



NUCLEAR RECEPTOR BINDING DOMAIN: FIGURE FROM:  
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# DOSE-RESPONSE APPROACHES FOR NUCLEAR RECEPTOR- MEDIATED MODES OF ACTION

WORKSHOP

SEPTEMBER 27 - 29, 2010

NATIONAL INSTITUTE FOR ENVIRONMENTAL  
HEALTH SCIENCES (NIEHS)  
RESEARCH TRIANGLE PARK, NC

...EXPLORING THE DEVELOPMENT OF BIOLOGICALLY-BASED DOSE-RESPONSE APPROACHES FOR  
NUCLEAR RECEPTOR MEDIATED TOXICITY...

## **Sponsors and Support**

We would like to thank our sponsors and supporters who provided financial and in-kind support to make this workshop a success. The workshop would not have been possible without the cooperative efforts of federal and state government agencies, universities, industry, consulting firms, and non-profits. We are most appreciative of the **National Institute of Environmental Health Sciences (NIEHS)** for hosting the workshop. Our special thanks to Dr. Linda Birnbaum and all the NIEHS staff who have been extremely helpful and supportive.

### **Alliance for Risk Assessment**

**American Chemistry Council's Center for Advancing Risk Assessment**

**Science and Policy**

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**The Hamner Institutes for Health**

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**U.S. EPA, National Health and Environmental Effects Research Laboratory**

**U.S. EPA, Office of Chemical Safety and Pollution Prevention**

**U.S.EPA, Office of Water**

September 27, 2010

Dear Workshop Participants:

Welcome to the Dose-Response Approaches for Nuclear Receptor-Mediated Modes of Action Workshop. This interactive workshop will explore the development of dose-response approaches for nuclear receptor-mediated liver cancer.

The purpose of this workshop is to convene a group of experts to explore systematic frameworks and data evaluation approaches for assessing dose-response implications of toxicants acting via nuclear receptor-mediated modes of action (MOA). Expert panels will use a case study approach to explore the development of dose-response approaches for nuclear receptor-mediated liver cancer in light of the current MOA understanding for three nuclear receptors: the aryl hydrocarbon receptor (AHR); the constitutive androstane receptor/pregnane X receptor (CAR/PXR), and the peroxisome proliferator-activated receptor-alpha (PPAR $\alpha$ ). Although data for specific toxicants that act through these receptors are being used to develop the case studies, the goal of this workshop is to assess dose-response implications of nuclear receptor activation in general terms at the level of individual biological response, and is not intended to provide updated or alternative population level risk assessments for specific chemicals.

A very diverse group of sponsors have provided monetary and in-kind support for the workshop and we are grateful for their generosity and support to advance science in this area -- Alliance for Risk Assessment, American Chemistry Council's Center for Advancing Risk Assessment Science and Policy, Chlorine Chemistry, CropLife America, CXR Biosciences, DuPont, The Hamner Institutes for Health, Indiana University Department of Environmental Health, Society of Toxicology, Society for Risk Analysis, 3M Company, Toxicology Excellence for Risk Assessment, U.S. EPA's National Health and Environmental Effects Research Laboratory, U.S. EPA's Office of Chemical Safety and Pollution Prevention, and the U.S.EPA's Office of Water.

Considerable work was done prior to the workshop by our three case study panels. These individuals have provided hours of thought and preparation to insure the success of this workshop. We thank the case study expert panel members for their hard work and in particular the co-chairs of each of the panels for organizing and leading the efforts: Drs. Bob Budinsky and Dieter Schrenk for AHR, Drs. Cliff Elcombe and Doug Wolf for CAR/PXR, and Drs. Jim Klaunig and Chris Corton for PPAR $\alpha$ . And finally, we recognize the vision and guidance of the workshop Steering Committee which has met for the last year to organize and plan the workshop.

Our goal over the next three days is to identify consensus and divergent opinions about the implications of receptor biology mode of action data for dose response assessments. We anticipate the case study panels will identify data gaps in the underlying MOA data and dose-response modeling tools for nuclear receptors and provide recommendations for further inquiry on these issues and the implications for health risk assessment methods.

We hope you enjoy the workshop.

Sincerely,

Melvin E. Andersen, The Hamner Institutes for Health  
Workshop Co-Chair

Julian Preston, U.S. EPA NHEERL  
Workshop Co-Chair





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Julian Preston, Ph.D,  
U.S. EPA NHEERL

**Workshop Organizer**

Toxicology Excellence for Risk Assessment (TERA)



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## **Tab 1 - Workshop Program**

## Agenda

**Monday, September 27<sup>th</sup>, 2010**

### Plenary Session [webcast]

- 8:00 - 8:30** Introduction and Opening Remarks - *Julian Preston*
- 8:30 - 8:45** NIEHS Welcome - *Steven R. Kleeberger*
- 8:45 - 9:15** Nuclear Receptor Key Events for Liver Tumorigens: *James Klaunig*
- 9:15 - 9:45** Recent Developments in Molecular Pharmacology of Nuclear Receptors: *Donald McDonnell*
- 9:45 - 10:15** Insights From Dose-response Curves: Guides for Toxicology from Glucocorticoid Receptor-Regulated Gene Induction: *S. Stoney Simons*
- 10:15 - 10:30** **BREAK**
- 10:30 - 11:00** Relationship Between Receptor Biology and Dose-Response Assessment: *Melvin Andersen*
- 11:00 - 11:30** Mode of Action/Human Relevance (Key Events) Analysis: *M.E. (Bette) Meek*
- 11:30 - 12:00** Q&A - *Plenary Speakers*
- 12:00 - 12:30** Introduction of Case Studies and Charge: *Julian Preston*
- 12:30 - 1:30** **LUNCH (on own at NIEHS cafeteria)**

### Concurrent Case Study Sessions [no webcast]

- 1:30 - 3:30** Case Study Sessions  
ROOM A: CAR/PXR, *Cliff Elcombe, Chair*  
ROOM B: PPAR $\alpha$ , *James Klaunig, Chair*  
ROOM C: AHR, *Robert Budinsky, Chair*
- 3:30 - 3:45** **BREAK**
- 3:45 - 5:30** Case Study Sessions, continued
- 7:00** **DINNER - Hotel**

**Tuesday, September 28<sup>th</sup>, 2010**

**Concurrent Case Study Sessions [no webcast]**

**8:00 - 10:00** Case Study Sessions, continued  
ROOM A: PPAR $\alpha$ , *James Klaunig, Chair*  
ROOM B: CAR/PXR, *Cliff Elcombe, Chair*  
ROOM C: AHR, *Robert Budinsky, Chair*

**10:00 - 10:15 BREAK**

**10:15 - 12:00** Case Study Sessions, continued  
ROOM A: PPAR $\alpha$ , *James Klaunig, Chair*  
ROOM B: CAR/PXR, *Cliff Elcombe, Chair*  
ROOM C: AHR, *Robert Budinsky, Chair*

**12:00 - 1:00 LUNCH (on own at NIEHS cafeteria)**

**1:00 – 3:00** Case Study Sessions, continued  
ROOM A: PPAR $\alpha$ , *James Klaunig, Chair*  
ROOM B: CAR/PXR, *Cliff Elcombe, Chair*  
ROOM C: AHR, *Robert Budinsky, Chair*

**3:00 - 3:15 BREAK**

**3:15 - 5:30** Case Study Sessions, completed  
ROOM A: PPAR $\alpha$ , *James Klaunig, Chair*  
ROOM B: CAR/PXR, *Cliff Elcombe, Chair*  
ROOM C: AHR, *Robert Budinsky, Chair*

**Wednesday, September 29<sup>th</sup>, 2010**

**Plenary Session [webcast]**

**8:00 – 8:15** Revisit Objectives and Charge Questions: *Julian Preston*

**8:15 - 9:00** Report from Case Study 1: *Rapporteur*

**9:00 - 9:45** Report from Case Study 2: *Rapporteur*

**9:45 - 10:30** Report from Case Study 3: *Rapporteur*

**10:30 - 10:45 BREAK**

**10:45 - 12:45** Panel Discussion of Case Studies

**12:45 - 1:00** Closing Remarks, Action Items: *Melvin Andersen*

## Scope and Objectives

### Background and Problem Formulation

The presence of threshold effects for non-cancer and (in appropriate cases) cancer has been the dominant paradigm for the practice of risk assessment. The application of dose-response modeling approaches that include a threshold has been recently questioned (White, et al, 2009; NAS, 2008). Specifically, a position has been articulated that for population-level risk analyses, in the absence of mode of action (MOA)-based dose-response models, the most appropriate low-dose extrapolation approach for both cancer and non-cancer end points is linear extrapolation to zero from the range of observed responses (i.e., no sub-threshold or non-adverse effect dose exists). This low-dose linear extrapolation is based on the suppositions that the effects of population variability and additivity to background disease and exposures invalidate the concept of a threshold for most chemicals (White, et al, 2009), in contrast to existing toxicology literature on which the threshold-based approach has been built (e.g., Casarett and Doull, 2008, page 23).

The scientific basis for addressing the issue of linearity versus non-linearity becomes especially relevant when viewed through the lens of the NAS report from 2007...*Toxicity Testing in the 21<sup>st</sup> Century: A Vision and a Strategy*. The NAS vision calls for a paradigm shift of both the toxicity testing and interpretive tools for assessing the risks to humans exposed to chemicals at environmental, low-level exposures. This computational systems biology approach would progress from development and validation of toxicity pathway assays to an understanding of dose-response relationships at the level of the gene. In particular, perturbations of gene-receptor effects would be examined in the context of real world, environmentally-relevant exposures.

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 World Health Organization (WHO) International Programme on Chemical Safety (IPCS) and International Life Sciences Institute (ILSI) have developed and refined a mechanistic approach to assessing the MOA based on guidelines for evaluation of key events (Sonich-Mullin et al. 2001, Meek et al. 2003, Boobis et al. 2006, 2008, 2009; IPCS 2007). The MOA has been defined as a “biologically plausible sequence of key events leading to an observed effect supported by robust experimental observations and mechanistic data” (Boobis et al. 2009). The MOA for a particular toxicologic effect is evaluated using a weight of evidence (WOE) approach following the Bradford-Hill causal association analysis (Sonich-Mullin et al. 2001; EPA 2005). In addition, this approach should include analysis of dose-response concordance, as the definition of a key event requires that it be a quantifiable and critical step in the progression of the toxicologic effect (Boobis et al. 2009). Each key event in a proposed MOA, based on its dose-response relationship, should be evaluated in the context of the overall dose-response relationship for the toxicologic effect, as well as evaluated for uncertainties including inter- and intra-species differences (Julian et al. 2010).<sup>1</sup>

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<sup>1</sup> The term critical effect used for noncancer toxicity, which is defined as the first adverse effect or its known precursor, can be seen as the adverse effect of first concern, or as the penultimate key event prior to this adverse effect.

## Workshop Objectives

The purpose of this workshop is to convene a group of experts to explore systematic frameworks and data evaluation approaches for assessing dose-response implications of toxicants acting via nuclear receptor mediated modes of action. The effort is intended to explore the current state of knowledge regarding receptor biology, key events related to the MOA for toxicity mediated through a nuclear receptor, and the implications of this knowledge to inform the development of dose-response assessments for health risk assessment. This workshop uses a case study approach to explore the development of dose-response approaches for nuclear receptor-mediated liver cancer in light of the current MOA understanding. This workshop will explore the biology and dose-response implications of toxicology information for three nuclear receptors: the aryl hydrocarbon receptor (AHR); the constitutive androstane receptor/pregnane X receptor (CAR/PXR), and the peroxisome proliferator-activated receptor-alpha (PPAR $\alpha$ ). Although data for specific toxicants that act through these receptors are being used to develop the case studies, the goal of this workshop is to assess dose-response implications of nuclear receptor activation in general terms at the level of individual biological response, and is not intended to provide updated or alternative population level risk assessments for specific chemicals.

A main objective of this workshop is to review the biology and dose-response relationships of receptor-driven gene changes, leading to the key events, which define a MOA relevant to an adverse human health outcome. The focus is on applying a systematic framework approach 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 based on the response of nuclear receptors perturbed by low-level, environmentally relevant exposures.

For each case study receptor, the group discussions and output should aim to address the following general discussion questions:

1. Is the existing biological knowledge for liver tumors induced through a specific nuclear receptor sufficiently understood to identify the mode of action and its component key events, associative events, and modulating factors? If not what are the key data gaps?
2. Is the existing biological knowledge of the MOA for liver tumors induced through a specific nuclear receptor sufficiently understood to reasonably exclude on a qualitative or quantitative basis the human relevance of rodent liver tumors induced through this receptor? If the data are not adequate to evaluate species concordance of the MOA what are the key data gaps?
3. If the data are sufficient to identify a MOA and support its relevance to humans, what are the dose-response implications of the key events in the MOA and associative events and modulating factors? Are the data adequate to develop biologically based dose-response or other biological-informed models for this receptor, and if not what are the key data gaps? In the absence of such data, is a linear low-dose modeling the most appropriate default, based on the underlying science of nuclear receptor biology?

## Workshop Data Evaluation Approach

Definitions of key terms from guidance documents as applied in this workshop are provided here to set a uniform approach for evaluation of the three different case studies.

### *Mode of Action versus Mechanism of Action*

Mode of action is different from mechanism of action in that mode of action is a “less detailed biochemical description of events” that provides “sufficient evidence to draw a reasonable working conclusion concerning the agent’s influence on key processes” and “permits information on precursor events to be evaluated and incorporated into the risk assessment process in a realistic way” (Dellarco and Baetcke 2005). As defined by the US EPA 2005 Cancer guidelines:

**Mode of Action:** “The term *mode of action* is defined as a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation. The toxicokinetic processes that lead to formation or distribution of the active agent to the target tissue are considered in estimating dose but are not part of the mode of action as the term is used here. There are many examples of possible modes of carcinogenic action, such as mutagenicity, mitogenesis, inhibition of cell death, cytotoxicity with reparative cell proliferation, and immune suppression. Mode of action is contrasted with *mechanism of action*, which implies a more detailed understanding and description of events, often at the molecular level, than is meant by mode of action.”

### *Key Events and Definitions of Other Biological Processes*

The case study panels will use the IPCS Human Relevance Framework (IPCS 2007) and the modified Hill approach for causality (EPA, 2005) to evaluate the MOA for liver tumors induced in rodents by activators of three different nuclear receptors. Because the underlying mechanistic knowledge of the nuclear receptors may be well-characterized, additional knowledge of biological processes beyond the major key events that is available can be used to refine our understanding of the overall dose-response behavior for this endpoint. To more fully use current mechanistic understanding, which is beyond a broad level of knowledge required for developing a mode of action hypothesis, possible refinements to the current IPCS (2007) mode of action and human relevance framework as developed by ILSI and others are being used to characterize the nature of the biological steps involved. The Workshop Steering Committee and case study teams have agreed on the following definitions for use in evaluating key steps in a proposed MOA:

**Key Event:** An empirically observable causal precursor step to the adverse outcome that is itself a necessary element of the mode of action. Key events are required events for the MOA, but often are not sufficient to induce the adverse outcome in the absence of other key events.

**Associative Event:** Biological processes that are themselves not causal necessary key events for the MOA, but are reliable indicators or markers for key events. Associative events can often be used as surrogate markers for a key event in a MOA evaluation or as indicators of exposure to a xenobiotic that has stimulated the molecular initiating event or a key event.

**Modulating Factor:** There are many factors or biological responses that are not necessary to induce the adverse outcome, but could modulate the dose-response behavior or probability of inducing one or more key events or the adverse outcome. Such biological factors are considered modulating factors. Example: excessive body weight loss at a high dose.

The MOA framework, as being applied in this workshop, contains three major areas of application for evaluation. The first is the MOA evaluation, or a weight of evidence approach to establish a MOA in experimental animals. For each defined mode of action, the key events must be identified using modified criteria employed by U.S. EPA (2005) and IPCS (2007) adapted from the Hill considerations for assessing causality (Hill 1965). The Hill definitions below have been supplemented with modifications presented by EPA (2005) for clarification and relevance to cancer MOA evaluation for this workshop. The IPCS 2007 framework incorporates the below areas of consideration as well.

**Strength:** Is the measured effect many-fold different or marginally different from controls?; “The finding of large, precise risks increases confidence that the association is not likely due to chance, bias, or other factors.” (EPA 2005)

**Consistency:** “Has it been repeatedly observed by different persons, in different places, circumstances, and times?” (Hill 1965); “An inference of causality is strengthened when a pattern of elevated risks is observed across several independent studies. The reproducibility of findings constitutes one of the strongest arguments for causality.” (EPA 2005)

**Specificity:** Is the association (disease/effect) limited to one particular site, organism, etc. with no apparent other explanations? Hill cautions not to over-emphasize this criteria; As originally intended, this refers to increased inference of causality if one cause is associated with a single effect or disease (Hill, 1965). “Based on our current understanding that many agents cause cancer at multiple sites, and many cancers have multiple causes, this is now considered one of the weaker guidelines for causality. Thus, although the presence of specificity may support causality, its absence does not exclude it.” (EPA 2005).

**Temporality:** Does the observed key event precede the ultimate adverse outcome in a logical manner? Conversely, does the adverse outcome cause the observed effect as a secondary consequence (i.e. the observed effect would then not be causal); “A causal interpretation is strengthened when exposure is known to precede development of the disease. Because a latent period of up to 20 years or longer is often associated with cancer development in adults, the study should consider whether exposures occurred

sufficiently long ago to produce an effect at the time the cancer is assessed. This is among the strongest criteria for an inference of causality.” (EPA 2005).

**Biological gradient:** Is there a dose-response or concentration-response gradient for the effect? This is contingent on having a reliable measurement of exposure; “A clear exposure-response relationship (e.g., increasing effects associated with greater exposure) strongly suggests cause and effect, especially when such relationships are also observed for duration of exposure (e.g., increasing effects observed following longer exposure times). There are many possible reasons that an epidemiologic study may fail to detect an exposure-response relationship. Thus, the absence of an exposure-response relationship does not exclude a causal relationship.” (EPA 2005). A second aspect of the dose-response relates to dose-response concordance between the key event and adverse outcome, such that, “...If a key event and tumor endpoints increase with dose such that the key events forecast the appearance of tumors at a later time or higher dose, a causal association can be strengthened” (EPA 2005).

**Plausibility:** “It will be helpful if the causation (key event) we suspect is biologically plausible. What is biologically plausible depends on the biological knowledge of the day.” (Hill 1965); “An inference of causality tends to be strengthened by consistency with data from experimental studies or other sources demonstrating plausible biological mechanisms. A lack of mechanistic data, however, is not a reason to reject causality” (EPA 2005).

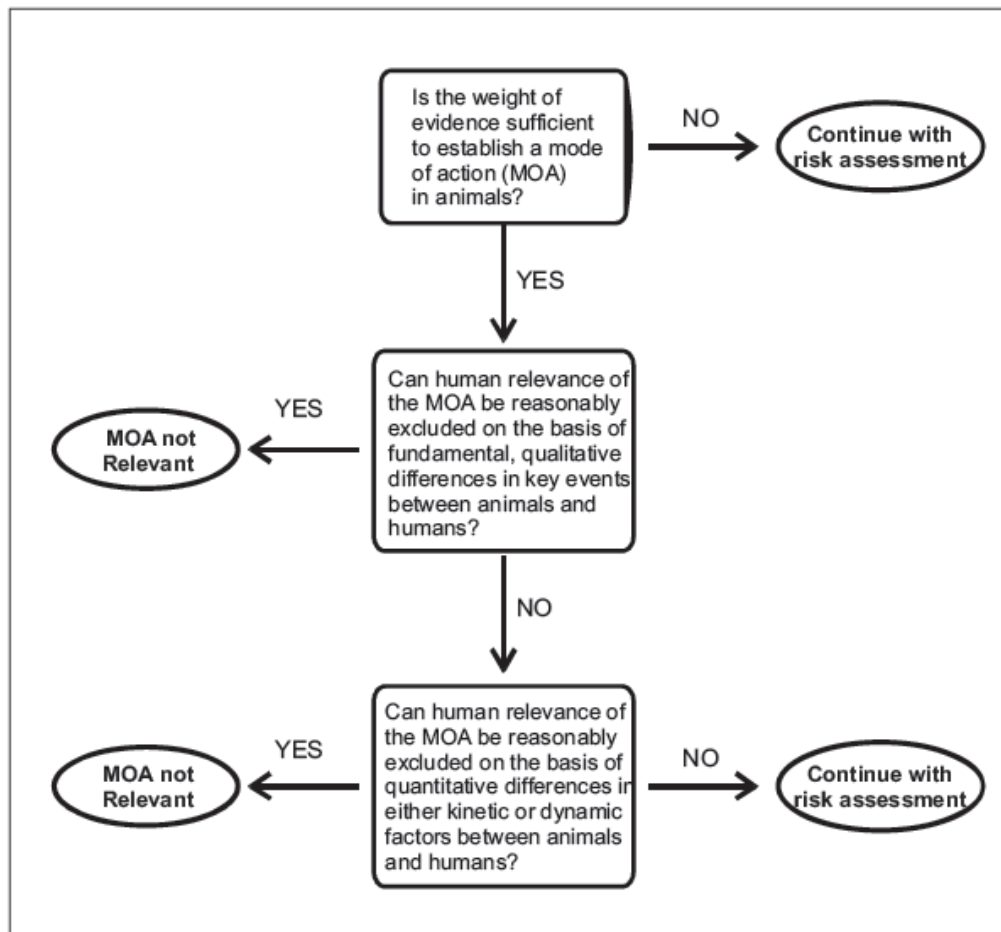
**Coherence:** The cause-and-effect interpretation of our data should not seriously conflict with the generally known facts of the natural history and biology of the disease; “An inference of causality may be strengthened by other lines of evidence that support a cause-and-effect interpretation of the association. Information is considered from animal bioassays, toxicokinetic studies, and short-term studies. The absence of other lines of evidence, however, is not a reason to reject causality” (EPA 2005).

### *Human Relevance Framework (HRF) (from IPCS 2007)*

If the MOA is successfully demonstrated for experimental animals, the IPCS (2007) Human Relevance Framework is used to evaluate whether data are adequate to reasonably exclude the human relevance of the demonstrated MOA. The IPCS HRF is presented as an approach to answering a series of three questions, leading to a documented, logical conclusion regarding the human relevance of the MOA underlying animal tumors (see Figure 1).

1. Is the weight of evidence sufficient to establish a mode of action (MOA) in animals?
2. Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?
3. Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans?

Questions 2 and 3 involve qualitative and quantitative considerations, respectively, in a concordance analysis of human information in relation to the animal MOA and its key events. These are followed by an explicit description of confidence in the evaluation, identification of specific data gaps, and the implications for risk assessment.



**Figure 1.** IPCS general scheme illustrating the main steps in evaluating the human relevance of an animal MOA for tumour formation. The questions have been designed to enable an unequivocal answer *yes* or *no*, but recognizing the need for judgement regarding sufficiency of weight of evidence. Answers leading to the left side of the diagram indicate that the weight of evidence is such that the MOA is not considered relevant to humans. Answers leading to the right side of the diagram indicate either that the weight of evidence is such that the MOA is likely to be relevant to humans or that it is not possible to reach a conclusion regarding likely relevance to humans, owing to uncertainties in the available information. In these cases, the assessment would proceed to risk characterization. It should be noted that only at this stage would human exposure be included in the evaluation.

### *Dose-Response Assessment Implications of MOA*

The preferred dose-response approach is development of a biologically based dose-response (BBDR) model. For many chemicals, insufficient data exist for the development of a BBDR model. In such cases, the traditional approach is to use assumptions based on the MOA to revert to either linear or non-linear dose-response methods (EPA 2005). Alternative approaches that

use biological understanding to inform the selection of empirical dose-response shapes are also a possibility that has been applied by some investigators.

The concept of using MOA information to inform dose-response behavior is embedded in the current frameworks being applied in this workshop. “Understanding dose–response can have a profound effect on hazard characterization and therefore is an important component of the MOA analysis, particularly when non-linear processes or dose transitions are inherent in the relevant biology. Similarly, quantifying hazard in the context of dose informs the process of risk assessment by suggesting extrapolation models that are consistent with our understanding of the biology” and the defined MOA (IPCS 2007). The incorporation of data for early biological responses and from systems biology emphasis are also being actively considered as a topic of application in this workshop. For example, Boobis et al. (2009) observed in describing dose-response applications of the MOA approach:“It is essential that computational approaches be used in parallel with data gathering exercises in order to be able to interpret the molecular data in a whole cell (or tissue) context. In this regard, molecular modeling techniques are an essential component of assessing low-dose responses where it is very difficult to obtain all the necessary informative experimental data.”

## Workshop Materials Development

Expert panels were formed for each of the three case studies and these teams have developed discussion questions highlighting key issues related to alternative MOA hypotheses and dose-response implications of each nuclear receptor. Data were compiled on an initial list of possible key events in preparation for full discussion at the workshop. During the workshop, each expert panel will discuss the available information on biological events, the potential MOA, human relevance of the MOA, and dose-response implications. MOA and concordance tables will be used as a tool to facilitate the documentation of the panel discussions. Summaries on some key issues were also prepared and made available to provide the panel members and observers a brief synopsis of the key highlights for discussion by the panel. Suggested background reading and key references are also provided to aid in workshop preparation.

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## Case Study Agendas

### AHR Case Study Agenda

Monday, September 27<sup>th</sup>, 2010

#### AHR Case Study Team Opening Remarks and Introductory Topics

- 1:30 - 2:00** Assembly and Opening Remarks: *Co-Chairs*
- 2:00 - 2:30** AHR – Current State of Knowledge and Role in Biology/Physiology: *Thomas Gasiewicz and Gary Perdew*
- 2:30 - 3:00** The Mode-of Action for AHR Mediated Carcinogenesis – Insights from Chronic Rodent Bioassays of TCDD and Related Compounds: *Nigel Walker and Michael DeVito*
- 3:00 - 3:30** Overview of Histopathology During TCDD -Mediated Tumor Promotion and Hepatocarcinogenesis: *Amy Brix*
- 3:30 - 3:45** Break
- 3:45 - 4:15** AHR-Activation Inhibition of (Focal) Apoptosis: *Dieter Schrenk*
- 4:15 - 4:45** Zonal Activation of CYP1A Proteins by AHR – Implications for AHR-mediated Tumor Promotion and Hepatocarcinogenesis: *Melvin Andersen and Sudin Bhattacharya*
- 5:15 - 5:30** Closing Remarks and Discussion for Day 1: Co-Chairs and AHR Expert Panel Members
- 7:00** **DINNER - Hotel**

Tuesday, September 28<sup>th</sup>, 2010

- 8:00 – 8:30** Introductory Comments and Follow-up Business from Day 1: *Co-Chairs*
- 8:30 - 8:45** Gene Array Analysis of TCDD-induced mRNA Expression from Primary Rat and Human Hepatocytes: *Russell Thomas and Craig Rowlands*
- 8:45 - 9:15** Quantitative Considerations for Interspecies and High-to Low Dose Extrapolation: *Lesia L. Aylward*

**9:15 - 10:15** IPCS Framework Analysis of the MOA for TCDD-mediated Liver Tumor Promotion and Hepatocarcinogenesis: *Robert Budinsky*

**10:15 - 10:30** **BREAK**

**10:30 - 11:00** Biomathematical Considerations for Dose-response Assessment: *Ted Simon and Bruce Allen*

**11:00 - 12:00** Addressing Dose-response, Concordance, and Key Events Tables

**12:00 - 1:00** **LUNCH**

**1:00 - 5:30** Case Study Discussion Questions with Breaks as Needed

## CAR/PXR Case Study Agenda

Monday, September 27<sup>th</sup>, 2010

### CAR/PXR Case Study Team Opening Remarks and Introductory Topics

- 1:30-2:00** Introductions and Opening Remarks: *Cliff Elcombe and Doug Wolf*
- 2:00-2:45** CAR – Current State of Knowledge and Role in Biology/ Physiology:  
*Curtis Omiecinski and Wen Xie*
- 2:45– 3:00** **BREAK**
- 3:00-3:45** CAR- and PXR-Mediated Liver Growth in Rodents: Review of Key Events for  
Phenobarbital-Induced Rodent Liver Tumor Formation: *Brian Lake*
- 3:45-4:10** Summary of Additional Literature: Histopathology and Nomenclature: *Russell  
Cattley*
- 4:10-4:35** Epigenetics and Carcinogenesis: Emphasis on Phenobarbital-Induced Alterations  
in DNA Methylation and Gene Expression: *Jay Goodman*
- 4:35-4:50** **BREAK**
- 4:50-5:05** CAR-Specific Data on Other Events: A Role for Oxidative Stress?: *Remi Bars*
- 5:05-5:30** Species Differences and Other Factors Impacting on Risk Assessment:  
*Cliff Elcombe*
- 7:00** **WORKSHOP DINNER - Hotel**

Tuesday, September 28<sup>th</sup>, 2010

### Dose-Response Modeling Considerations

- 8:00 - 8:15** Review of Day 1 and Plans for Day 2
- 8:15 - 8:45** Microarray and Biological Pathway of Phenobarbital Transcriptomic Research:  
*David Geter and Susan Hester*
- 8:45 - 9:45** IPCS Framework Analysis of MOA for Phenobarbital-Induced Mouse Liver  
Tumors (Interactive Presentation/Discussion): *Douglas Wolf and Richard Peffer*

**9:45-10:00 BREAK**

**10:00 - 10:30** IPCS Framework Analysis of MOA for Phenobarbital-Induced Mouse Liver Tumors (Interactive Presentation/Discussion, continued): *Douglas Wolf and Richard Peffer*

**10:30 - 11:15** Biologically Based Dose-Response Modeling for Hepatocarcinogenic Effects of Phenobarbital: *Rory Conolly and Kenny Crump*

**11:15 - 12:00** Begin Case Study Discussion

**12:00-1:00 LUNCH**

**1:00-5:30** Case Study Discussion with Breaks as Needed

## PPAR $\alpha$ Case Study Agenda

### Monday, September 27<sup>th</sup>, 2010

- 1:30-2:00** Assembly, opening remarks and charge; PPAR $\alpha$ -mediated liver growth in rodents: review of key events in PPAR $\alpha$  activator-induced rodent liver tumor formation: *Chris Corton and Jim Klaunig – Co Chairs*
- 2:00-2:45** PPAR $\alpha$  Activation as a Key Event: *Jeff Peters*
- 2:45– 3.00** **BREAK**
- 3:00-3:35** PPAR $\alpha$ -Mediated Liver Growth in Rodents: *Michael Cunningham*
- 3:35- 3:50** PPAR $\alpha$ -Mediated Liver Pathology and Adaptive Changes: *Jim Popp*
- 3:50-4:20** Role of Oxidative Stress and NFKb: *Chris Corton*
- 4:20-4:35** PPAR $\alpha$ -Specific Data on Other Events That May Be Involved in Carcinogenesis: *Jim Klaunig*
- 4:35-4:50** **BREAK**
- 4:50-5:05** Species Differences and Other Factors Impacting Risk Assessment: *Tim Hummer and Phillip Bentley*
- 5:05-5:30** Consideration of objectives for Day 2 including “homework” assignments
- 7:00** **DINNER - Hotel**

### Tuesday, September 28<sup>th</sup>, 2010

#### Human Relevance Framework Discussions

- 9:00- 9:15** Review of Day 1, comments and questions : *Jim Klaunig, discussion leader*
- 9:15-10:30** IPCS Framework Analysis of MOA for PPAR $\alpha$ -induced Rodent Liver Tumors: *Jennifer Seed*
- 10:30-10:45** **BREAK**

**10:45-11:30** IPCS Framework Analysis of MOA for PPAR $\alpha$ -induced Rodent Liver Tumors,  
Continued: *Jennifer Seed*

**11:39-12:00** Biologically Based Dose-Response Modeling for Hepatocarcinogenic Effects of  
PPAR $\alpha$  activators: *Lorenz Rhomberg*

**12:00-1:00** **LUNCH**

**1:00-5:30** Continue Discussion with Breaks as Needed

## Steering Committee and Case Study Panels

### Steering Committee

#### Co-Chairs

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ATS, CIH, DABT, PhD  
The Hamner Institutes

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U.S. EPA

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American Chemistry Council

Michael Dourson, DABT, PhD  
Toxicology Excellence for Risk Assessment

Robert Budinsky, PhD  
Dow Chemical Company

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University of Dundee Medical School

Michael Cunningham,  
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NIEHS-NTP

James Klaunig, ATS, PhD  
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Vicki Dellarco, PhD  
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Assisted by:  
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Tom Gasiewicz, PhD  
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## CAR/PXR Panel Members

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Remi Bars, PharmD, PhD  
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Brian Lake, DSc  
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Stephen Ferguson, PhD  
CellzDirect/ Life Technologies

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Wen Xie, PhD, MD  
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Amber Goetz, PhD  
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## PPAR Panel Members

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### Rapporteurs

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Jeffrey Peters, PhD  
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Michael Cunningham, PhD,  
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James Popp, PhD, DVM,  
ATS, DACVP  
Strataxon LLC

Timothy Hummer, PhD,  
DABT  
U.S. FDA-CDER

Lorenz Rhomberg, PhD  
Gradient Corporation

Jennifer Seed, PhD  
U.S. EPA-OCSP

### **Workshop Organization and Support**

Andrew Maier, PhD, DABT, CIH  
Toxicology Excellence for Risk Assessment

Alison Willis, BS  
Toxicology Excellence for Risk Assessment

Jacqueline Patterson, MEn  
Toxicology Excellence for Risk Assessment

## Alliance for Risk Assessment (ARA)

The *Dose-Response Approaches for Nuclear Receptor-Mediated Modes of Action* workshop is a project of the Alliance for Risk Assessment (ARA). The ARA is a collaboration of organizations that fosters the development of technical chemical risk assessment products and services, through a team effort of specialists and organizations dedicated to *protecting* public health by improving the process and efficiency of risk assessment, and to increasing the capacity for developing risk values to meet growing demand.

The work of ARA focuses resources to help meet the needs of state, local, and tribal risk assessors. All projects under the ARA are vetted by a Steering Committee of diverse stakeholders to promote scientific relevance and avoid duplication of effort. Learn more at: [www.allianceforrisk.org](http://www.allianceforrisk.org).

The *Dose-Response Approaches for Nuclear Receptor-Mediated Modes of Action* workshop will be conducted in conjunction with the *Beyond Science & Decisions* workshop series currently underway. The ARA would like to invite your participation in the upcoming workshop:

### **Beyond Science and Decisions: From Issue Identification to Dose-Response Assessment - Workshop II**

**Date: October 11-13, 2010**

**Location: Crystal City, Virginia**

**(In tandem with the Federal & State Risk Assessment & Toxicology Conference)**

#### **Background & Purpose:**

To continue the discussion set forth by *Science and Decisions: Advancement of Risk Assessment* (NAS, 2009), and conducted under the aegis of the Alliance for Risk Assessment (ARA), a series of three meetings is envisioned over the course of a year. The ultimate goal is consensus among participants on guidance highlighting key considerations for applying a variety of dose-response techniques to different risk assessment settings, as characterized in early problem formulation or issue identification. The workshops will focus on purpose-specific dose-response assessment, which integrates relevant available data and is illustrated by case studies. The workshop series will include brainstorming and selection of case studies at the first meeting, evaluation of the case studies during the second, and build consensus on purpose-specific dose-response assessment methods during the third. More information is available at [http://www.allianceforrisk.org/ARA\\_Dose-Response.htm](http://www.allianceforrisk.org/ARA_Dose-Response.htm).

#### **General Workshop Objectives:**

- Build off of the NAS (2008) report on improving the risk assessment process to develop practical guidance for use by risk managers at a variety of levels (e.g., states, regional managers, people in a variety of agencies, and in the private sector) for risk assessment techniques applicable to specific problem formulations.
- Implement a multi-stakeholder approach to share information, ideas and techniques in support of developing practical problem-driven risk assessment guidance.

#### **Who Should Attend?**

The workshop will be open to all interested in the advancement of risk assessment methods and science. Risk assessors from state agencies are highly encouraged to participate. Registration will be available until October 5, 2010. Registration is free for State, Tribal, and Provincial risk assessors, and \$150.00 per person for others. Registration is available at: [www.allianceforrisk.org/workshop/WS2Registration.htm](http://www.allianceforrisk.org/workshop/WS2Registration.htm).

## Workshop Attendees (as of September 22)

### Onsite

|   |   |
|---|---|
| Barbara Abbott<br>U.S. EPA                                    | John Butenhoff<br>3M Company                |
| Bruce Allen<br>Allen Consulting                               | Danielle Carlin<br>NIEHS                    |
| Melvin Andersen<br>The Hamner Institutes for Health Sciences  | Erik Carlson<br>General Electric Company    |
| Lesa Aylward<br>Summit Toxicology, LLP                        | Russell Cattley<br>Amgen                    |
| Michael Babich<br>U.S. Consumer Product Safety Commission     | Melissa Chan<br>NIEHS                       |
| Mostafa Badr<br>UMKC  | Sue Chang<br>3M Company                     |
| Craig Barrow<br>Dow Chemical Company                          | Xiaoqing Chang<br>NIEHS                     |
| Remi Bars<br>Bayer CropScience                                | Rory Conolly<br>U.S. EPA                    |
| Richard Becker<br>American Chemistry Council                  | Christopher Corton<br>U.S. EPA              |
| David Bell<br>European Chemicals Agency                       | Jordon Crago<br>Great Lakes WATER Institute |
| Philip Bentley<br>Novartis Institutes for BioMedical Research | Kenny Crump<br>U.S. EPA                     |
| Amy Brix<br>Experimental Pathology Laboratories               | Michael Cunningham<br>NIEHS                 |
| Robert Budinsky<br>Dow Chemical Company                       | Michael Dourson<br>TERA                     |
| John Butala<br>Toxicology Consultants, Inc.                   | Danielle Duma<br>NIEHS                      |

June Dunnick  
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Abigail Jacobs  
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Annie Jarabek  
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James Klaunig  
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LFR Molecular Sciences

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Madisa Macon  
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Donald McDonnell  
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Meril Limited

Susan Belman  
Thompson Hine

Virunya Bhat  
NSF International

Heather Burleigh-Flayer  
PPG Industries, Inc.

Richard Canady  
ILSI

Patricia Casano  
GE – Corporate Env'al Programs

Vicki Dellarco  
U.S. EPA

Caroline English  
NSF International

Penelope Fenner-Crisp

Carla Kinslow  
Texas Commission on Environmental  
Quality

Matthew LeBaron  
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Efrat Rubinstein  
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Joanna Shoenfelt  
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Mireille Tallandier  
Laboratories Fournier

Shirley Wang  
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Kuen-Yuh Wu  
National Taiwan University

Melanie Young  
U.S. EPA

## Tab 3 – Plenary Presentations

## Plenary Abstracts and Speaker Biographical Information

### Nuclear Receptor Key Events for Liver Tumorigens: James Klaunig

Indiana University

#### ABSTRACT

Risk assessment and hazard identification of chemicals that induce cancer is dependent on the assumption that similar mechanisms for the induction of cancer in rodents undergo the same biological processes seen in humans. An essential part of the risk assessment process is the evaluation of a chemical (or chemical grouping) mode of action in rodents and the relevance of this mode of action to humans. Guidance documents on the methodology by which this can be accomplished has been developed and further refined over the past decade from the initial proposals that were initiated by the International Programme on Chemical Safety (IPCS) and the International Life Sciences Institute (ILSI)/Risk Sciences Institute (RSI). Several articles describing and illustrating the application of this framework has been published. In addition, studies examining the utility of the mode of action framework for specific chemicals or chemical classes have been reported. In rodent bioassays the liver is the most common target organ for chemical that are carcinogenic in the rodent. Several modes of action have been identified for liver carcinogenesis, in rodent models as well as in humans. In the rodent models a number of Modes of action have been identified based in part on cellular targets and activity. Overall the Modes of Action of rodent liver carcinogens can be divided into two groupings based on their interaction with genomic DNA: either DNA reactive and non-DNA reactive. The DNA reactive agents usually require metabolic activation for the agent to reactive with genomic DNA. The Modes of action of non DNA reactive liver carcinogens can be further subdivided into receptor mediated and non-Receptor mediated groups. The non- receptor mediated Modes of Actions include cytotoxic, infectious, metal overload, oxidative stress and other categories. While the receptor mediated Modes of Action include PPARalpha, CAR, PXR, AH and estrogen categories. Despite unique features of each of the non-DNA reactive modes of action, common casual Key Events are seen, specifically mechanisms that produce an increase in cell growth (or decrease in cell death) selectively on preneoplastic hepatocytes. This is not unexpected since the basic general processes of cancer formation are the same (mutation followed by cell growth) with all tumor development. Besides discerning the Key Events of each non-DNA reactive Mode of Action in the liver, it is important to consider the temporal nature of the events as well as the role that dose and reversibility plays in these causal events.

#### BIOGRAPHICAL INFORMATION

Dr. Klaunig is the Professor and Chair of Environmental Health at Indiana University Bloomington. He previously was the Robert Forney Professor of Toxicology and Director of Toxicology at the IU School of Medicine as well as the founding Director of the Center for Environmental Health and Associate Director of the IU Cancer Center. He received his BS in Biology from Ursinus College, Collegeville Pa, and a Ph.D in Experimental Pathology from the University of Maryland, Baltimore, MD. After postdoctoral studies in chemical carcinogenesis He entered the professorial ranks at the University of Toledo Medical School in 1982 in the Departments of Pathology and Pharmacology. Following a sabbatical year at the Chemical Industry Institute of Toxicology in 1991 he accepted the position of Professor and Director of

Toxicology in IU School of Medicine and also concurrently served the State of Indiana as the Director of the State department of toxicology (forensic toxicology) and as the State Toxicologist from 1991 to 2003. His service to the state was recognized by the awarding of the Sagamore of the Wabash. He has received several honors for his research and service including the Kenneth P. Dubois Award from the Midwest Society of toxicology, the Otis R. Bowen, IU Distinguished Leadership Award and the IU Board of Trustees' Teaching Excellence Award from Indiana University, the Freehold High School alumni award, the George Scott award from the Toxicology Forum. He is a Fellow in the Academy of Toxicological Sciences. He served as a member of the USEPA Science Advisory Board, the National Toxicology Program Advisory Board, the Board and the Executive Committee of the Health and Environmental Sciences Institute (HESI), and the Board of Directors of the Toxicology Forum. He is currently a member of the NIH Cancer Etiology study section. He has served the Society of Toxicology on both elected and appointed committees over the past 25 years. He has published over 200 peer reviewed manuscripts and book chapters and has mentored many MS, Ph.D., and postdoctoral fellows. He has also served as an Associate Editor of Toxicological Sciences and as the Editor in Chief of Toxicologic Pathology. Dr. Klaunig's research interests are dedicated to understanding the mechanisms of chemically induced toxicology and carcinogenesis with emphasis on human health and genetic and environmental factors affecting human risk. His research has been supported by the NIH, USEPA, DOD, ACS, and nonfederal sources.

## **Recent Developments in Molecular Pharmacology of Nuclear Receptors: Donald McDonnell**

Duke University

### **ABSTRACT**

*Identification and exploitation of targetable proteins/processes in the AR signaling pathway involved in prostate cancer pathogenesis. Donald P. McDonnell, Daniel E. Frigo, Ching-yi Chang, Anthony R. Means, and John D. Norris Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC*

Significant progress has been made in defining the molecular mechanism(s) by which cells distinguish between AR agonists and antagonists and how some receptor modulators can manifest their actions in a cell-selective manner. The most important of these are (1) differences in the relative expression level of receptor subtypes or isoforms, (2) the impact which the bound ligand has on the structure of its cognate receptor, and (3) the complement of coactivators and corepressors in a target cell which can interact with the activated receptor. These advances in our understanding of the molecular pharmacology of nuclear receptors have provided insights into the processes underlying drug resistance to antihormonal therapies and have provided direction to efforts aimed at the development of improved cancer therapeutics. We have developed and applied a cofactor interaction/AR conformation-based screen to define important mechanistic distinctions between existing antiandrogens and to direct the discovery of new classes of antagonists that function by unique mechanisms and which exhibit favorable activities in cellular models of hormone refractory prostate cancer. These findings highlight the primacy of receptor conformation in determining the pharmacological activities of bound ligands and provide direction for the pharmaceutical exploitation of this receptor in prostate cancer and other androgenopathies.

### **BIOGRAPHICAL INFORMATION**

Donald P. McDonnell, Ph.D. is Professor of Pharmacology and Cancer Biology and Glaxo-Wellcome Professor of Molecular Cancer Biology at Duke University Medical Center. He earned his Ph.D. in cell biology from Baylor College of Medicine in 1987. His research is focused on the pharmaceutical exploitation of nuclear receptors as therapeutic targets in cancer. More specifically, his group has used biochemical, genetic and chemical biological approaches to define the key regulatory steps in androgen, progesterone and estrogen signaling pathways and have developed small molecule approaches to modulate these processes in cancer. In this manner, they have not only developed several drugs that are currently being evaluated in the clinic as cancer therapeutics, but have also defined biomarkers and predictors of response that can help to target the use of these new drugs. Furthermore, we have spent a considerable amount of effort in understanding the molecular mechanisms underlying resistance to conventional endocrine therapies and have used this information to improve the delivery of existing drugs.

# Insights From Dose-response Curves: Guides for Toxicology from Glucocorticoid receptor-Regulated Gene Induction: S. Stoney Simons, Jr.

National Institutes of Health

## ABSTRACT

Two major questions in evaluating the toxicity of an agent are: (1) does low dose toxicity increase linearly or non-linearly with a threshold and (2) can one identify critical “toxic reaction steps” to use in a high-throughput screening of suspected harmful agents, as recommended by the National Academy of Sciences report of 2007 on toxicity testing. Our continuing studies of dose-response curves with regard to steroid hormone action in general, and glucocorticoid action in particular, have yielded results that address both questions. Specifically, we have reported that numerous cofactors/reagents can affect both the potency ( $EC_{50}$ ) and the maximum activity ( $A_{max}$ ) of steroid-regulated gene expression in a gene-selective manner. These studies led to a new experimentally supported, theoretical model of steroid hormone action that yields previously inaccessible mechanistic information. Graphical analyses of the modulation of both  $A_{max}$  and  $EC_{50}$  by a given factor are capable not only of identifying the type of factor activity (activator, non-competitive inhibitor, etc) but also of indicating where a factor acts as opposed to binds. This model is supported by correctly predicting the activity of two previously characterized glucocorticoid receptor cofactors, Ubc9 and TIF2. This model also makes the novel predictions that Ubc9 acts after, and TIF2 acts before, the concentration limiting step (CLS), which is the steady-state equivalent of the rate limiting step in enzyme kinetics. Finally, this model and associated graphical analysis are applicable to any process that displays a first-order Hill plot dose-response curve. Therefore, these results are relevant for the “bench-to-world” questions of toxicologists by providing an experimentally confirmed theoretical model supporting a non-linear/threshold model of low dose toxicity and offering one method for identifying critical downstream, toxicity-inducing reactions that can be further examined in high-throughput screens.

## BIOGRAPHICAL INFORMATION

Dr. S. Stoney Simons Jr. is Chief, Steroid Hormones Section, Clinical Endocrinology Branch, NIDDK, of the National Institutes of Health. Dr. Simons' training is in organic chemistry and molecular endocrinology. He received his A.B. in Chemistry from Princeton University (magna cum laude, Phi Beta Kappa) and his Ph.D. in Organic Chemistry from Harvard University before obtaining postdoctoral training in steroid receptors and molecular endocrinology from Gordon Tompkins at UCSF. Dr. Simons then went to the NIH and NIDDK, where he rose to his current position of Section Chief. Dr. Simons has conducted extensive research on the mechanism of steroid hormone action at a molecular level, with an emphasis on glucocorticoid receptors. Starting from his development of the first affinity label of glucocorticoid receptors, his laboratory has developed a long-standing interest and expertise in a quantitative understanding of numerous basic features of steroid hormone action and their biochemical foundations. These interests include the ability of novel proteins to cause differential gene expression by modulating receptor transcriptional properties, new targets of these novel proteins and

coregulators, selective modifications of gene induction vs. repression, and determinants of agonist vs. antagonist activity. Dr. Simons has served on several grant and journal review boards. He has published over 140 papers and been granted 5 patents in the field.

# Relationship Between Receptor Biology and Dose-Response Assessment: Melvin Andersen

The Hamner Institutes of Health

## ABSTRACT

Co-ordinate expression of multiple genes by receptor-activation in cells involves binding of active promoters at multiple genes spread across many chromosomes, leading to sequential alterations of gene expression groupings with diverse time courses. Initial models of gene induction by TCDD through binding with the Ah receptor (AhR) were quite simple, focusing on increases in specific genes, e.g., CYP1A1 or CYP1A2 (Leung *et al.*, 1990a; Leung *et al.*, 1990b). Receptor activation through a Hill-function drove transcription or combined transcription-translation based on occupancy of the Ah receptor or occupancy of a putative Ah receptor response element by the AhR-TCDD complex (Andersen *et al.*, 1993; Kohn *et al.*, 1993). Observations of apparently quantal gene induction with PPAR, CAR and AhR activation in cells in vitro and in the liver in vivo where gene products appeared either fully induced or in a basal state (Bars *et al.*, 1989; Bars *et al.*, 1992; Tritscher *et al.*, 1992; Bars *et al.*, 1993) provided motivation for showing how auto-upregulation of a nuclear receptor via positive feedback might give rise to switch-like induction (Andersen and Barton, 1999). At the same time as the pharmacokinetic and pharmacodynamic modeling of TCDD-AhR gene induction was progressing, other investigators were showing how genetic regulatory networks could produce complex dynamic behaviors, including bistability (a more formal description of switching between cellular states) and oscillations (Smolen *et al.*, 1998; Smolen *et al.*, 2000). The importance of mitogen activated protein kinase (MAPK) cascades in generating non-linear responses was also being worked out for multiple signaling pathways (Huang and Ferrell, 1996; Ferrell and Bhatt, 1997; Ferrell and Machleder, 1998). Over the past decade, the field of computational systems biology of cellular signaling pathways has blossomed through improved analysis tools (Strogatz, 2000; Aldridge *et al.*, 2006; Alon, 2007a) and through new approaches to data collection, especially use of technologies to query responses of individual cells (Batchelor *et al.*, 2009; Geva-Zatorsky *et al.*, 2010). These methodologies have provided a new appreciation of the circuitry involved in cellular responses to stressors (Tyson *et al.*, 2003) and the likely dose-response behaviors expected for activation of groups of genes and proteins by receptor-mediated signaling (see for example, [http://www.thehamner.org/education-and-training/drm\\_workshop.html](http://www.thehamner.org/education-and-training/drm_workshop.html)). Signaling motifs, conserved organization of regulatory network components, are being identified (Tyson and Novak, 2010). The organization of these motifs control sequential cascades of gene expression in so-called developmental networks, such as receptor-mediated induction of gene batteries in tissues (Davidson *et al.*, 2002; Alon, 2007b). This overview highlights the dynamics of gene regulatory networks with concepts such as ultrasensitivity, bistability, hysteresis, and biological attractor states. One design characteristic common to the three receptors to be discussed over the next three days appears to be upregulation of gene batteries functioning to control active ligand concentration within an appropriate range using negative feedback processes associated with increases in expression of Phase I, II and III enzymes (Zhang and Andersen, 2007). These negative feedback processes require ultrasensitive motifs to insure robust control characteristics. A second outcome of receptor activation in liver is functional, phenotypic

change such as cell replication, hypertrophy, and organellar proliferation. Dose-response modeling of these responses requires careful consideration of the kinetics of the ligand, the structure of the response circuitry, and the relationship of the circuit dynamics to both induction and altered phenotype. Bioinformatic tools have recently been used to extract pathway information for neurite outgrowth with G-protein coupled receptors (Bromberg *et al.*, 2008a; Bromberg *et al.*, 2008b; Zorina *et al.*, 2010). These tools should be useful in uncovering the function and connection among batteries of genes controlled by ligand activation of hepatic receptors and outlining the role of regulatory networks and MAPK cascades in controlling cell/tissue phenotype. Application of these new computational methodologies will be essential for creating mechanistic dose response models for receptor-mediated processes, for evaluating the association of pathway activation with phenotypic responses in cells, multi-dimensional tissue cultures, and the intact liver, and improving risk assessment for low dose exposures to various receptor-activating compounds.

### **BIOGRAPHICAL INFORMATION**

Dr. Melvin Andersen is Director, Program in Chemical Safety Sciences, The Hamner Institutes for Health Research. Over a 35-year career in toxicology and risk assessment, his research has focused on computational approaches for dose response modeling and human health risk assessments for environmental chemicals. His current program is geared towards implementing key recommendations from the 2007 NAS report, "Toxicity Testing in the 21st Century: A Vision and A Strategy", especially use of computational systems approaches for dose response modeling of *in vitro* assays. Dr. Andersen is board certified in industrial hygiene (since 1978), in toxicology (since 1982) and a Fellow of the Academy of Toxicological Sciences. He is senior author or co-author of 400 research papers and book chapters and has edited books on physiologically based pharmacokinetic modeling (2005) and computational toxicology (2010). In 2002, Dr. Andersen was recognized as a 'highly cited' scientist by the Institute for Scientific Information. He holds a Bachelor of Science degree in Chemistry (Brown University, Providence, RI; 1967) and Ph.D. in Biochemistry and Molecular Biology (Cornell University, Ithaca, NY; 1971).

## **Mode of Action/Human Relevance (Key Events) Analysis: M.E. (Bette) Meek**

University of Ottawa

### **ABSTRACT**

Frameworks to systematically consider the weight of evidence for hypothesized modes of action (key events) in animals and their relevance to humans promote transparency in hazard characterization in risk assessment and dose-response analysis. These frameworks also encourage early assimilation and consideration of mechanistic data as a basis to be more predictive in risk assessment, facilitate peer input and review and identify critical research needs. Iterative use of such frameworks assists communication between assessors and researchers on key data gaps; they are also helpful in transitioning to the use of data from more progressive and predictive mode of action based testing strategies. Recent developments in the evolution of these frameworks will be presented. Lessons learned as a function of increasing experience in their application relevant to the case studies being considered at the workshop will be emphasized and examples provided.

### **BIOGRAPHICAL INFORMATION**

Dr. M.E. (Bette) Meek has a background in toxicology receiving her M.Sc. in Toxicology (with distinction) from the University of Surrey, U.K. and her Ph.D. in risk assessment from the University of Utrecht, the Netherlands. She is currently the Associate Director of Chemical Risk Assessment at the McLaughlin Centre for Population Health Risk Assessment, University of Ottawa, completing an interchange assignment from Health Canada. She has extensive experience in the management of chemical assessment programs within the Government of Canada, most recently involving development and implementation of process and methodology for the health assessment of Existing Substances under the Canadian Environmental Protection Act (CEPA) and previously, for programs for contaminants in drinking water and air.

With colleagues within Canada and internationally, she has contributed to or led initiatives to increase transparency, defensibility and efficiency in health risk assessment, having convened and participated in initiatives in this area for numerous organizations including the International Programme on Chemical Safety, the World Health Organization, the International Life Sciences Institute, the U.S. Environmental Protection Agency, the U.S. National Academy of Sciences and the U.S. National Institute for Environmental Health Sciences. Relevant areas have included frameworks for weight of evidence analysis including mode of action, chemical specific adjustment factors, physiologically-based pharmacokinetic modeling, combined exposures and predictive modeling. She has also authored over 175 publications in the area of chemical risk assessment and received several awards for contribution in this domain.



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