

NUCLEAR RECEPTOR BINDING DOMAIN: FIGURE FROM:
[HTTP://WWW.MAN.POZNAN.PL/CBB/RESEARCH.HTML](http://www.man.poznan.pl/cbb/research.html)

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

AHR Case Study

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

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TAB 1 - Background

TAB 1 - Background

AHR Panel Members

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AHR Case Study Agenda

Monday, September 27th, 2010

AHR Case Study Team Opening Remarks and Introductory Topics

- 1:30 - 2:00** Assembly and Opening Remarks: *Dieter Schrenk and Robert Budinsky*
- 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 28th, 2010

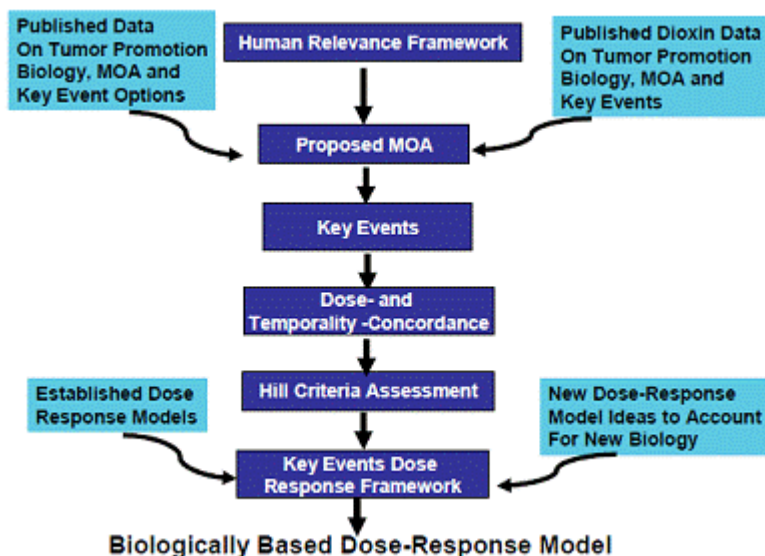
- 8:00 – 8:30** Introductory Comments and Follow-up Business from Day 1: *Co-Chairs*
- 8:30 - 8:45** Gene Array Analysis of TCDD-induced mRNA Expression from Primary Rat and Human Hepatocytes: *Russell Thomas and Craig Rowlands*

- 8:45 - 9:15** Quantitative Considerations for Interspecies and High-to Low Dose
Extrapolation: *Lesa 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

Introduction for AHR Case Study

Background – the Human Relevance Framework and a Weight-of-Evidence Approach

Each nuclear receptor case study will be reviewed and discussed by its respective expert panel. The panel will evaluate dose-response modeling approaches based on the available data that supports key events, their associative events, and any modulating factors leading to rodent liver tumors. A weight of evidence approach will follow the human relevance framework approach endorsed by the U.S. EPA, the more recent key events dose response framework that has been developed by ILSI, and the evidence will be examined against charge question (See Figure 1 and the discussion below).



The process in Figure 1 is being proposed as an approach for conducting the case-study review and discussion. Beginning with what we know about how tumor promotion works, and the Human Relevance Framework template serving as the scaffolding for querying the published dioxin evidence, an MOA and Key Events hypothesis has been developed (see below for further details). Dose response and temporality concordance between the key events, the associative events, and the modulating factors will be examined with respect to the development of rodent liver tumors. Hill's causal associations, e.g., coherence, biological plausibility, will be discussed. Finally, the quantitative modeling of the integrated biology of all the key event data will be assessed using established dose response models (e.g., the 2-stage clonal growth model from initiation-promotion studies) and newer dose-response strategies for including more detailed biological data leading to a biologically based dose-response model picture of how the AHR promotes liver tumors (Simon et al., 2009, Luke et al., 2010, Ong et al., 2010 as published studies that have looked at the AHR activation-liver tumor outcome or have described modeling approaches for key events in the proposed AHR activation MOA).

The AHR case study summary is organized according to:

- A “Statement of the Case” which provides an overview of the critical toxicological studies including the: a) cancer bioassays, b) Initiation-promotion studies, c) *in-vitro* and *in-vivo* Mode-of-Action/Mechanistic studies, c) Human data, e.g., epidemiology, and d) Dose-response modeling data including genomics data.
- AHR Charge Questions
- Presentations on specific topics relevant to the AHR MOA and dose-response modeling issues

Statement of the Case

TCDD, and a few other polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) have been studied in cancer bioassays and shown to be liver tumor promoters. In rats, the dose-dependent development of adenomas, hepatocellular carcinomas, and bile duct tumors is only observed in female rats suggesting a sex-dependent, i.e., estradiol, key event. The development of hepatic tumors is associated with the onset of liver toxicity and occurs late over the time course of the 2-year study. In mice, liver tumors occur in both males and females. Liver toxicity, e.g., inflammatory changes, occurs relatively early in mice in contrast to the slower development of hepatopathy in rats.

For the 2004 NTP bioassays, extensive pathology working group review of liver histopathology was conducted. Some of the findings of this activity were published in Hailey et al., (2005). Hailey et al. 2005 describes the histopathological evidence of hepatopathy observed in female rats over the 2-year NTP cancer bioassay at interim sacrifices of 14, 31, and 53 weeks. An earlier pathology working group examination of the Kociba et al., (1978) study was published by Goodman and Sauer (1991). Histopathological data from sub-chronic and chronic studies in mice are also available for examining this important Key Event.

A number of hepatic initiation-promotion (IP) studies have been conducted with TCDD and a few other PCDDs/PCDFs. IP studies provide a variety of data for examining Key Events, Associative Events and Modulating Factors. One of the possible limitations of the IP studies with respect to dose-response modeling is the artificial influence of using a strong initiating agent and a mitogenic treatment, i.e., hepatectomy, whereas the following proposed AHR MOA is based on AHR activation in background initiated hepatocytes or altered hepatic foci.

The epidemiological evidence for liver and biliary cancer mortality is negative to equivocal. There is no causal evidence of increased liver cancer mortality among the various cohorts of dioxin-exposed workers and the Seveso Italy population. However, the negative epidemiological studies do not rule-out, at this time, the possibility that excessive and sustained AHR activation could act as a tumor promoter in human liver.

AHR Case Study Discussion Questions

Introduction and Definitions

This case study reviews AHR-mediated mode of action (MOA). The objective of this effort is to use the weight of evidence for the key events (including those derived from molecular, cellular and genomic data) and accompanying dose-response data to better characterize the likely dose-response behavior for apical outcomes (e.g., liver tumors) induced through activation of AHR. The goal is to recommend dose-response modeling approaches that most accurately reflect the underlying biology, when the data are available, or identify needed data.

The discussion questions have been developed building on the IPCS Human Relevance Framework (IPCS 2007) and the modified Hill Criteria for Causality (EPA, 2005) for evaluating the MOA for AHR activation. Because the underlying mechanistic knowledge of the AHR is relatively well-characterized, additional knowledge of biological processes beyond the major key events is available to refine our understanding the overall dose-response behavior. To capture the impacts of this degree of mechanistic understanding refinements to the current IPCS (2007) framework, as being developed by ILSI and others, are being used to characterize the nature of the biological steps involved.

Important definitions included in the charge to the expert panel include:

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.

Discussion Questions

Establishing Key Events in the Mode of Action (MOA) for Nuclear Receptor-Mediated Hepatomegaly and Tumorigenicity

1. What is the mode of action (MOA) for AHR-mediated rodent liver tumors for a model AHR activator (e.g., dioxins or related compounds), as evaluated using the IPCS Framework for Human Relevance and the modified Hill Criteria applied to MOA (IPCS and EPA MOA Framework)?
 - a. What are the putative key events in the MOA for tumorigenicity?
 - b. Are there other important biological responses that are associated with these key events (i.e., associative events) that could be markers of AHR activation?
 - c. Are there other important biological responses that are not key events themselves, but might modulate the key events (i.e., modulatory events) in the MOA?
 - d. Are there key events for the MOA for liver tumors that are not mediated via AHR activation?

Evaluation of Receptor-Mediated Gene Expression Changes (Early Biological Responses)

2. What are the fundamental biological steps in ligand-activation of the AHR necessary to affect gene regulation?
 - a. Is the existing molecular biology for gene regulation sufficiently understood to support this as a key event in the MOA?
 - b. Does this event meet the requirements of the IPCS Human Relevance and MOA Frameworks to be supported as a key event (e.g., the modified Hill Criteria)?
 - c. Based on the available data (qualitative or quantitative) can the human relevance of the key event be reasonably excluded per the IPCS Human Relevance Framework?
 - d. What are the key data needed to support AHR activation as a key event; what are the data needs to establish human relevance of this key event?
3. Are the existing data sufficient to determine a dose-response relationship for gene regulation?
 - a. Are the existing descriptions of mathematical and statistical models for characterizing the fundamental biological steps complete?
 - b. Is the existing description of concentration or dose-response data for these steps sufficient for dose-response modeling?
 - c. What data are available on dose-response for nuclear receptor-regulated gene regulation?
 - d. What are the data needed for dose-response characterization and modeling?

4. Is there an amount of ligand that would be insufficient for activating the AHR for induction of changes in gene regulation?
 - a. Are there empirical data that show an amount of ligand that is insufficient to activate the AHR such that there is no observable change in gene regulation? Has a no effect level (NOEL) been demonstrated?

Evaluation of Downstream Biological Responses

5. Subsequent to ligand-activation of the specific nuclear receptor, what are the fundamental biological changes necessary to cause downstream responses?
 - a. Are the downstream responses that lead to the apical outcome sufficiently understood to define the sequence of key events to identify the MOA? Do these downstream responses meet the requirements of the IPCS Human Relevance and MOA Frameworks to be supported as key events (e.g., the modified Hill Criteria)?
 - b. Based on the available data (qualitative or quantitative) can the human relevance of the downstream key events be reasonably excluded per the IPCS Human Relevance Framework?
 - c. What are the data needed to support the sequence of downstream events as key events; what are the data needs to establish human relevance of these key events?
6. Are the existing data sufficient for downstream key events to determine a dose-response relationship?
 - a. Is the existing description of mathematical and statistical models for characterizing these key events complete?
 - b. Is the existing description of concentration or dose-response data for these key events sufficient for dose-response modeling?
 - c. What are the key data needed to characterize the dose-response relationship?
7. Is there an amount of ligand that would be insufficient for activating the AHR such that there would be no induction of these key events or associated biological responses? Has a NOEL been demonstrated for these downstream key events?

Evaluation of the Relationship Between the MOA and the Apical Outcome

8. Does current knowledge of early and downstream key events as well as important associative and modulating events support choice of appropriate dose-response models for liver tumors induced through AHR activation? If not, what are the missing data and what research is needed to fill the remaining data gaps?

Additional Issues that Impact the MOA for the AHR

9. What framework or guidance can be suggested that describes a minimum series of assays, tests, experiments, or studies that would specifically confirm this MOA.

10. What framework or guidance can be suggested to rule out alternative MOA hypotheses for a compound that activates the AHR and induces liver tumors?
11. Is there a need to, and what are the recommendations for, updating or modifying the IPCS Frameworks for MOA and Human Relevance such that they could better include current knowledge and approaches based on application to AHR-mediated toxicity?

What are the most appropriate data needs for informing future risk assessments for AHR ligands?

Tab 2 - Presentations

Tab 2 - Presentations

AHR Case Study Group Draft Presentation Abstracts and Outlines

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AHR – Current State of Knowledge and Role in Biology/Physiology: Gary Perdew¹ and Thomas Gasiewicz²

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Based on the ability of polycyclic aromatic hydrocarbons (PAHs) to induce particular drug metabolizing enzymes, e.g. aryl hydrocarbon hydroxylase, a new genetic locus, the *Ah* locus, was defined in the 1970s. This ultimately led to the discovery of the Ah receptor (AhR) as the product of this locus, and our understanding that this protein mediates not only the induction of the genes encoding these enzymes and a number of other proteins, but the toxicity of a family of halogenated aromatic hydrocarbons (HAHs) with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin being the most potent. Events leading to these responses involve binding of the PAH and HAH ligands to a cytosolic AhR that is complexed with several other proteins including heat shock protein 90 dimer, p23, and X-associated protein 2 (XAP2). This binding elicits a conformational change in the AhR that results in cytoplasmic to nuclear translocation, interaction with another protein, Arnt, recognition of regulatory elements, AhR-responsive elements (AhREs), in responsive genes, recruitment of other critical co-regulatory proteins, and modulation of gene expression. While this is the well-accepted paradigm for many of the biologic and toxic responses to xenobiotic ligands, research in recent years suggest that this is likely to be more complex for different responses. For example, agonist or antagonist responses to different ligands are clearly dependent on differences in ligand binding affinity, efficacy, as well as the relative ability of the ligands to be metabolized. Differences in types of “AhREs” and in the nucleotides flanking these, as well as the positioning of these AhREs relative to other transcription factor binding sites, may determine the relative strength of the response and if the effects on certain genes are stimulatory versus inhibitory. Furthermore, the set of target genes regulated by the AhR may not be well conserved across species. While, in general, the domain structure of the AhR is conserved across species, differences in sequence at the ligand binding and C-terminal transactivation domains appear to play a large role in designating species differences in the dose-dependent ability to respond and the type of response. Since discovery of AhR, many publications have documented differences in its expression in a variety of cells and tissues, and changes in this expression and activity in response to a number of agents or conditions. These include AhR degradation by ubiquitin/proteasome-mediated processes, regulation of *Ahr* expression by developmental (epigenetic) and tissue-specific factors, and/or cell cycle- and differentiation state-dependent regulation of activity. There is also data suggesting that the AhR may elicit responses through mechanisms that are not AhRE-dependent, but dependent on the direct interaction with other proteins such as NF- κ B subunits, estrogen receptor and retinoblastoma protein. On the other hand, the use of mouse models that have mutations at the AhR nuclear localization sequence and or the DNA-binding domain suggest that many toxic responses are absolutely dependent on the ability of the AhR to translocate to the nucleus and bind AhREs. Finally, our view of

the AhR as it relates to the toxicology of xenobiotic AhR ligands and the biology of human disease is also very much dependent on what the normal function of the AhR may be. Clearly, evidence from both an assessment of endogenous ligand candidates and characteristics of AhR null-allele mice suggest a number of cell- and tissue processes in which the AhR is required for various normal physiological functions. Including, T-cell development, inflammatory processes, growth and regulation of reproductive tissues, angiogenesis, neurological development and function, and bone marrow development and maintenance.

The Mode of Action for AHR mediated carcinogenesis-Insights from chronic rodent bioassays of TCDD and related compounds: Nigel Walker

NIEHS/National Toxicology Program

Abstract

This talk will cover the following topics:

- Overview of cancer bioassays of TCDD and related compounds
- Common target sites in chronic rodent bioassays
- Comparison of tissue burdens in Dow-Kociba vs NTP TCDD bioassays
- Hepatic toxicokinetics issues related to target sites
- Comparative dose response for hepatocarcinogenicity of TCDD
- Dose responses for hepatic effects
- Early-, mid-term and late-term effects
- Possible modes of action for liver effects

The cancer bioassay for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in Sprague Dawley rats conducted by the Dow Chemical company in the mid 1970s been used extensively for conducting quantitative cancer risk assessments for human exposure to TCDD (Kociba et al., 1978). More recently the National Toxicology Program (NTP) conducted a series of chronic cancer bioassays of TCDD (http://ntp.niehs.nih.gov/files/521_Web.pdf) and related dioxin-like compounds (DLCs) in female rats as part of its evaluation of the utility of the dioxin TEF methodology for assessing cumulative cancer risk (Walker et al., 2005). This included studies of several different DLCs known to act via the AHR (TCDD; 2,3,7,8-PeCDF; PCB126 and PCB118) and included extensive evaluation of the time-dependent development of both biochemical and pathological responses. The comparison of similarities and difference between the data from these bioassays provide important insights into the possible mode(s) of action of these AHR agonists.

When comparing across these bioassays, common target sites for these persistent AHR agonists were the primarily the liver, but also the lung, and oral cavity. While the liver was the primary target site, the most notable qualitative difference between the NTP and the Dow studies was the increased incidence of both hepatocellular and biliary tumors seen in the NTP studies, whereas tumors in the Dow study were only hepatocellular in nature. Given that hepatic stem cells can differentiate to both biliary and hepatocellular lineages, this suggests that hepatic stem cell proliferation may be an important component of the MOA. With regards to this MOA the increased incidence of oval cell (stem cell) hyperplasia was one of the earlier effects seen and observed at lower doses. While more marked hepatic “toxicity” was seen, and have been a contributor to the MOA, this generally occurred at the higher doses and with longer durations of exposure.

References

Kociba et al. 1978. Toxicol. Appl. Pharmacol. 46, 279-303

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Walker et al 2005, Environ. Health Perspect. 113, 43-48

Overview of Histopathology During TCDD -Mediated Tumor Promotion and Hepatocarcinogenesis: Amy Brix

Experimental Pathology Laboratories

Note: Title change pending

Abstract to be provided

AHR-Activation Inhibition of Focal Apoptosis: Dieter Schrenk

University of Kaiserslautern

Abstract

Because of lack of genotoxicity/mutagenicity of TCDD, carcinogenicity in rodent liver elicited by TCDD and some other AHR agonists (Schrenk et al., 1994) is considered to be due to tumor promotion. Suppression of apoptosis of putative preneoplastic hepatocytes was suggested as a key event in rodent liver tumor promotion (Stinchcombe et al., 1995). Inhibition of apoptosis can be mimicked in vitro in rat hepatocyte cultures treated with certain pro-apoptotic factors. Likewise, TCDD suppresses apoptosis initiated by UV irradiation (Woerner and Schrenk, 1996). The suppression of apoptosis on a concentration-response scale coincides with induction of CYP1A1 (Woerner and Schrenk, 1998). Inhibition of apoptosis by TCDD can be abrogated in the presence of an AHR antagonist. The inhibitory action of TCDD is caspase-independent, and leads to suppression of a yet unidentified DNase residing in intact nuclei (Chopra et al., 2009). Under pro-apoptotic stimuli causing inhibition of protein synthesis (Chopra et al., 2010), TCDD is no longer anti-apoptotic suggesting the need for protein synthesis for the anti-apoptotic effect. It is suggested that persistent AHR agonists act as liver tumor promoters in rodents via suppressing apoptosis of preneoplastic lesions (Schrenk et al., 2004). This effect is likely to exert a practical threshold. It remains to be elucidated if suppression of apoptosis is strictly linked to other AHR—dependent events such as induction of CYP1A1. We suggest that significant, long-term CYP1A1 induction may serve as a surrogate marker for increased risk of hepatocarcinogenesis in rodent liver.

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Zonal Activation of CYP1A Proteins by AhR – Implications for AhR-Mediated Tumor Promotion and Hepatocarcinogenesis: Melvin Andersen and Sudin Bhattacharya

The Hamner Institute

Abstract

Many hepatic components, especially CYP proteins, have specific regional distributions in the liver (Oinonen and Lindros, 1998) that are altered by enzyme inducers. Induction of CYP1A1 by ligand binding to AhR in individual hepatocytes and in the intact liver showed abrupt dose-response behaviors, more similar to a switch than a graded response following simple mass action principles (Bars *et al.*, 1989; Bars *et al.*, 1992; Tritscher *et al.*, 1992). Behaviors of this kind have also been noted with CYP2B induction by ligands such as octamethyltetrasiloxane (Bars *et al.*, 1992). Some 15 years ago, PK and PD models for TCDD and the AhR were developed to describe hepatic induction using a representation of the liver that had five-distinct regions moving from the periportal through the centrilobular region (Andersen *et al.*, 1997a; Andersen *et al.*, 1997b). The regional areas/volumes were estimated from least and greatest path lengths through the acinar structure implemented with polar coordinate measures (Figure 1). This representation produced 5 regions with volumes of 13, 26, 34, 20 and 7% going from periportal to centrilobular regions (Figure 2). Immunohistochemistry results (Tritscher *et al.*, 1992) were visualized by using different binding affinities for the AhR in each region and a variable Hill-coefficient for induction. A three-fold difference in K_d between regions and a very large Hill-coefficient of 5 produced a good representation of the immunohistochemistry. Studies with CYP2B induction were described with a similar model structure. An interesting observation with CAR activation was a difference in the dose-response for protein induction and increases in tissue weight. The ED50 for the proportionate increases in CYP2B was significantly lower than was the ED50 for liver weight gain (Sarangapani *et al.*, 2002). Other studies have noted different regional responses to TCDD in rat liver. Hepatocellular proliferation was increasingly periportal in distribution with increasing TCDD doses (Maronpot *et al.*, 1993). Oval cells are located in the periportal regions (Figure 3) and may be the associated with the observed pattern of proliferation (Gaudio *et al.*, 2009). These regional differences in responsiveness to induction and proliferation may be involved in tumor formation. Our earlier PK work was developed to examine a proposed hypothesis for TCDD hepatocarcinogenesis related to negative selection or escape from mito-inhibition (Bell, 1976; Andersen *et al.*, 1995; Conolly and Andersen, 1997). These ideas remain plausible. Based on advances in computational systems approaches to understanding gene regulation (Alon, 2007b; Alon, 2007a), other studies could also be focused more on the understanding ultrasensitive pleiotropic activation of gene batteries and the associations between activation of these groups of genes and phenotypic outcome, such as proliferation or apoptosis. The high degree on non-linearity (ultrasensitivity in more current jargon) and regional induction merits further examination to better understand the processes leading to switch-like activation of individual cells and how such ultrasensitive signaling motifs might affect carcinogenesis. The PK models for these behaviors need to account for the different regional characteristics of the liver.

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**TETRACHLORODIBENZO-P-DIOXIN IN A RAT-TUMOR PROMOTION MODEL -
QUANTIFICATION AND IMMUNOLocalIZATION OF CYP1A1 AND CYP1A2 IN
THE LIVER. *Cancer Research* 52, 3436-3442.**

**Gene Array Analysis of TCDD-induced mRNA Expression from
Primary Rat and Human Hepatocytes: Russell Thomas¹ and Craig
Rowlands²**

¹The Hamner Institute ²Dow Chemical Company

Abstract to be provided

Quantitative Considerations for Interspecies and High to Low Dose Extrapolation: Lesa L. Aylward

Summit Toxicology, Falls Church, VA

Abstract

The mode of action framework includes examination of dose and species concordance as components of the risk assessment process, with an examination of both qualitative and quantitative factors that impact extrapolations. This talk provides an overview of two quantitative factors potentially impacting extrapolation: 1) structural differences in the ligand-binding and transactivation domains of the AhR among various strains and species that impact ligand binding affinity and AhR activation, and 2) impact of CYP1A2 protein induction on high- to low-dose extrapolation and estimation of tissue dose metrics.

The specific impacts of polymorphisms in the structure of the AhR have been examined in the classic C57 vs. DBA mouse model, in AhR knock-out and knock-in mouse models, and via modeling of AhR conformation. The human AhR exhibits a DBA-type polymorphism in the ligand-binding domain that impacts binding affinity for TCDD by approximately 10-fold or more compared to the higher affinity C57 AhR structure. A shift of a similar magnitude in the dose-response curves for enzyme induction and other responses is consistently observed. Polymorphisms among human AhR have been identified, but in limited data available to date, none demonstrate higher affinity or greater responsiveness compared to the human wild type. Interspecies differences in gene activation patterns have also been observed.

CYP450 enzyme induction is one of the most well-understood outcomes resulting from binding of TCDD to the AhR. CYP induction can be regarded in several contexts. It can be regarded as a sensitive marker of biological activation of the AhR, and thus, an “associative” event. It might also be regarded as a key event if increased enzyme activity results in altered biological processes leading to pathological outcomes. Induction of CYP1A2 has an additional impact on the assessment of dose response for hepatic responses and the assessment of internal doses as a function of external dose due to the ability of CYP1A2 protein to bind TCDD and some other dioxin-like compounds. As CYP1A2 protein is induced, an increasing proportion of TCDD is located in the liver in preference to distribution in lipids in the remainder of the body, as demonstrated by the concentration-dependent distribution patterns observed in rodents, and to a limited extent, in human tissue samples. The role of that dose-dependent protein binding in either increasing available TCDD in the liver, or alternatively, binding TCDD and making it unavailable for binding to the AhR has not been fully elucidated.

Quantitative information regarding AhR polymorphisms and CYP1A2 induction will be presented in the context of tissue concentration data for key responses observed in laboratory animals as well as in humans exposed via the food chain and via occupational and accidental exposures.

Key Questions and Issues

1. Is the information on quantitative and qualitative interspecies differences between AhR ligand binding and gene activation relevant to interspecies extrapolation of TCDD dose-response information?
 - a. If so, how should the reduced ligand binding affinity for TCDD of the human AhR (and demonstrated shifts in dose-response curves for enzyme induction) be incorporated?
 - b. What level of information is needed to judge the impact of the observed differences in gene activation profiles among species and incorporate relevant differences into interspecies extrapolations?
2. How does CYP1A2 protein binding affect the dose-response for various observed hepatic responses on a whole weight tissue concentration basis?
 - a. Are responses related to the free tissue concentration of TCDD rather than the whole weight liver concentrations (CYP1A2 bound plus free)?
 - b. What information is needed to model the intracellular kinetics (competitive binding between CYP1A2 and AhR)?
 - c. What experimental approaches can inform this issue?
 - d. What information is available to compare human and rodent CYP1A2 induction and impacts on interspecies extrapolation?

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IPCS Framework Analysis of MOA for TCDD-Mediated Tumor Promotion and Hepatocarcinogenesis: Robert Budinsky

Dow Chemical Company

Abstract

A Mode of Action (MOA) for sustained AHR activation, based on AHR ligands such as TCDD, has been developed using the Human Relevance Framework. The Human Relevance Framework was originally designed under the International Programme on Chemical Safety (IPCS) and has undergone refinement and incorporated into the USEPA's 2005 Cancer Guidelines. The framework sets forth a weight-of-evidence methodology for evaluating a proposed MOA, its key events, the dose- and temporality-concordance aspects of the key events, and a Bradford Hill causal association assessment. The proposed MOA for dioxin-induced rodent liver tumors is tumor promotion through sustained aryl hydrocarbon receptor (AHR) activation resulting in dysregulation of liver cell function. The proposed key events include: 1) chronic AHR activation; 2) inhibition of apoptosis in spontaneously initiated hepatocytes; 3) hepatopathy, and; 4) regenerative repair. AHR activation selectively inhibits apoptosis in spontaneously initiated liver cells thereby providing a growth advantage for the clonal expansion in both the number and volume of altered hepatic foci. Sustained AHR activation eventually results in hepatopathy and necrosis that leads to regenerative-repair. Regenerative repair is triggered by the release of mitogenic growth factors that stimulate cell division in normal hepatocytes and further promote altered hepatic foci allowing the eventual acquisition of growth autonomy and tumor characteristics. In addition to these key events, there are a number of associative events, e.g., biomarkers that correlate with key events, that can be used for an assessment of the quantitative, dose-, and temporal-concordant relationships between the proposed key events and the tumor outcome. For example, CYP1A1 induction is a well-characterized biomarker for AHR activation and could serve as a useful associative event. Dose-concordance is established by the fact that these key and associative events occur at dosages and tissue concentrations of a smaller magnitude than the cumulative dose/tissue concentrations required for tumor expression. Temporal concordance is established by key and associative events that occur prior to, or in sequence with, each other and ultimately with tumor expression. This proposed MOA, key events, and associative events satisfy Hill's causal associations. For example, numerous initiation-promotion studies consistently show a strong association between TCDD administration and tumor promotion. The biological plausibility of the key events is supported by and coherent with the well-recognized biology of carcinogenicity and tumor promotion. A possible alternative MOA for TCDD that must be carefully considered is genotoxicity. However, numerous studies have conclusively demonstrated that TCDD is neither mutagenic or genotoxic. An additional issue for consideration is the impact of modulatory factors on AHR activation and the resulting effects on the shape of the dose-response relationships for the tumor response, key events and associative events. These modulatory factors include the zonal expression of the AHR and the molecular events involving AHR activation, as well as adaptive responses that mitigate the tumor promotion key events. The integration of the linear and the non-linear dose-responses for the key events, associative events and modulatory

factors results in an overall non-linear relationship between sustained AHR activation and the development of rodent liver tumors.

Biomathematical Considerations for Dose-response Assessment: Ted Simon

Ted Simon, LLC.

One of the goals of this workshop is to develop a set of criteria for identifying critical aspects of the mode of action (MOA) and dose-response for biological effects mediated by nuclear receptors. For the aryl hydrocarbon receptor (AHR), the most critical choice for dose response assessment of ligands such as TCDD is the choice of endpoint related to the Key Events and Associative Events in the MOA, and the Modulatory Factors that may alter the MOA. This presentation will cover:

- Choice of Endpoint
- Mode of Action, Key Events, Associative Events and Modulatory Factors
- Toxicokinetic and PBPK Models
- Toxicodynamics, Genomics, Proteomics and Phenotypic Changes
- Statistical Models vs. Biologically-Based Dose Response (BBDR) Models

Cancer bioassay results in female SD rats indicate a strongly dose-related increase in both hepatic toxicity and cancer incidence, but are these appropriate endpoints for humans? Species differences in both toxicokinetics and toxicodynamics need to be accounted for in any dose-response assessment. A number of PBPK models for TCDD have been developed that can reproduce human and animal data with varying fidelity. Regarding toxicodynamics, the primary difference between humans and animals is the reduced ligand binding affinity of the human AHR. Humans and animals likely exhibit additional differences in gene and protein expression mediated by the AHR. In rats, various Key Events and Associative Events in the MOA for TCDD can be fit with Hill functions. The early events in the MOA have a Hill coefficient close to unity, but the Hill coefficient increases for downstream effects suggesting that events other than ligand binding are necessary for the expression of phenotypic changes. Various Modulatory Factors likely alter the occurrence of Key Events and observed phenotypic changes.

Can the key events in the MOA, such as inhibition of apoptosis or cell proliferation, be understood in terms of systems biology? Regenerative-repair driven mitogenesis, resulting from sustained AHR activation-induced hepatopathy, is a Key Event and a significant factor in the dose-response for tumors. How can this Key Event be included in a dose-response model for sustained AHR activation? Zonal activation, which is centrilobular in nature, could be an important Modulatory Factor in how AHR activation acts on a chronic time scale to alter the background occurrence of initiation in single hepatocytes and subsequent development of altered hepatic foci. The scale of the model is another consideration.

- Hepatocyte scale – transcription, translation, enzyme effects
- Hepatic lobule scale – hepatic zonation, blood flow through the lobule
- Scale of genomic and proteomic changes
- Scale of phenotypic changes
- Organism scale – PBPK model

The appropriate scale of the model is still unclear and opens the debate about parsimony and simplicity, on one hand, versus realism and complexity on the other. Finally, can the differences between humans and animals be accounted for in an accurate and protective fashion in a BBDR model?

Tab 3 - Tables

Tab 3 - Tables



AHR Data Tables

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Table 1. AHR Temporality Concordance Table

	Timing				
	Hours	Days	Weeks	Months	Years
AHR Activation					
Associative Event <ul style="list-style-type: none"> • CYP1A1 mRNA • CYP1A2 mRNA • EROD • MROD/POD/4AH • CYP1B1 • Genomics 					
Intrafocal Apoptosis Inhibition					
Associative Event <ul style="list-style-type: none"> • P53 Phosphorylation • ↑Mdm2 • AGR2 					
Hepatopathy					
Associative Event <ul style="list-style-type: none"> • Steatosis • Inflammation • Mitochondrial Toxicity • ROS 					
Regenerative Repair					

<ul style="list-style-type: none">• BrdU• PCNA• Hyperplasia					
Associative Event <ul style="list-style-type: none">• Cell-to-Cell Communications					

Table 2. AHR Dose-Response Concordance Table

Key Event	Liver Conc. (ng/kg)	Species and Strain	Doses Tested	NOEL	LOEL	Reference	Comment
AHR Activation							
Associative Event <ul style="list-style-type: none"> • CYP1A1 mRNA • CYP1A2 mRNA • EROD • MROD/POD/4A H • CYP1B1 • Genomics 							
Modulating Factor <ul style="list-style-type: none"> • Zonal-Dependency • SNPs • Other Polymorphs • ARNT • AHRR • Co-regulatory proteins • NAHRAs 							

Intrafocal Apoptosis Inhibition							
Associative Event <ul style="list-style-type: none"> • P53 Phosphorylation • ↑Mdm2 • AGR2 • Apoptotic Networks 							
Modulating Factor <ul style="list-style-type: none"> • Zonal Dependency • Rate of spontaneous AHF formation • G1-S Cell Cycle Lock 							
Hepatopathy							
Associative Event <ul style="list-style-type: none"> • Steatosis • Inflammation • Mitochondrial Toxicity • ROS 							
Modulating Factor							

<ul style="list-style-type: none"> • Zonal Dependency • ↑GST and UGT • ↑ Nrf2 • FasL Activation of apoptosis 							
Regenerative Repair <ul style="list-style-type: none"> • BrdU • PCNA • Hyperplasia 							
Associative Event <ul style="list-style-type: none"> • Cell-to-Cell Communications 							
Modulating Factor <ul style="list-style-type: none"> • Cell Cycle Delay • DNA Repair, e.g., ↑ TiPARP 							
Possible Alternate Key Events							



And also thanks to: DuPont; U.S. EPA Office of Chemical Safety and Pollution Prevention, U.S. EPA Office of Water
