Quantitative Key Event Relationships in the Adverse Outcome Pathway (AOP) for AHR-Mediated Rodent Liver Tumor Promotion

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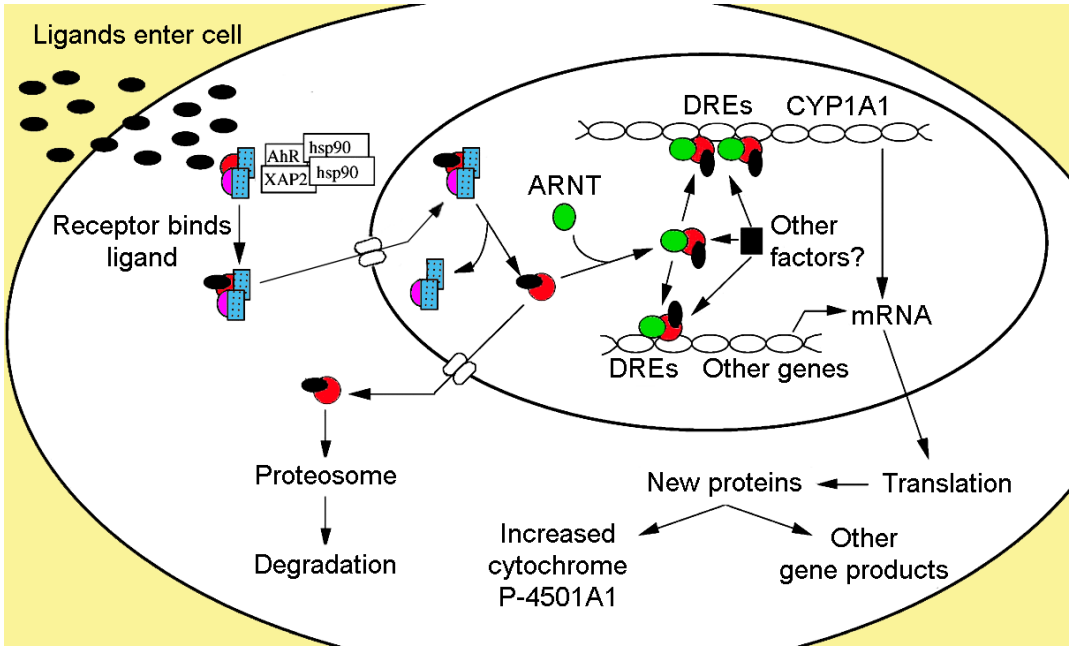
Road Map

- Biology of the Aryl Hydrocarbon Receptor and the Associated Tumor Response
- Description of the AOP
- Expressing the MIE in terms of both Dose and Time, i.e. Area-Under-the-Curve or AUC
- Quantitative Considerations of KE Occurrence and KE Relationships
- Lessons learned
- Path forward for AOPs

The Aryl Hydrocarbon Receptor (AHR)

- The AHR is a ligand-activated transcription factor and part of the basic helix-loop-helix (bHLH) Per-Arnt-Sim (PAS) superfamily
  - Activated by a variety of exogenous chemicals
    - Dioxins, PCBs, Dibenzofurans
    - Other planar polyaromatic hydrocarbons
    - Natural phytochemicals, flavinoids and indoles
    - Multiple endogenous ligands proposed, e.g. FICZ

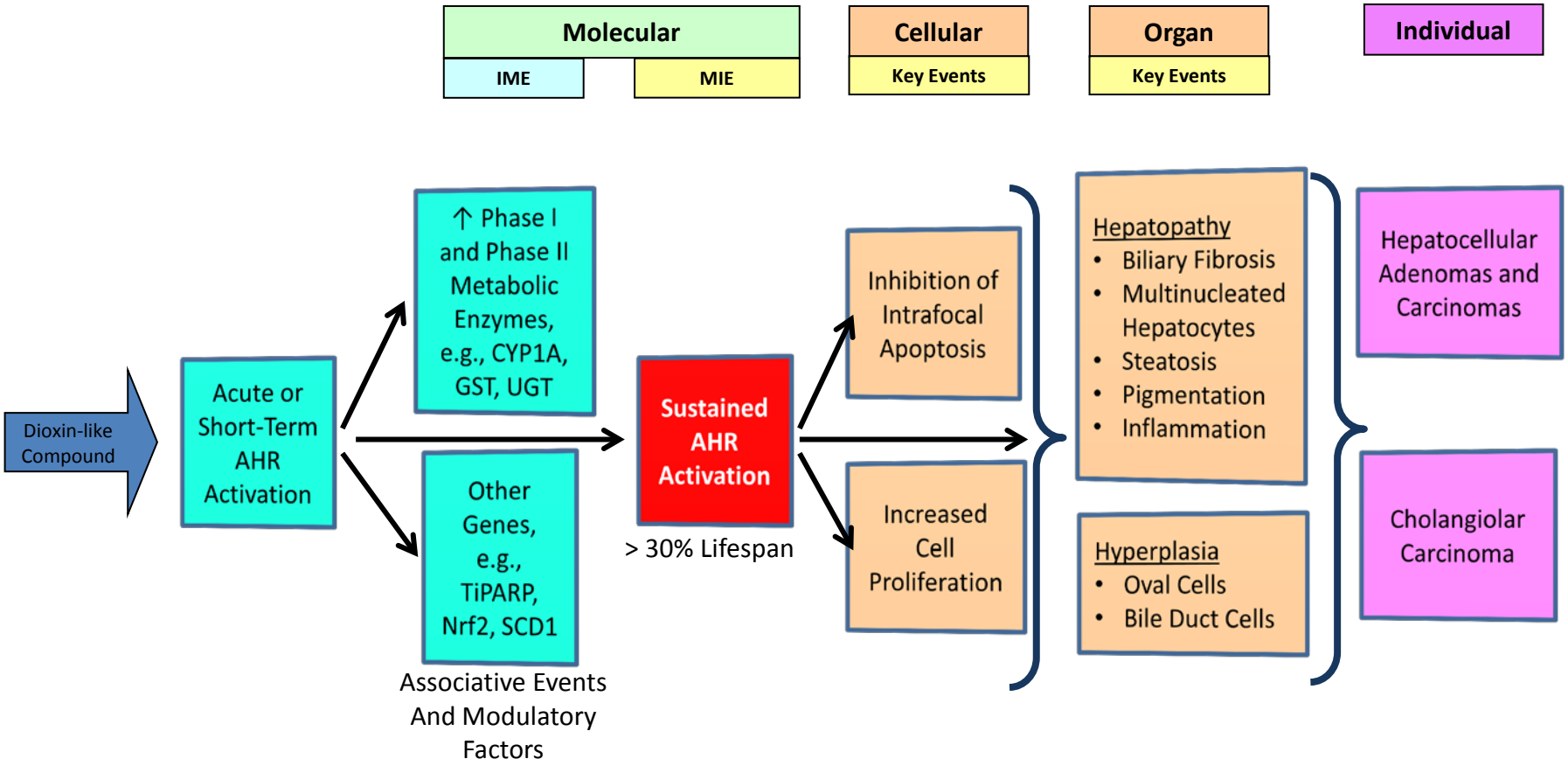


  - Regulates a diverse array of genes
    - Phase I metabolic enzymes (e.g., Cyp1a1, Cyp1a2)
    - Phase II metabolic enzymes (e.g., Ugt1a2, Gsta1)
    - Others (e.g., Tiparp, p27Kip1, Bach2)

AHR mediated Liver Tumors

- The NTP cancer bioassay in Sprague-Dawley rats observed increased incidences of several cancers (Walker et al. 2007) including
  - hepatocellular adenoma -- gingival squamous carcinoma (oral)
  - cholangiocarcinoma -- cystic keratinizing epithelioma (lung)
- AHR activation is considered to be the initial key event for dioxin-induced tumor
- However, many ligands activate the AHR and do not produce tumors, e.g. indole-3-carbinol in broccoli, omeprazole
- It is assumed that activation of AHR is the initial key event in the Mode of Action promoting tumorigenesis
- BUT is acute or short-term AHR activation the MIE or just an early event?
- What about sustained AHR activation?

AHR AOP for Rat Liver Tumor Promotion



Basic AHR AOP for Rat Liver Tumor Promotion

Toxicant	Macro-Molecular Interactions	Cellular Response	Organ Response	Organism Response
Well-defined Halogenated Co-planar	Sustained AHR Activation	Changes in Apoptosis , Proliferation and Cellular Homeostasis	Hepatopathy, Hyperplasia	Liver Tumors
HAHs Natural ligands	Classical Non-classical AHR Pathways	Gene Changes BrDU Apoptosis Lipid Metabolism	Parenchymal Non-Parenchymal	Parenchymal Non-Parenchymal

MIE → KE#1 → KE#2 → AO

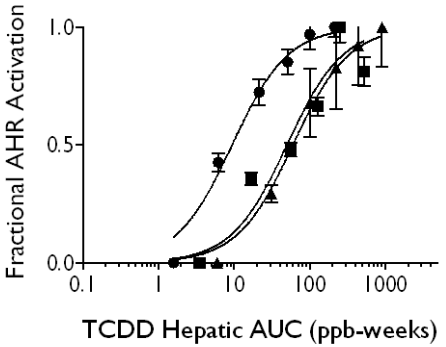
Molecular Initiating Event

- Sustained AHR Activation
- Area-Under-the Curve (AUC) for AHR
- Substances with rapid metabolism (e.g. bergamottin in Earl Gray tea and grapefruit) do not produce tumors
- Poorly metabolized or persistent chemicals (e.g., TCDD) produce tumors
- How to quantify the MIE as an AUC?

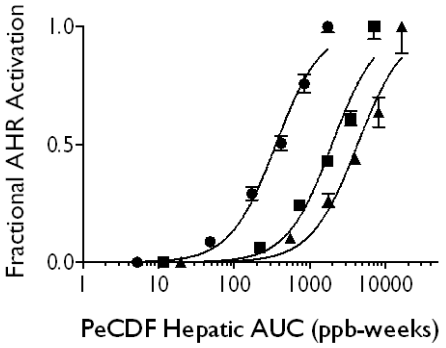
AUC Concept

- The dose-response for AHR activation measured by EROD (CYP1A1 induction) using hepatic AUC of the DLC in ppb-weeks as the dose-term was similar at 14, 31 and 53 weeks in three NTP bioassays for TCDD, 4-PeCDF and PCB126.
- Expressing the response as the fractional AHR Activation (0-1 scale) shows the response is similar over the three time points (to the right).
- These graphs can be combined. The dose term will be the AUC of hepatic TEQ and the response will be sustained activation (SA) as the AUC of fractional AHR activation.

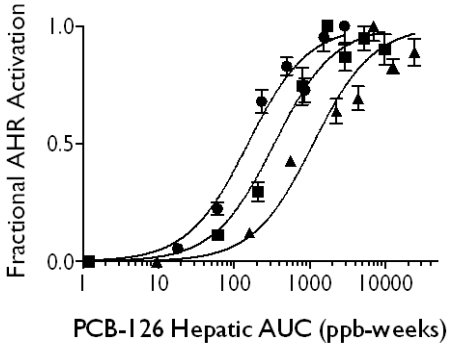
TCDD

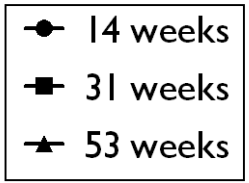


4-PeCDF



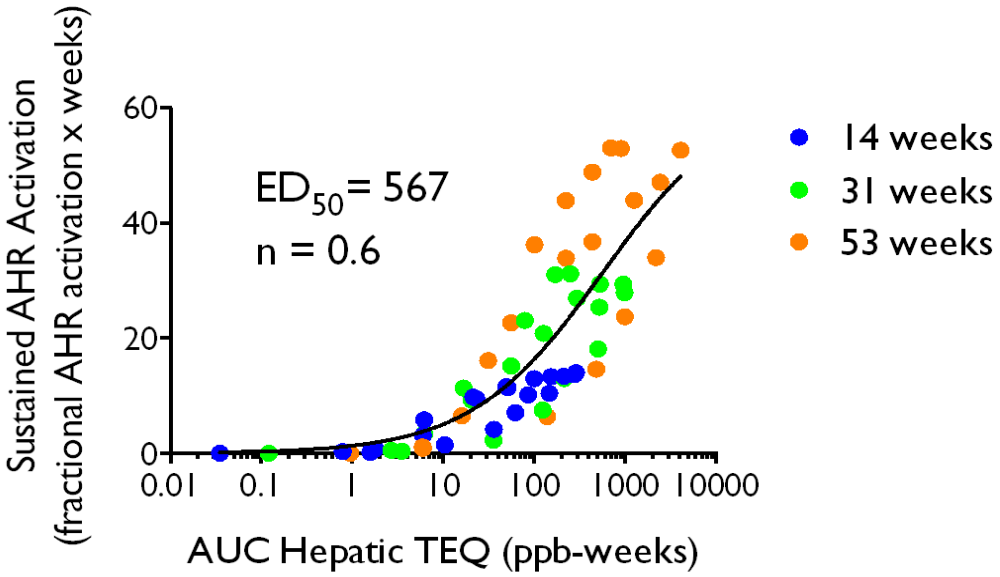
PCB126



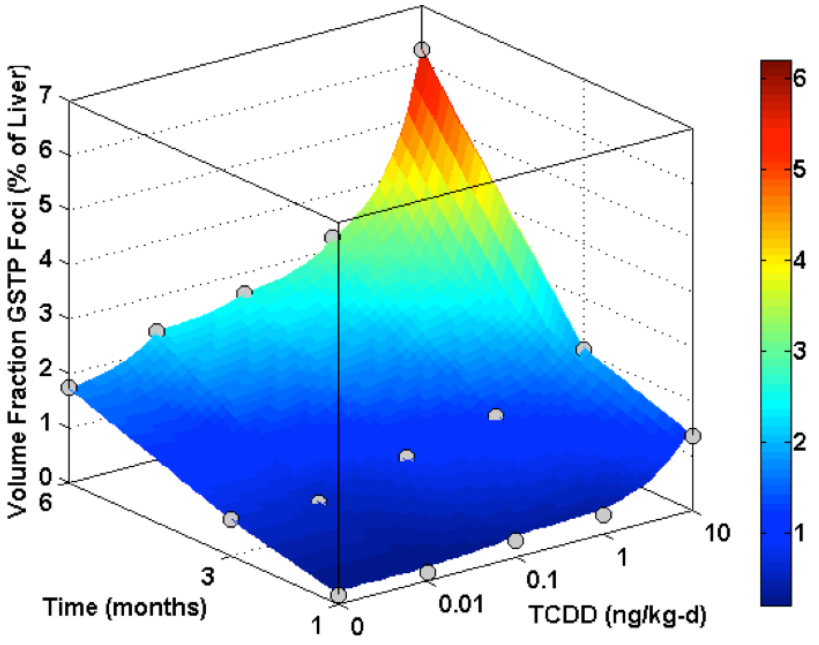


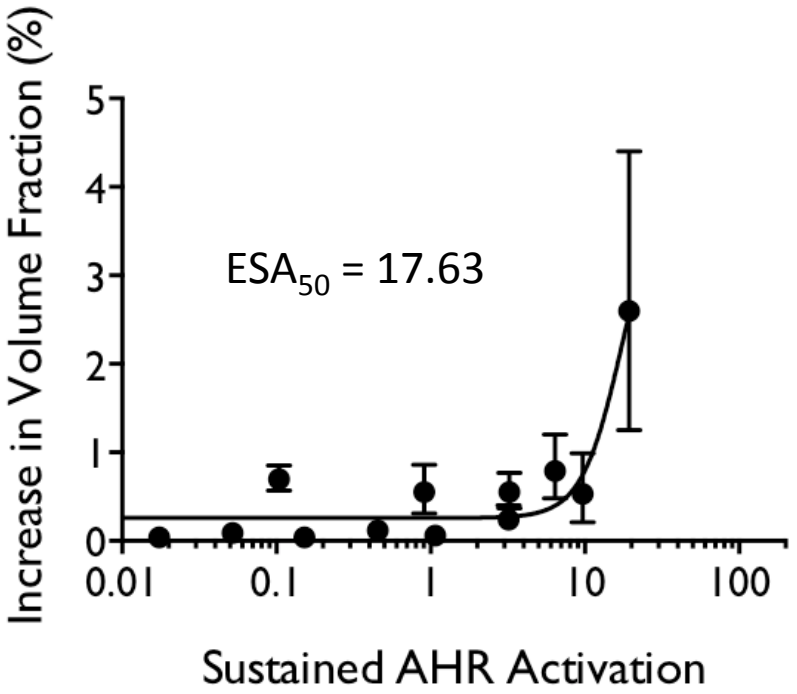
Relating the MIE to AUC for Dose

- Sustained Activation (SA) = AHR Activation Level x Time
- Fitting the dose response SA to TEQ Hepatic AUC is consistent with a Hill dose-response model
- The relationship of SA to AUC allows us to examine the “dose-response” of downstream events to SA in a quantitative fashion



KER: MIE → KE1, SA →Alteration of Cellular Growth Homeostasis





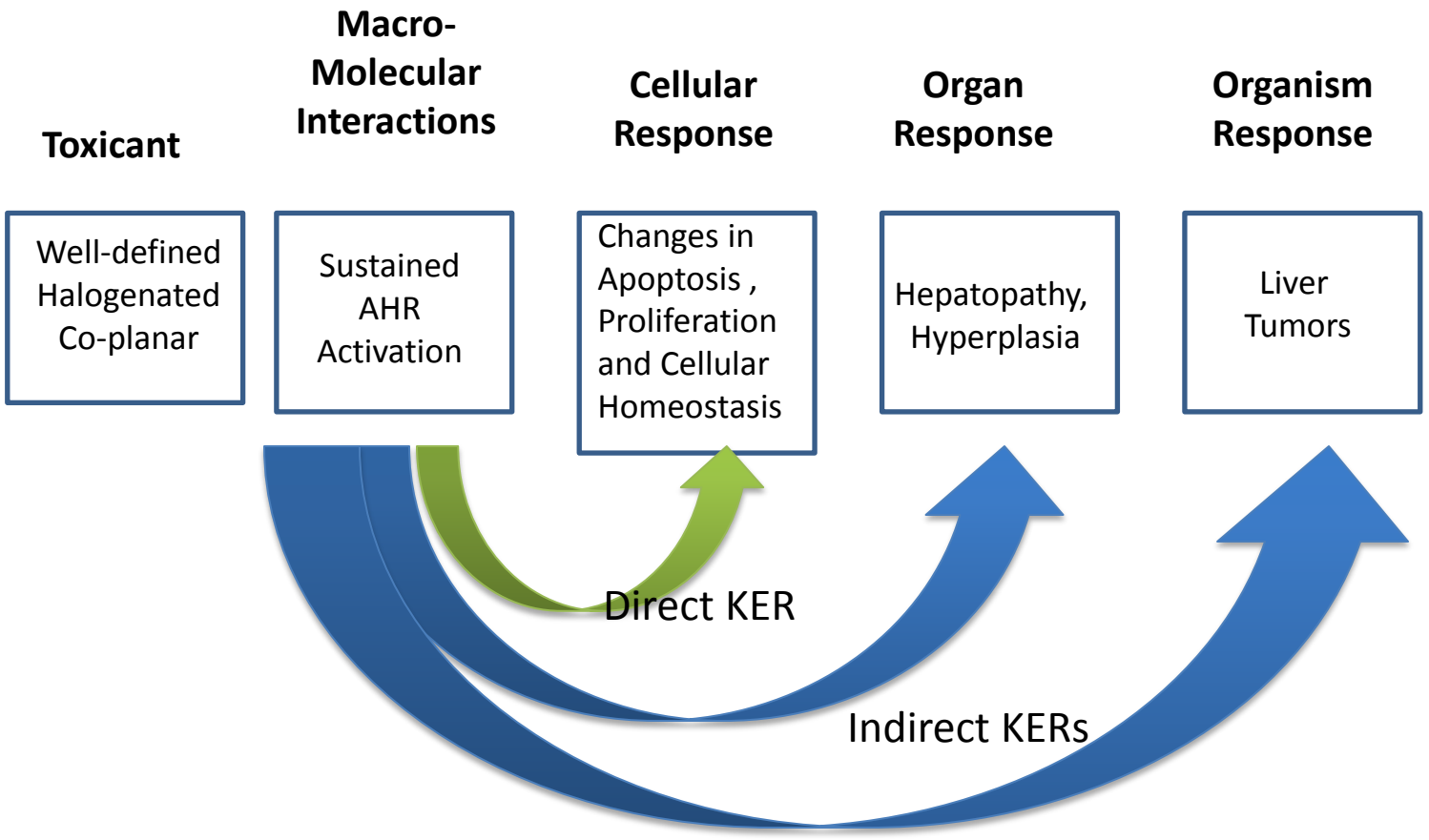
- 3D Dose-time Plot of Volume Fraction Increase of GSTP-positive Foci

Budinsky et al., 2014, Crit Rev Toxicol 44(1):83)

- Volume Fraction Increase of ATPase-deficient Foci vs. SA
- ESA_{50} is a measure of the "potency" of the MIE

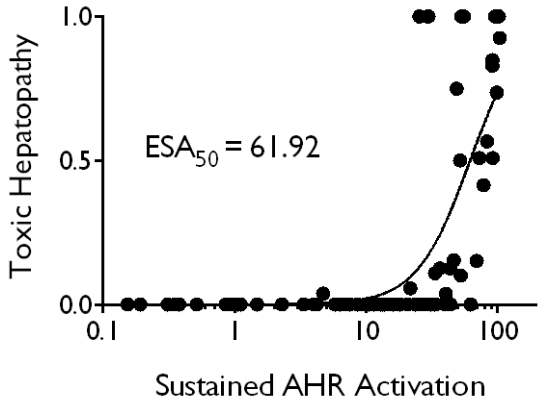
Teeguarden et al. 1999, Toxicol Sci 51(2):211

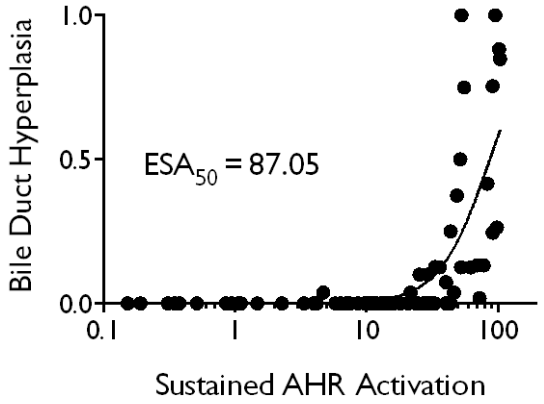
Basic AHR AOP for Rat Liver Tumor Promotion

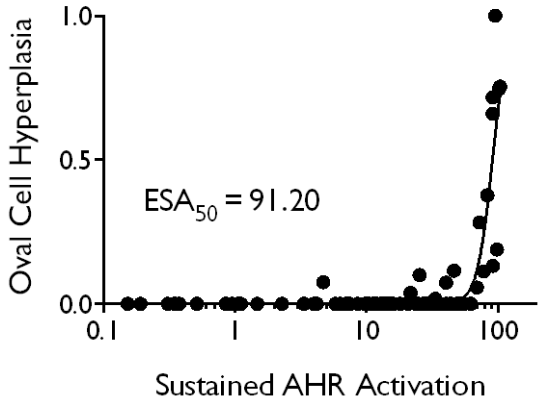


KER: MIE → KE2, SA → Hepatotoxicity, Hepatopathy

- Indirect KER between MIE and KE2
- Possibility of examining the direct relationship of KE1→KE2→AO because of many initiation-promotion studies for dioxin-like chemicals
- How do changes in cellular growth homeostasis leading to organ-level proliferation and tumors?

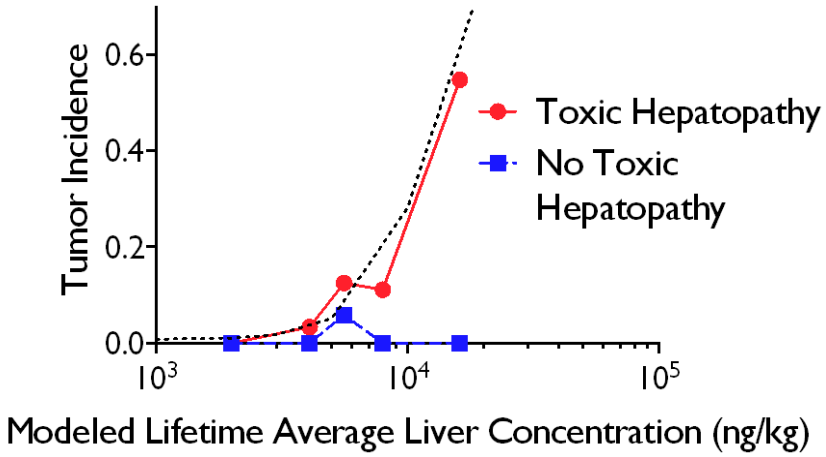


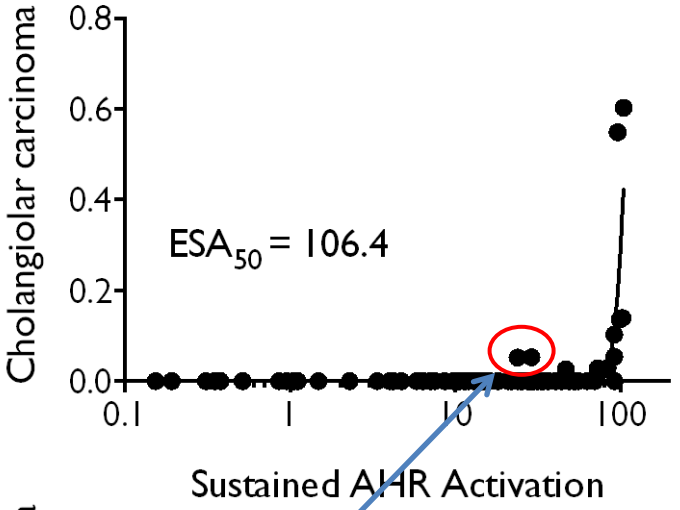


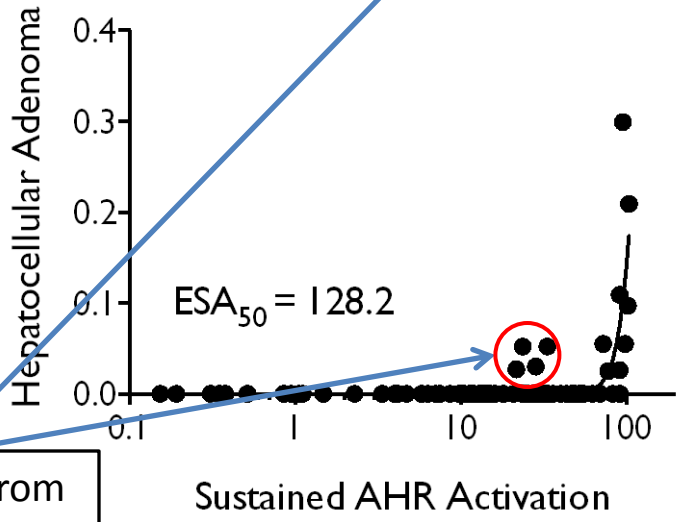


KER: KE2 → AO, MIE → AO

SA →Hepatotoxicity → Tumor Formation







Left: Evidence of Direct KER but no quantitative relationship that could be predictive

Right: Indirect KER but quantitative prediction may be possible

Circled responses from stop-exposure group

Dose-Temporality Concordance Table #1

Dose	Temporal →														
Dose ALC (ng/kg)	Key Event 1 (Immediate)	Key Event 2 (Days to Weeks)	Key Event 3 (Months)				Key Event 4 (Months)						Tumors	
			Proliferation/Hyperplasia				Toxicity							
	AHR Activation/ Transcrip. (XME) (1,2,3,4)	↓ Apoptosis (5,6,10)	AHF Vol. (5,8-12)	BrdU LI (1,7,12	Bile duct (BDH) (1,11,12)	Oval Cell (OCH) (1)	Multi-nucleate Hepatocytes (MNH) (1,11) (weeks)			Diffuse Fatty Change (DFC) (1) (weeks)			Hepatic Adenoma (1)	Cholangio-carcinoma (1)
							14	31	53	14	31	53		
<100		+												
100-1000	++++	+	+	+										
1000-2000	++++	+	++	+	+									
2000-5000	++++	+		+	+									
5000-10000	++++		++	+	++			+	+			+	+	+
>10000	++++	++	+++	++	++	+	+	++	+	+	+	+	+	+

The potential predictive value of SA emphasizes the importance of the dose-time concordance table (Budinsky et al., 2014, Crit Rev Toxicol 44(1):83; Meek et al., 2014, J Appl Toxicol 34(6):595; Simon et al. 2014, Crit Rev Toxicol 44 Supp 3:17)

Lessons Learned: Additional Nomenclature for the earliest events may be needed

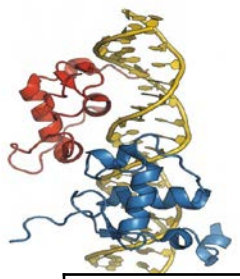
- For AHR AOPs,
  - receptor binding and acute transcriptional changes represent the Initial Molecular Event but this may not the necessary event for the AO (Patlewicz et al. 2013, Reg Toxicol Pharmacol 65(2):259)
  - the Molecular Initiating Event is sustained AHR activation
- AUC Concept will likely be important
- The idea of distinguishing early events from the MIE is generally applicable across many AOPs and may be necessary for applying AOPs
- Quantitative dose-response analysis will likely be useful in these efforts

The AHR AOP Rodent Liver Tumor Team

- Rick Becker (ACC)
- Bob Budinsky (Dow)
- Grace Patlewicz (DuPont)
- J. Craig Rowlands (Dow)

Questions?

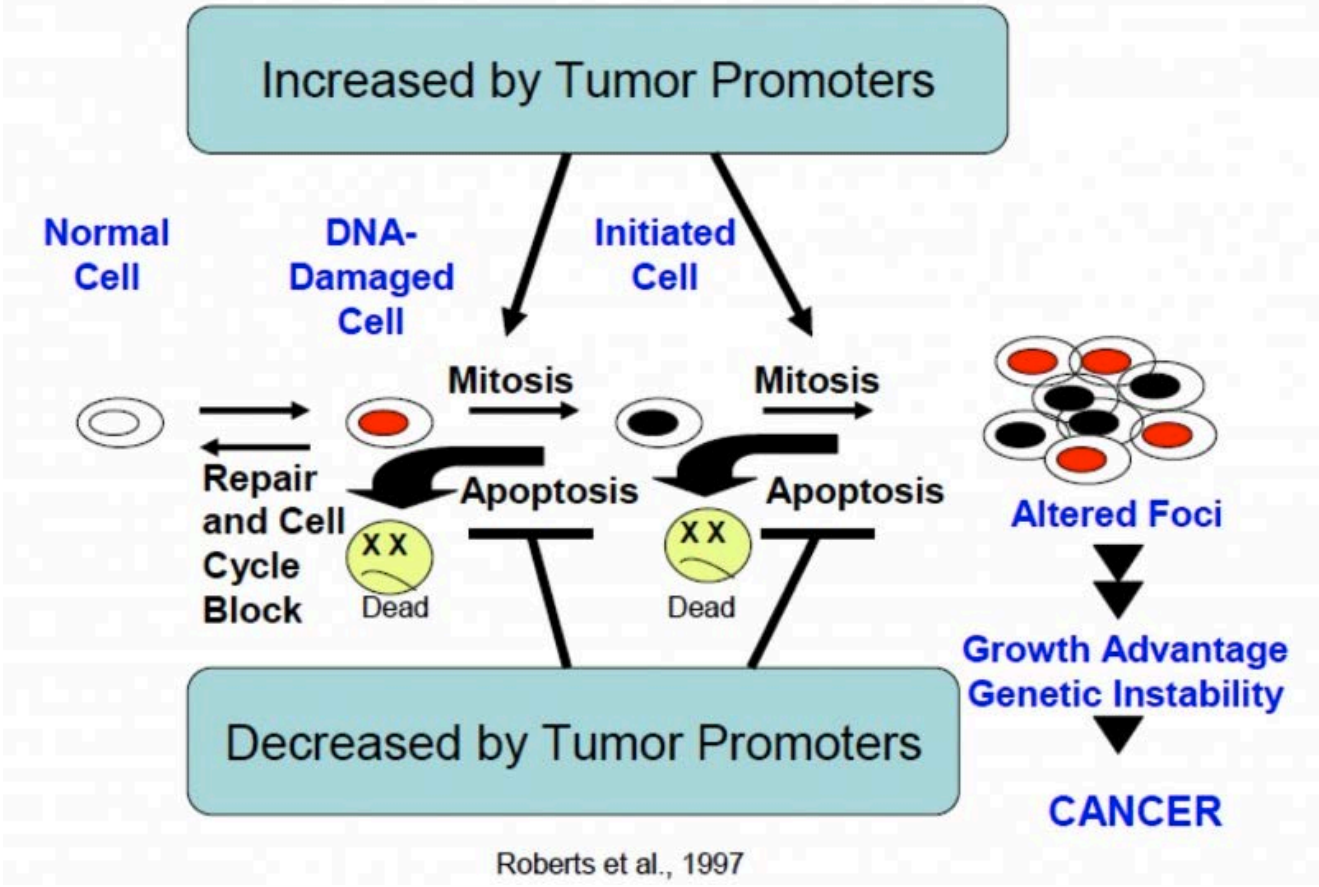
and Thank You for Your Attention



Experimental Support for the Key Events of the AOP

Key Events	Experimental Support	Strength of Evidence
IME	**<u>AHR Activation:</u>** Binding Affinity; Constitutively Active AHR; AHR Polymorphisms; AHR-KO Animal Models	Very Strong
Molecular Initiating Event	**<u>Subchronic Changes In Transcription</u>** Genomic Pathways/Networks Linked To Key Events Role of cytokines??	Weak to Moderate (a Data Gap?)
Key Event #1	**<u>Inhibition of Intrafocal Apoptosis</u>** In-vivo (Initiation-promotion) and in-vitro primary hepatocyte evidence.	Moderate to Strong
Key Event #2	**<u>Increased Cell Proliferation/Hyperplasia</u>** ↑BrdU-labeling, oval cells and bile duct hyperplasia	Very Strong
Key Event #2	**<u>Hepatopathy</u>** Constellation of histopathological changes consistently observed	Very Strong
Adverse Effect	**<u>Liver Tumors</u>** ↑Hepatocellular adenomas, cholangiomas and cholangiolar carcinomas	Very Strong

KER: MIE → KE1, SA →Alteration of Cellular Growth Homeostasis



Roberts et al., 1997

- Reduction in Apoptosis may provide a selective advantage to initiated cells in altered hepatic foci
- Increase in cell proliferation may also be occurring