REPORT OF A WORKSHOP ON DOSE-RESPONSE APPROACHES FOR NUCLEAR RECEPTOR-MEDIATED MODES OF ACTION

September 27-29, 2010
NIEHS, Research Triangle Park, NC

Workshop Steering Committee:

Melvin Andersen, Co-Chair, The Hamner Institute
Julian Preston, Co-Chair, U.S. Environmental Protection Agency
Richard Becker, American Chemistry Council
Robert Budinsky . Dow Chemical Company
Michael Cunningham, National Institute of Environmental Health Sciences
Vicki Dellarco, U.S. Environmental Protection Agency
Michael Dourson, Toxicology Excellence for Risk Assessment
Cliff Elcombe, CXR Biosciences
James Klaunig, Indiana University
Michael Honeycutt, Texas Commission on Environmental Quality

Workshop Organized by: Toxicology Excellence for Risk Assessment (TERA)

Contact: Jacqueline Patterson (Patterson AT tera.org)

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Workshop Scope and Objectives

A public workshop on nuclear receptor mode of action and dose-response was held at the National Institute of Environmental Health Sciences (NIEHS) in Research Triangle Park, North Carolina on September 27-29, 2010. Linear extrapolation without threshold for cancer, and sub-threshold doses for non-cancer and (in appropriate cases) cancer have been the dominant paradigms in human health risk assessment, at least in the US, but the application of dose-response modeling approaches with a threshold has been recently questioned (White et al., 2009; NAS, 2008). The growing body of molecular toxicology information is allowing us to explore the presence or absence of sub-threshold doses for a number of receptor-mediated modes of action (MOA). The workshop goal was to explore the development of dose-response approaches for nuclear receptor-mediated liver cancer, within a MOA Human Relevance Framework (HRF). Case studies were used to address activation of the aryl hydrocarbon receptor (AHR); the constitutive androstane receptor/Pregnane X receptor (CAR/PXR), and the peroxisome proliferator-activated receptor-alpha (PPARα).

A main objective of the workshop was to evaluate the biology and dose-response relationships of receptor-driven gene changes and other early biological responses as putative key events in the MOA for adverse human health outcomes. The focus was on applying a systematic framework approach (IPCS, 2007; Boobis et al., 2009) for relating early biological processes or key events (e.g., gene expression changes) to downstream adverse endpoints to develop MOA-informed conclusions about dose-response behavior. Such a systematic approach is intended to be useful for determining the appropriate dose-response assessment techniques to apply to health risks driven by the response of nuclear receptors perturbed by low-level, environmentally-relevant exposures.

The Conduct of the Workshop

A Steering Committee was formed in late 2009 to plan and organize the workshop. Dr. Julian Preston of the U.S. EPA and Dr. Melvin Andersen of The Hamner Institutes were selected as workshop co-chairs. The Steering Committee, assisted by staff from Toxicology Excellence for Risk Assessment (TERA), developed an agenda and workshop process to explore mode of action and dose response for three nuclear receptors. The evidence for each MOA and its key events and dose-response implications was explored using a case study approach based on data for selected ligands for each receptor. The AHR panel discussions focused on available data on dioxins and related chemicals, the CAR/PXR panel used phenobarbital as the model receptor-activator with data that best support MOA evaluation, and the PPARα panel discussed pertinent information on diethylhexyl phthalate and clofibrate.

Chairs and co-chairs were recruited to form and lead the three case study teams. The AHR case study team was lead by Robert Budinsky of Dow Chemical and Dieter Schrenk of the University of Kaiserslautern; the CAR/PXR case study team was led by Cliff Elcombe of CXR Biosciences and Douglas Wolf of the U.S. EPA; the PPARα case study team was led by James Klaunig of Indiana University and Christopher Corton of the U.S. EPA. For each case study a diverse and multi-disciplinary team of experts from academia, industry, government and other organizations was formed several months prior to the workshop. These teams prepared initial materials to facilitate discussions, including background information and presentations on key issues and

data. Throughout the process the Steering Committee and case study leadership emphasized the need to refrain from reaching conclusions prior to the workshop so that all views would be carefully heard and considered. Substantial efforts were made to engage experts with diverse points of view and opinions to better explore the issues.

The format for the workshop included a half-day plenary session with speakers addressing key issues and background information, one and one-half days of concurrent case study sessions, and a final half-day with reports from the case study sessions and a number of experts who circulated among the sessions to identify common themes. The goal was to identify areas of consensus and identify divergent opinions regarding dose-response implications of receptor biology after incorporation of mode of action data. An outcome was the identification of data needs to better describe the MOA to improve dose-response modeling tools for nuclear receptors.

The workshop was supported by the Alliance for Risk Assessment, ACC's Center for Advancing Risk Assessment Science and Policy, CropLife America, CXR Biosciences, Dow Chemical, DuPont, The Hamner Institutes for Health, Indiana University, Society of Toxicology, Society for Risk Analysis, 3M Company, Toxicology Excellence for Risk Assessment , U.S. EPA National Health and Environmental Effects Research Laboratory, U.S. EPA Office of Chemical Safety and Pollution Prevention, and U.S.EPA Office of Water.

While developing dose-response approaches was a goal of the workshop, the panels spent significant time discussing the key events (including associative events and modulating factors) to develop or update assessments of the mode of action for rodent liver tumorigenesis due to activation of each receptor and the relevance of this MOA to humans.

Plenary Session

The workshop began with a series of talks by experts in three key areas: receptor biology, mode of action analysis, and dose-response modeling for risk assessment. Workshop organizers recognized that the panels and other attendees would collectively cover these areas of expertise, but few would have a comprehensive understanding of all three of them. Therefore, the plenary talks were intended to provide an overview of primary considerations to take to the case study analyses, such that all workshop participants would have a basic understanding of the aspects of the science needed to fully evaluate the discussion questions for their respective case studies.

Dr. Steven Kleeberger of the NIEHS welcomed the group and opened the workshop. In his opening remarks he used recent scientific developments from his own laboratory to emphasize the role of new research and evolving science to better inform our understanding of the environmental and health impacts of chemicals that act through receptor driven mechanisms.

Dr. James Klaunig of Indiana University reviewed mechanisms associated with hepatic carcinogenesis in the rodent, and nuclear receptor key events for liver tumorigens. He described many different MOA hypotheses, including DNA and non-DNA reactive MOAs, and receptor and non-receptor mediated MOAs. He reviewed key events and data necessary to support each potential MOA. He noted that activation versus receptor binding is a key concept. He also

highlighted the importance of the temporal nature of key events and the role of dose and reversibility in the pathways that lead to liver tumor formation.

Dr. Donald McDonnell of Duke University presented recent work in his laboratory on developments in molecular pharmacology of nuclear receptors, and specifically AR and prostate cancer. He discussed evolutions in our understanding of the complex mechanisms involved in nuclear receptor activation. The science has moved beyond the view of a simple model of ligand binding followed by DNA response element binding as the mechanism underpinning gene expression changes. Dr. McDonnell noted that receptor conformation is determined by the nature of the bound ligand; differences in receptor conformation dictate co-activator binding preferences; and the biological activity of different nuclear reactor-cofactor complexes are not equivalent. As a result, cofactor interaction profiles might be used as a predictor of response. Ligand binding affinity alone is unlikely to be a good predictor of diverse biological activities since the biological activation profile of a receptor is not the same in all cell types.

Dr. S. Stoney Simons of NIEHS presented on insights from dose-response curves developed from glucocorticoid steroid receptor-regulated gene induction. He noted two major questions: 1) does low dose toxicity increase linearly or non-linearly with a threshold; and 2) can critical reactions for toxicity be identified by high-throughput screening as recommended by the National Academy of Sciences report on toxicity testing (NAS 2007). He presented information on a new model of steroid hormone action that analyzes the modulation of A_{max} and EC_{50} by a given factor that allows for both the identification of factor activity and where a factor acts, as opposed to where it binds. He also presented an approach for integrating a series of complex biological steps into dose-response functions, where certain conditions (e.g., fundamental Hill function coefficients) are demonstrated empirically from the dose-response data. Such novel approaches provide insights into how tools may be developed to better describe the interplay between biology and dose-response prediction.

Dr. Melvin Andersen of The Hamner Institutes reviewed progress in the last several decades on the relationship between receptor biology and dose-response assessment. He concluded that dose response analysis of receptor-mediated processes requires much more than evaluation of binding affinities of ligands to native receptors. He discussed feedback processes, kinase cascades, ligand persistence, and other circuit elements that provide nonlinear response characteristics for control of groups of genes and phenotypic outcomes. Computational and experimental tools show promise to permit this type analysis with surface for nuclear receptors such as AhR, CAR and PPARα.

Dr. Bette Meek of the University of Ottawa presented details of the IPCS/ILSI mode of action / human relevance framework. She emphasized that MOA involves identification of several key events between exposure and effect. but that it is not necessary to identify every step in the process. Early key events are essential, but not necessarily sufficient; the analysis should focus on key events that have been measured and are likely influential in determining risk. With the key events identified, one would evaluate whether the weight of evidence is sufficient to establish the MOA in animals and then identify fundamental qualitative and quantitative differences in key events between animals and humans. Dr. Meek enumerated key questions to be addressed in a MOA/HRF analysis: At relevant doses, what are the early chemical-related key

event(s) that are potentially influential? Are the later key events consistent with knowledge of human disease? Is there an animal model that best informs in this context? What are relevant biomarkers in human studies? Dr. Meek noted that framework can help transition between research and risk assessment, enabling the relating of testing results from high throughput technologies to traditional endpoints in a mode of action context and permitting movement away from hazard characterization to more mode of action based predictive testing. Refinements and improvements to the framework are underway to better characterize uncertainty versus yes/no decisions; better integration of dose-response and temporal concordance for key events with subsequent dose-response analysis for risk characterization; integrating chemical-related information with disease process; and moving to a more systems-biology understanding of toxicity.

Overarching Themes and Major Findings from Roving Experts and Panel Discussion

Theme 1: Importance of Discussion Among Diverse Experts

- Participants commented that it was a good learning experience for all - an opportunity for risk assessors and modelers to better understand the complexity and promise of the molecular biology, and for biologists to gain an understanding of the types of data needed to characterize hazard and risk for humans and how the risk assessors might use the laboratory scientists' work. The sharing of information among a unique mix of risk assessment scientists, biologists and mathematical modelers within each receptor group, leading to a greater understanding of the complexities of each area and allowed some consensus to develop.

- The open workshop process involving dozens of very diverse experts supported a more robust outcome than a typical approach of a small group of scientists working in isolation. Each team went through similar stages - initial discussions to identify and compile data and then discussion on diversity of data and opinions. In each case this was followed by a period of intense exchange, where it was not evident that there would be any agreement; however, with continued discussion and providing one another with insights, each group was able to reach agreement on key conclusions.

- A number of risk assessors commented on how they now have a better appreciation of how the biological science can contribute to a better understanding of mode of action and noted that the current risk assessment practice sometimes loses sight of the underlying biology when calculating dose-response from the range of observation to the range of inferences. The challenge is to understand that risk assessors are not able to estimate human health risk with observation data alone, but will need to use inference, based on knowledge of biological processes. Biological insights are very helpful in selecting the appropriate, or developing new, mathematical models for extrapolation. These assessors hoped that more workshops bringing together the biologists and risk assessors will continue in the future.

Theme 2: Challenges and opportunities due to the current knowledge of nuclear receptor-mediated toxicity

- Many participants went away with an appreciation for the promise of new techniques to provide evidence for human relevance. For example, using data from knock-out and -in animals, is pivotal to establish greater confidence in our understanding of the biological processes underlying the receptor response. Identifying key events *in vivo*, but then determining what *in vitro* information can help further define the MOA or provide some dose-response information. Gene expression data did not have quite the value in the workshop discussions that some expected, but the case study groups were beginning to appreciate the value of this type of data and how to fit it into the framework.

- There was a dynamic tension in the discussions, between a desire to understand every step in the biological processes and the full mechanism of toxicity, and the need for a less comprehensive understanding to establish a mode of action for risk assessment purposes. Each team grappled with questions of whether an additional study was needed because of a lack of data or because it could help understand the mode of action.

- One important gap noted within the context of the case studies was an understanding of mechanisms at the level of induced genes as key events that drive the downstream intermediate and apical events. The similarity in the overall MOA for downstream events was clear among the three case studies. In contrast, important differences were heavily related to the specific nature of the early key events (events between receptor activation, gene expression change and early changes in cell fate). Because of difficulty in identifying the changes in gene expression that are important for driving the apical response, associative events were often used as markers for receptor activation. This gap in knowledge may hinder use of new dose-response methods derived from systems biology and early effect key event data.

- Plenary speakers noted the value of not just overall tissue reaction, but cell-by-cell interactions, and distinguishing between many cells reacting to small extent or a small number of cells to large extent and the role in key events. There needs to be a greater understanding or indication of how many molecules are needed to evoke an effect, the biologists should clearly communicate whether the apical effect can be evoked by one molecule of ligand.

- Case study teams found the limited human data were helpful to evaluate plausibility for humans, but there were not sufficient human data for dose-response. The teams were able to consider species beyond rats and mice to predict human response.

Theme 3: The application of current MOA/HRF for nuclear receptor-mediated outcomes and potential refinements to the approach.

- The MOA Human Relevance Framework worked well to organize the available data and focus attention and discussions on the underlying biology. MOA framework discussions took more time than anticipated and as a result the teams did not devote as much time to discussion of dose response. However, it was essential to lay out the key and associative events and evaluate how the modulating factors relate to adverse health outcomes.

Focusing on the key intermediate events, and discussing how to collect *in vivo* and *in vitro* information, is critical to designing a path forward to be able to ultimately build dose-response models.

- Workshop participants noted that for the MOA analysis, one does not need to identify every step, rather a number of key events is determined and then the hazard-based dose-response is evaluated between the key and apical events. A key question was "how many key events are needed?" and participants thought that a value of information approach could be used to better inform the dose-response implications of proposed research. Moreover, the inclusion of associative events and modulating factors into the framework application was viewed as useful to allow for the consideration of mechanistic data to inform dose-response beyond the data for key events.

- Participants noted the importance in identifying an appropriate biomarker or indicator of the key event, how to use key event data as a surrogate for the apical endpoint of interest, and ultimately how to use the key events' data to describe dose-response.

- Participants were concerned about human polymorphisms or differences in sensitivity and response among a human population. One pointed out that good mechanistic information on a key event could be used to help define variations in populations and another noted that there is much pharmaceutical data that might inform understanding of human variability. However, others cautioned that variability in early responses may be very different from variability in later responses (e.g., 100-fold difference in induction would not expect to see 100-fold difference in later sequelae). Others saw promise of putting key events through high throughput screening to provide a sense of variability, and that there are data coming out of mouse diversity panels. One participant cautioned that with a given MOA, if one sees a large difference between animals and humans it could appear to be a qualitative species difference, but it may just be different quantitative parameter values for each species.

- Other refinements to the mode of action approach might more fully address the concept of there being multiple pathways or components within a single integrated MOA description for a single apical endpoint. It was also noted that in part this issue may be reflected in the common presentation of the MOA as a "series" of key events, rather than a web or network of interrelated events as might better reflect the biology.

- Participants also discussed general considerations around dose-response implications. Research on new empirical approaches may help inform our understanding of likely low-dose behaviors. For example, one participant noted that if responses fit first order Hill plots, then lower doses should follow first order behavior into the extrapolation range. Other speakers commented on examples of dose-response approaches that use data from early key events directly or through linked dose-response functions that connect early and later key events. A participant noted that the question of likely dose-response behavior in the low dose region may in part reflect the perspective of which direction we are looking from (high dose down or low dose up) in terms of the biology.

- Since the current frameworks might be enhanced by describing these dose-response considerations further it was noted that there are ongoing developments in refining the MOAE/HRF concepts to incorporate some of the issues raised during this workshop related to dose-response behavior. For example, the current ILSI/ HESI dose response framework draft has been revised to include modulating factors and population issues. The group is now selecting case studies and will have a workshop in January to describe how the whole project is evolving. The results of this nuclear receptor workshop are important to consider in this evolving framework.

Conclusions from the Case Study Panels

The AHR expert panel, for the first time in an expert panel format, rigorously applied the MOA/HRF framework and agreed on an MOA. Similarly, the CAR expert panel identified relevant data and applied the framework with emphasis on the qualitative and quantitative aspects of human relevance. For PPARα, the expert panel built upon previous applications of the MOA/HRF framework using significant new data that allowed for refinement of the key event descriptions and updated considerations related to human relevance. Each panel identified data needs and suggested improvements for application of the MOA/HRF framework. The public workshop had broad support and funding from industry, government, universities, scientific societies, and research organizations.

Materials from the workshop are available at http://www.tera.org/peer/nuclearreceptor/. Publications for the workshop and case studies are being prepared for submittal.

Moreover, a perhaps expected benefit of the workshop was the sharing of information among a unique mix of risk assessment scientists, biologists and mathematical modelers within each receptor group, leading to a greater understanding of the complexities of each area and allowing some consensus to develop.

References

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