as a basis for risk assessment, when such data are available. In this way, an MOA evaluation complements the chemically-agnostic AOP information with chemical-specific ADME information.

For most real-world applications, AOP networks are functional units of predictions. Although AOPs are modular in organization, they are not isolated. Rather, they interact, providing the capacity to build larger networks and facilitate greater understanding. The linear sequential representation of an AOP from the MIE to the outcome is a desirable way to simplify and present data, although it is recognized that biology is complex and this representation may not be ideal in all cases. For example, identical MIEs with similar KEs and KERs can result in different outcomes in different species. This can occur, for example, when the MIE and KE/KERs occur in different tissues or cells because of differences among species in levels of gene expression (or protein activity, metabolism, etc.).

The use of AOPs does not necessarily require detailed systems-level understanding of every gene, protein or pathway perturbed by a chemical. Instead, it is necessary to understand the context well enough to know if there may be multiple MIEs and how perturbation of those MIEs impact downstream key events within the AOP network. Although MIEs are unlikely to be predictive of complex toxic effects, understanding the pathway at the macro level provides the risk assessor with a lot more information that is normally used currently in default approaches to hazard assessment.

The panel discussed whether it is appropriate to include the two branches (oxidative stress and inhibition of cellular DNA damage response) in the AOP under consideration, or whether the two branches should be considered two separate AOPs. This question could be reframed as whether there is one MIE (binding to protein sulfhydryls), or whether this should be considered two MIEs – binding to sulfhydryls of DNA binding proteins vs. binding to oxidative stress-related proteins. If each branch is able to elicit downstream key events independently from the other, that would suggest two MIEs, and thus two AOPs. Alternatively, if the activation of both

branches by a single chemical is required to elicit the downstream key events, it would be best described as a single AOP. The goal for non-branching AOPs is to keep things simple, but in this case, the branch logically is part of the pathway. Using the structure of the AOP Wiki should help in addressing this issue. It was noted that one MOA may be reflected in two AOPs.

An important discussion evolved regarding the different species of arsenic that can adduct sulfhydryl-rich proteins, specifically iAs^{+3} and trivalent monomethyl arsenic (MMA^{+3}). It was noted that dimethyl arsenic (DMA) is a product of iAs^{+3} metabolism, and is found at higher concentrations as a metabolite observed in rodents, and therefore not as important in humans as in rodents. Moreover, rat hemoglobin binds DMA avidly, leading to a higher internal dose of this metabolite than would occur in humans. It was also noted that arsenic methyl transferase (AS3MT) knockout mice, which are unable to methylate arsenic, still exhibit cytotoxicity, implying that iAs by itself can be sufficient to induce cytotoxicity. This does not mean that MMA^{+3} or DMA^{+3} do not play a role, *in vivo*, but that the relevant metric is likely to be the total load of trivalent arsenicals (*i.e.* iAs and MMA^{+3} or DMA^{+3}). However, all of this information pertains to arsenic and/or arsenite metabolites and therefore is required for a MOA analysis but not the chemically-agnostic AOP. In the context of an AOP, arsenic is only one chemical stressor that can be used to evaluate and characterize the nodes and edges of the AOP, and contributes along with other evidence to the WOE supporting individual KEs and KERs. This weight of evidence can be considered as part of the overall MOA-human relevance or species concordance analysis. The structured framework of the AOP Wiki and Effectopedia is designed to instill the rigor needed for in depth consideration of the data supporting this AOP (for carcinogenesis) in a systematic fashion. Population of the Wiki and Effectopedia for the associated AOP, will facilitate subsequent preparation of a MOA for arsenite carcinogenesis mediated by a vicinal dithiol protein binding.

In response to a question from the audience about the implications of this or similar AOPs for dose-response assessment, panel members stated that one needs to do the comparative MOA weight of evidence analysis and quantitation of the KERs before a judgment can be made. Dose-response implications of the ADME for the chemical need to be considered in that MOA analysis. Another panel member added a different perspective, suggesting that the available data indicate that arsenic acts via a proteotoxic MOA, suggesting nonlinearity. The author stated that this latter perspective is consistent with the conclusions of EPA's panel on MOA (Eastern Research Group, 1997). Since the case study was about the chemical-agnostic AOP, not the arsenic MOA, this issue was not further discussed , however. The author also noted that both branches of the AOP (both DNA damage and cell proliferation) are needed for carcinogenicity; this represents a circumstance where a simple linear AOP is not sufficient to capture the biological complexity so that both branches should be included in a single AOP**.**

An audience member asked about the use of KEGG (Kyoto Encyclopedia of Genes and Genomes)[2] diagrams showing the molecular pathways to aid in evaluating AOPs. A panel member responded that KEGG elements could be used as building blocks for KEs, rather than

[2] KEGG is a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies.

KEs themselves.

In response to an audience question about the nature of the final node in an AOP, panel members stated that the final adverse outcome is defined in terms that are relevant in a regulatory context. The initial AOP concept came from the ecological risk assessment community, where the only unit of analysis is often at the population level. The emphasis in human health risk assessment is often on consideration of the sensitive groups within a population, which typically requires additional information on the variability of the individuals within a population, so adverse outcomes within an AOP that is relevant for human health assessment are typically at the organism level. Another panel member noted that both disciplines are now emphasizing community impacts.

Based on the preceding discussion, the panel agreed that a new header should be added to the *ARA* framework, for AOPs, and that the framework will cross-reference between MOAs and AOPs. In addition, a paragraph distinguishing between AOPs and MOAs will be developed by Bette Meek, Daniel Villeneuve, Stephen Edwards, and Annie Jarabek. The material under the AOP header will include pointers to the AOP Wiki and to the NAS scheme for biomarkers (1989).

Case Study: Vicinal Dithiol Binding Non Cancer Adverse Outcome Pathway	**Authored by: Harvey Clewell, Robinan Gentry, Jan Yager, Petra Begemann, and Tracy Greene**

The Adverse Outcome Pathway (AOP) method provides a means to organize existing and developing toxicological understanding into a format that can facilitate application of mechanistic information to risk-based decisions. Based on pre-meeting comments from a panel member, this AOP builds on the vicinal dithiol binding *cancer* AOP. The material presented in the presentation expanded on the case study by looking for consistency across targets and by looking more carefully at the third KER, between oxidative stress/proliferation/inflammatory signaling and the individual AOs.

As noted for the related cancer AOP, the panel thought that the overall case study was well done and panel members supported carrying the method forward into the framework, but they recommended a number of revisions before including it on the *ARA* dose response framework. Rather than focusing on revising the case study text, the panel proposed that the authors should enter the AOP into the web-based AOP-Wiki website (http://aopwiki.org/), following the guidance in the OECD User's Handbook, and that the *ARA* framework include a link to the Wiki. The focus on a noncancer endpoint, linked to a related cancer AOP, was noted as a particular contribution of the method.

A panel member noted that this AOP shares KEs with the cancer AOP up to and including the KE of oxidative stress, but that cell proliferation occurs only in the cancer AOP. This means that the AOP for the cancer and noncancer endpoints could be the same, except for the final step. A lot of general biology literature could be brought to bear for that final step.

The panel discussed the data related to some of the specific adverse outcomes. One panel member noted that there is a strong human data base supporting the role of oxidative stress and inflammation in cardiovascular disease. For some other endpoints, such as neurological effects,

the epidemiology data are weaker, with more issues of confounding, and the biological understanding is weaker. This suggests that it may be most effective for the authors to focus on two or three endpoints with a stronger database, in particular where the epidemiological evidence is stronger, and where the biology is better understood. Another panel member suggested thinking about what might be the targets of oxidative stress (i.e., the targets of one of the KEs in the pathway), and use this information to identify what further work is needed, including potential epidemiology studies. Panel members recommended focusing on the endpoints where the biology is well understood (e.g., cardiovascular disease and diabetes), before extending the analysis to the arsenic MOA using arsenic-specific data. Conversely, the authors noted that the endpoints where the concern is highest (neurological and immunological effects) may have weaker databases than that for cardiovascular disease, and so it may be important to include these endpoints in a MOA evaluation. A panel member recommended that the authors not include hypertension, since it is not necessarily related to endothelial damage. It was also noted that the AOP approach would be useful in helping to interpret the epidemiology data related to diabetes and arsenic, and determining whether observed associations are due to causality or reverse causality.

A panel member noted that the current critical effect for noncancer effects of inorganic arsenic (iAs) is skin lesions, and wondered whether the other endpoints considered in the case study had not received sufficient attention, or whether skin is the first target as the dose increases. An author replied that the skin effects were the original focus because they were readily observed and occurred reasonably quickly (within 6 months). Other, more subtle effects may also occur at low doses, and it is less clear which effects occur first at the lowest doses. A panel member noted that if the purpose is to document the MOA for the purpose of evaluating risk, then it may be best to focus on the events that occur first in terms of both dose and time.

It was noted that it would be useful to further engage the epidemiology community in AOP development. This could be done by emphasizing the use of early biomarkers that can be measured in the human population, but the challenge is to identify the correct biomarkers (exposure, response or susceptibility) and KEs and to ensure the predictive reliability of the biomarkers, using standard criteria. The additional mechanistic understanding obtained by using the AOP construct would help with the interpretation of negative epidemiology data. In addition, AOPs can help epidemiologists in addressing biological plausibility when an association is seen between a chemical exposure and a disease. Presentations at professional society meetings would be useful to further engage the epidemiology community, by illustrating, for example, how AOPs can be used to address combined exposures/cumulative risk.

Panel members noted that data gaps and research needs will become clearer as the authors work through the AOP framework. This will aid in identifying the key data gaps in both the general understanding of the biology and with regard to the MOA of iAs. A challenge for this case study was that much of the available research data did not necessarily address the correct critical questions related to hazard evaluation and risk assessment of arsenic. It is important to think about the MOA and involve people who would use the data when designing the studies (part of the problem formulation).

An audience member suggested that genotyping of sloughed uroepithelial cells in arsenic-exposed populations would be useful. An author responded that the exfoliated uroepithelial cells have undergone apoptosis, degrading their DNA, and so useful information cannot be obtained from these cells.

In response to an audience question about the implications of the AOP for the dose-response assessment for iAs, a panel member recommended moving away from the *defaults* of threshold versus non-threshold evaluations. Instead, AOPs aid in defining the dose-response for the MIE, and the response-response relationships for the KERs, with the ultimate goal of better describing the dose-response at lower doses. Once the biological pathway has been activated, dose is less relevant, unless the pathway requires continued exposure. Arsenic is also different from many industrial chemicals, because human populations have been exposed to arsenic at doses in the range of those where effects begin to occur. This suggests that the epidemiology and other data could be combined to meaningfully extrapolate the dose-response curve. Benchmark dose analyses could be done on the genomics data to aid in extending the dose-response curve as needed. One panel member suggested that one could talk conceptually about how the AOP affects the understanding of the iAs dose-response, based on whether a minimal number of molecules need to be damaged for an effect to occur.

An author noted that pharmacodynamics determines whether the effect accumulates. *In vitro* data are needed in order to evaluate human pharmacodynamic variability, as was done in the experiments that formed the basis for the case study.

A systematic assessment methodology for flame retardants (FRs) based on hazard and exposure- the FR framework	**Authored by: Smadar Admon, Marc Leifer, Joel Tenney, Tami Weiss-Cohen**

The FR Framework is an assessment tool for evaluating flame retardant (FR) products in their intended application during the use phase. The framework improves upon existing hazard-based approaches by incorporating an estimated exposure component based on the anticipated level of product contact and measurable potential emissions of flame retardants from the matrix in which they are incorporated (e.g. plastic, foams, textile's formulations). The purpose of the FR framework is to provide guidance to users of FRs in making more informed decisions regarding flame retardant selection. The framework can also be used as an alternative assessment tool when comparing different FRs in specific applications. Other life cycle stages of the FR are not included in the framework.

The panel consensus was that the case study method addressed a clear risk assessment need, and in particular helped to forward the science of alternatives assessment by including exposure considerations. Another advantage to the method was the inclusion of degradation products. The panel recommended a number of useful revisions to be made to the case study prior to it being included in the *ARA* dose response framework. The entire panel will review the revised case study to ensure that the recommended changes are made. After the changes are made, the method would be included as a qualitative screening method. A listing of, and links to, other alternatives assessment methods would also be added. One panel member suggested that the framework could point users to the OECD (2013) meta-review of alternatives assessment

methods. The current case study is similar to the Dutch Quickscan method, which could also be listed[3].

The panel discussed a number of areas where additional clarification and/or transparency is needed. A panelist recommended that the documentation should specify up-front the classes of flame retardants to which the method applies. The author noted that the general method would apply to other classes, but analytical methods would need to be developed for these other classes, and the relevant cutoffs for the different categories would change. The report should also state that the method relates only to the use stage. The case study states that it addresses the "worst" case" scenario; panel members noted several areas (e.g., dust exposure) where the current framework may not be the worst case. The panel suggested that the text clarify what is meant by "worst case," and that additional potential sources of exposure be considered. More documentation is needed regarding the endpoints chosen for inclusion. A panel member recommended that additional consideration be given to some endpoints that are not in this framework, but are considered by other methods, such as endocrine disruption and neurotoxicity in children. With regard to the potential for considering additional endpoints, such as endocrine disruption, the author noted that the framework is updated in an ongoing manner. Since there is no guidance for endocrine disruption, strong evidence for ED is considered high hazard.

A panel member suggested that the framework should address end of lifecycle exposure, and suggested that more attention be paid to how design processes may minimize the potential for worst case exposures, even in situations where methods for recycling, disposal, etc. may be inappropriate. However, the author stated that the end of life processes are beyond the company's control, and it is hard to make assumptions about the methods used. It was also noted that if a chemical is persistent and bioaccumulative, it ends up in the "unacceptable hazard" bin.

Greater transparency is needed regarding how uncertainty in the exposure and hazard areas, as well as data gaps and data quality, are considered in the decision criteria. A possible enhancement would be to rate uncertainty as high, medium, or low for the various endpoints, to give a sense of the comparative nature and size of the database. A panelist recommended that the authors conduct a *post hoc* analysis after several chemicals are evaluated using expert judgment, to try to identify the decision criteria that are being used, in a formal iterative feedback loop. Additional documentation is also needed for the case example in the appendix, to provide not only the conclusions, but the rationale for those conclusions.

One panelist noted that Health Canada developed a hierarchical scheme for using quantitative structure activity relationship (QSAR) models in their weight of evidence evaluations for hazard characterization. The agency developed a method for ranking output from different models and using the tools. This was part of the hazard characterization process for inherent toxicity for the Canadian domestic substances list (DSL)[4]; it would be useful to develop and document a similar scheme for the current framework.

[3] Archived at http://www.chemicalspolicy.org/archives.reports.europe.memberstates.netherlands.php

[4] See http://www.hc-sc.gc.ca/ewh-semt/contaminants/existsub/categor/index-eng.php

One panel member expressed concern about the initial categorization of exposure into frequent, sporadic and rare. The panel member noted that a primary pathway of exposure is from migration of the FR to the surface of the material, and from there to house dust, which can become widely dispersed in the house and lead to dermal and oral exposure. This means that exposure may be higher than otherwise expected for an object with "sporadic" direct contact. However, if the object containing the FR is truly isolated from living space, this is less of an issue.

One of the panel members initially suggested refining the exposure assessment in a number of ways to make the evaluation more quantitative, based on modeled exposure and hazard banding for a quantitative hazard estimate, to obtain a true risk context. The panel member suggested that readily available exposure models could be used to place the exposure in broad quantitative bands. However, the author stated that modeling exposure would be complicated, and the industry has a need for a rapid screening tool. Overall, the panel members thought that the method was appropriate for the stated problem formulation – a qualitative tool to aid in comparative analyses. However, a panelist noted that in doing this qualitative evaluation, it is important to look at worst-case situations.

The panel made a number of additional recommendations or suggestions:

- Additional documentation is needed for several of the criteria. For example, based on the author comment that the grouping of the blooming data was based on a distributional analysis of a number of FRs in different plastics and that the data fell into three groups, it would be useful to present the distributional analysis in the case study.
- All of the measurement protocols (e.g., for measuring blooming and leaching) need to be documented in sufficient detail so that someone else could reproduce the method.
- GHS categories that address effects on the respiratory tract from inhalation and aspiration should be included.
- It would be useful to modify the blooming protocol to include a wipe with a mild solvent to ensure that most of the FR on the surface is captured. The solvent used should not dissolve or penetrate the product matrix.
- Volatilization needs to be accounted for, both in the blooming analysis and in general. It was noted that the plan is to include volatilization in the framework, pending the development of appropriate methods.
- It may be useful to compare the framework with the method that some users in the supply chain currently apply (if different).
- The ISVOC program can be useful for making predictions of leaching from polymer matrices[5].
- It was suggested that UV degradation be considered, but the author noted that the typical product formulation includes an inhibitor of UV degradation.

Generic AOP for a Mutagenic MOA for Hepatocellular Carcinoma	**Authored by:** Lynn H. Pottenger, Martha M. Moore, and Rita Schoeny

[5] See http://www.epa.gov/nrmrl/appcd/mmd/i-sovc.html

	With support from Ted Simon and Rick Becker

Adverse Outcome Pathways (AOPs) are a means to organize information on the steps (key events) from an initial molecular perturbation (molecular initiating event or MIE), such as following a chemical exposure, to an adverse health outcome (AO). AOPs array the various biological events in a temporal sequence and can describe quantitative relationships between the key events (KEs) as the key event relationships (KERs). The focus of this case study is the mutagenic mode of action (MOA) for hepatocellular carcinoma (HCC). Aflatoxin B1 (AFB1) was selected as an illustrative chemical based on the following: (1) it is a data-rich substance with mechanistic *in vitro* data, *in vivo* dose data in animals, and human data; and (2) there is substantial, albeit imperfect, evidence that AFB1 causes HCC *via* a mutagenic MOA. Based on the AOP created for AFB1, it is possible to create a generic AOP for hepatocellular carcinoma development for substances acting *via* a mutagenic MOA.

The panel thought that the overall case study was generally well done and highly informative from the perspective of applying the OECD AOP guidance and WOE considerations. They supported carrying the method forward into the *ARA* framework, with some changes and restructuring to additionally distinguish between the AOP and MOA concepts. Rather than focusing on revising the case study text, the panel proposed that the authors should enter the AOP into the web-based OECD AOP Wiki website (http://aopwiki.org/), and then the ARA framework can include a link to the Wiki.

Much of the panel discussion centered on distinguishing between the MOA and AOP concepts, recommendations for presenting the analysis as an AOP, and refining the identification of the KEs. In particular, as this is an AOP, neither the name of the chemical nor the term "MOA" should be part of the title. Defining the AOP is different from asking through which AOP a chemical is acting. An original goal of the authors was to develop a generic AOP for cancer occurring from a mutation as an influential KE, possibly the MIE. To get to this point, the authors chose to work through the specific case study with AFB1. However, now that they have thought through the chemical-specific aspects, the panel advised that it is important to step back and identify what aspects are part of a chemical-agnostic AOP.

There was considerable discussion about how to identify KEs and whether to rename the key event that was initially identified as "mutations induced in critical genes." One panel member noted that the supporting data for the KE or KER do not need to be chemical-specific. If a chemical is causing cancer *via* a mutagenic MOA, then the need for a mutation to occur in a critical gene flows logically, and it is not a concern if that specific endpoint has not been measured. Another panel member noted that KEs are chosen because they are essential steps, for which supporting data exist. The panel suggested that the KE referring to mutation should be renamed as "*early* mutation induced in critical genes." The use of the term "early" distinguishes the event from secondary mutations. For clarity, it was also suggested that this KE could be called the most influential KE, rather than using the term "defining KE."

It was noted that several different MIEs can converge to the influential KE of "early mutations in critical genes," which would then be an intersecting node. Thus, the AOP presented could easily be expanded to additional AOPs, based on different MIEs. Four such potential MIEs were

identified: promutagenic adducts (*e.g*., the case study being presented, with AFB1), intercalation, cross-linking, and topoisomerase inhibition. It was suggested that the authors start by completing their current AOP, and consider including one additional MIE.

With regard to the indirect KERs, a panel member clarified that indirect KERs are part of the pathway itself (rather than being an alternative way to reach a KE), but they allow evidence to be presented that is not sequential. In other words, the indirect KERs reflect a way to present the data even if no study specifically evaluated the intermediate KEs. (For example, there may be an indirect KER between KE 1 and 3, where KE 2 was not evaluated.) In the case of the AOP being considered in this case study, good evidence is not available for the direct KERs connecting the middle KEs, but the existence of these KEs is supported by biological plausibility. As shown appropriately in the AOP diagram, data are available for the indirect KERs, adding support to the overall AOP. The data based on the indirect KERs can then be factored into the WOE consideration and confidence assessment.

The case study authors raised a question about a possible additional KE specifically to represent tumor progression/mutations; this would separate the influential KE "early mutation in critical genes" from later mutations that occur in tumor progression, and serve as an intersecting node for other AOPs. A panel member noted that tumor progression is implied in the biology, is not necessarily a KE, and does not meet the criteria of being an **early** and influential event. However, it was noted that other AOPs could feed into the pathway, with the tumor progression being a branch point. Therefore, panel members recommended that the tumor progression KE be incorporated as part of the linear flow of the pathway, rather than being left as currently shown as an offset. The authors could use the hallmarks of cancer to support the tumor progression KE, rather than needing to dig into the literature for support.

With respect to the adverse outcome, an audience member noted that the AOP is not specific to the liver, aside from naming the altered hepatic foci (AHF) as the outcome of clonal expansion. This means that the AOP could be named simply "carcinogenicity *via* the induction of mutations."

As one of the purposes of the case study was to emphasize that a carcinogen that is a mutagen is not necessarily acting *via* a mutagenic MOA, a panel member suggested that this concept be further developed with examples. An author noted that there are two AOPs under development for the OECD Wiki for mutagens that are not acting *via* mutagenic MOAs - propylene oxide and vinyl acetate[6]. Captan is another chemical that is mutagenic but does not act via a mutagenic MOA. A panel member noted that properly defining the KEs and KERs with appropriate caveats helps to avoid the impression that only the MIE is needed. For example, sustained activation of the aryl hydrocarbon receptor (AHR) is a key event in one AOP – not simply binding to the receptor.

[6] These AOPs were agreed for development before the idea of separating AOPs from MOAs for a chemical was fully clarified.

References

Boobis, AR; Doe, JE; Heinrich-Hirsch, B; Meek, ME; Munn, S; Ruchirawat, M; Schlater, J; Seed, J (2008). IPCS Framework for Analyzing the Relevance of a Noncancer Mode of Action for Humans, Critical Reviews in Toxicology 38:87-96.

Boobis, AR; Cohen, SM; Dellarco, V; McGregor, D; Meek, ME; Vickers, C; Willcocks, D; Farland, W (2006). IPCS Framework for Analyzing the Relevance of a Cancer Mode of Action for Humans. Critical Reviews in Toxicology 36:781-792.

Eastern Research Group, Inc. (1997). Expert Panel on Arsenic Carcinogenicity: Review and Workshop. USEPA (U.S. Environmental Protection Agency), NCEA (National Center for Environmental Assessment), Washington, DC, August.

Hanahan, D; Weinberg, RA (2000). The Hallmarksof Cancer. Cell 100:57–70.

Hanahan, D; Weinberg, RA (2011). Hallmarks of Cancer: The Next Generation. Cell 4;144(5):646-74.

Meek, M; Bucher, J; Cohen, S; et al. (2003). A Framework for Human Relevance Analysis of Information on Carcinogenic Modes of Action. Critical Reviews in Toxicology 33:581-653.

Meek, ME; Bolger, M; Bus, JS; Christopher, J; Conolly, RB; Lewis, RJ; Paoli, G; Schoeny, R; Haber, LT; Rosenstein, AB; Dourson, ML (2013). A Framework for Fit-for-Purpose Dose Response Assessment. Regul. Toxicol. Pharmacol. 66(2):234-40. Doi: 10.1016/j.yrtph.2013.03.012 Open Access/.

NRC (National Research Council of the National Academy of Science). (2009). Science and Decisions: Advancing Risk Assessment. National Research Council, National Academies Press, Washington, DC. AKA, "Silverbook".

NRC (National Research Council of the National Academy of Science). (2014). A Framework to Guide Selection of Chemical Alternatives. ISBN978-0-309-31013-0
Available at: http://www.nap.edu/catalog.php?record_id=18872.

OECD (Organisation for Economic Co-operation and Development). (2013). Current Landscape of Alternatives Assessment Practice: A meta-review. ENV/JM/MONO(2013)24.

OECD (Organisation for Economic Co-operation and Development). (2014). Users' Handbook Supplement to the Guidance Document for Developing and Assessing AOPs. Available at: https://aopkb.org/common/AOP_Handbook.pdf.

Seed, J; Carney, E; Corley, R; et al. (2005). Overview: Using Mode of Action and Life Stage Information to Evaluate the Human Relevance of Animal Toxicity Data. Critical Reviews in Toxicology 35:663-672.

Tickner, JA; Schifano, JN; Blake, A; Rudisill, C; Mulvihill, M (2015). Advancing Safer Alternatives Through Functional Substitution, Environmental Science & Technology 49: 742−749.

U.S. EPA (United States Environmental Protection Agency) (1994). Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. Office of Research and Development, Washington, DC.EPA/600/8-90/066F.

U.S. EPA (United States Environmental Protection Agency) (1994). Revised Guidelines for Carcinogen Risk Assessment Incorporating Mode of Action Data. Office of Water, Washington, D.C.

U.S. EPA (United States Environmental Protection Agency) (2005). Guidelines for Carcinogen Risk Assessment. EPA/630/P-03/001B, March. http://www.epa.gov/ncea/iris/backgr-d.htm.

Wiltse, JA; Dellarco,VL; (1998). U.S. Environmental Protection Agency's Revised Guidelines for Carcinogen Risk Assessment: Incorporating Mode of Action Data. Mutation Research. Sep 20;405(2):273-7.

Figure 1. AOPs vs. MOA Analysis