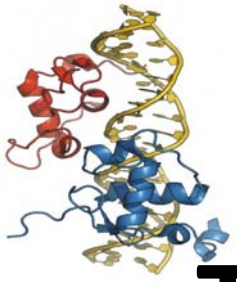
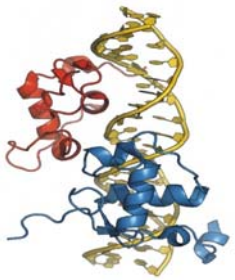


The HESI RISK21 Quantitative Key Events / Dose-Response Framework (Q-KEDRF)

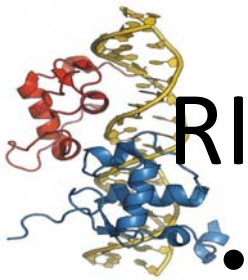
Beyond Science and Decisions: From Problem Formulation to Dose-Response Assessment

Alliance for Risk Assessment
Arlington, VA May 29, 2013
Ted Simon, Ph.D., DABT
Ted Simon, LLC



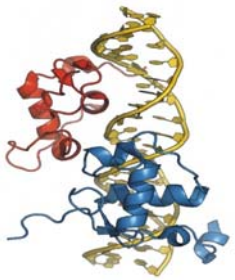
Mode of Action

- The unifying concept in risk assessment
- Defined in the 2005 Cancer Guidelines
- Promotes credible science-based risk assessment
  - Species extrapolation
  - Linear or non-linear low dose extrapolation
  - Sentinel or precursor events
- "Key Event" is the basis of MOA
  - Key Events are necessary for the adverse outcome



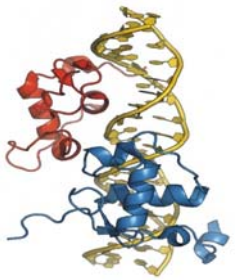
RISK21 and Dose-Response Subteam

- Strategy for using all available data to inform MOA
  - In vivo, in vitro, in silico, human, animal
- History of MOA
  - Male rat alpha 2u-globulin and nephrotoxicity
  - Rat bladder cancer and cell proliferation
- EPA definition
  - "*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. A "key event" is an empirically observable precursor step that is itself a necessary element of the mode of action or is a biologically based marker for such an element*"
- Julien et al. 2009 redefined MOA
  - *"fundamental biological events and processes that underlie the effect of a bioactive agent"*



Aspects of MOA - 1

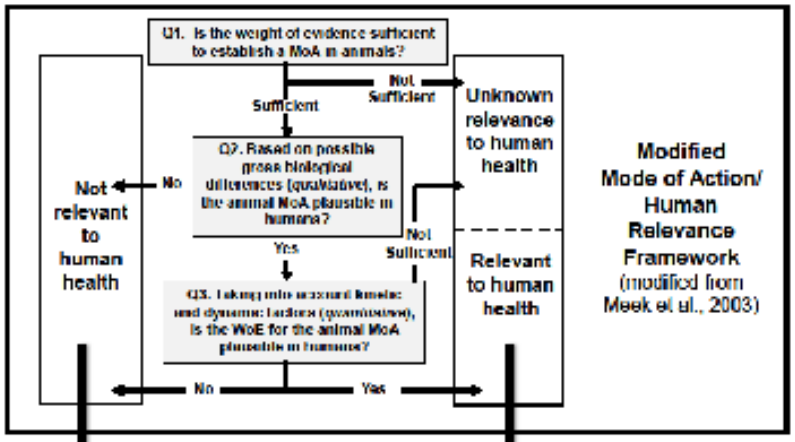
- Key Event (KE)
  - An empirically observable causal precursor step to the adverse outcome that is itself a necessary element of the MOA. KEs are necessary but usually not sufficient for the adverse outcome in the absence of other KEs.
- Associative Event (AE)
  - Biological processes that are themselves not KEs for the MOA but are reliable indicators or biomarkers for KEs. AEs can often be used as surrogates or biomarkers for a KE in a MOA evaluation; depending upon the nature of the biomarker, AEs may reflect exposure to a xenobiotic, the resulting effect, or both.



Aspects of MOA - 2

- Modulating Factors (MFs)
  - Biological and individual factors, including control mechanisms or host factors, that can modulate the dose-response relationship of one or more KEs, thus altering the probability or magnitude of the adverse outcome
  - Host Factors
    - Polymorphisms, disease state, hormonal status
  - Lifestyle Factors
    - Diet, exercise, pharmaceuticals, alcohol
  - Environmental Factors
    - Coexposures, occupation, hobbies

Q-KEDRF



Quantitative Key Events / Dose-Response Framework (Q-KEDRF)

B1. Use current methodologies, e.g., Margin of Exposure (MOE), on the most appropriate endpoint.

Dose-Response and Temporality of Key Events (pathways)

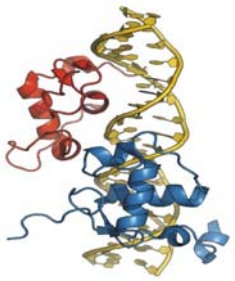
B2. What Key Events can be identified unequivocally? Are any Key Events represented by an Associative Event?

B3. What is the dose response and temporal relationship between the Key Events and the apical event?

B4. What are the Modulating Factors for Key Events of the human dose response? How do the Key Events and their Modulating Factors vary within the human population?

B5. Use quantitative dose response analysis to understand species differences with the goal of developing human toxicity criteria based on the MOA.

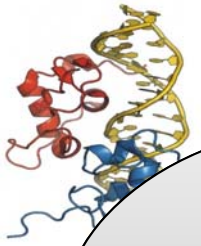
DOSE-RESPONSE (most relevant apical event)



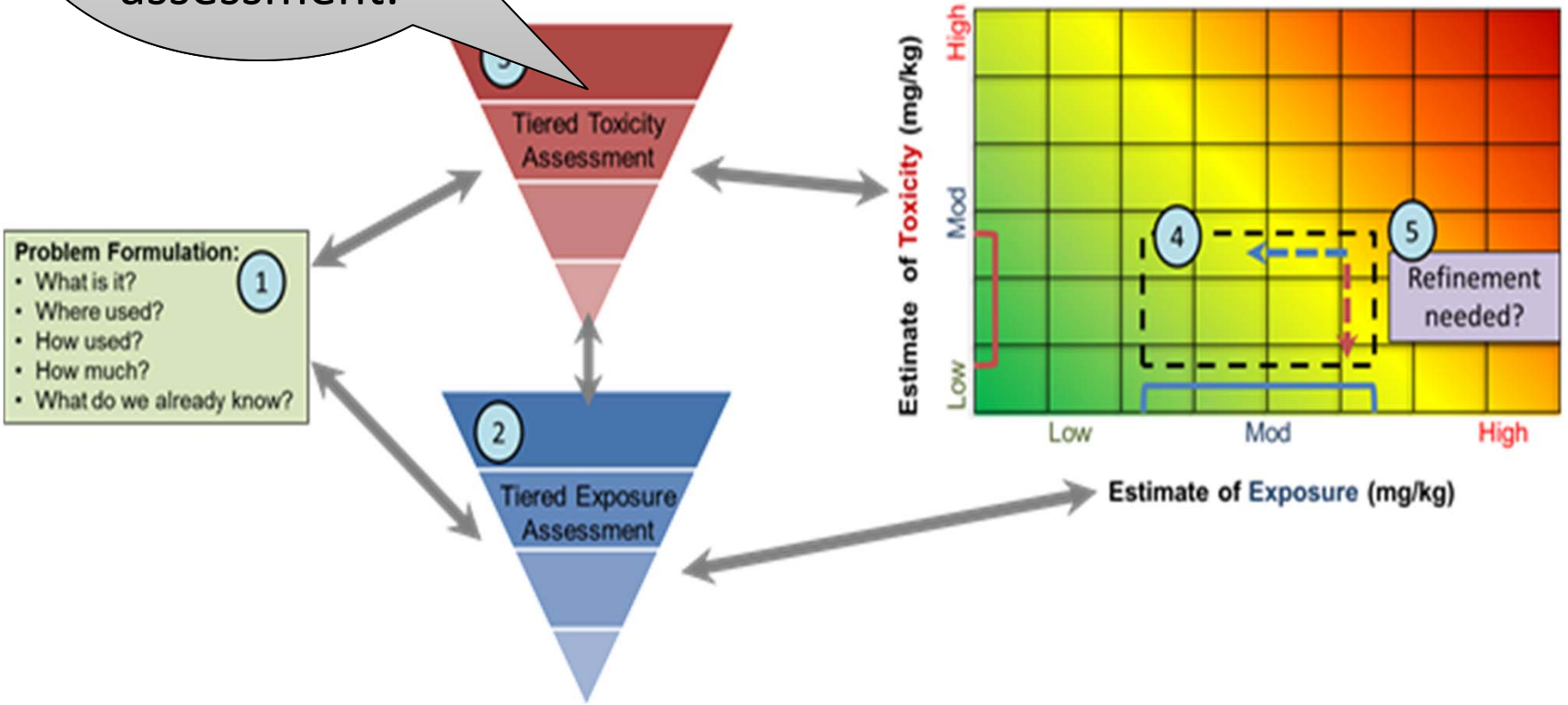
Questions Addressed by the Q-KEDRF

- What KEs can be identified unequivocally? Which are represented by AEs?
- What is the D-R and temporal relationship between various KEs and the apical event?
- What are MFs in humans for the various KEs? How do these MFs vary in the population?
- How do we use quantitative information to inform interspecies and low dose extrapolation?
- EXAMPLES PROVIDED!!

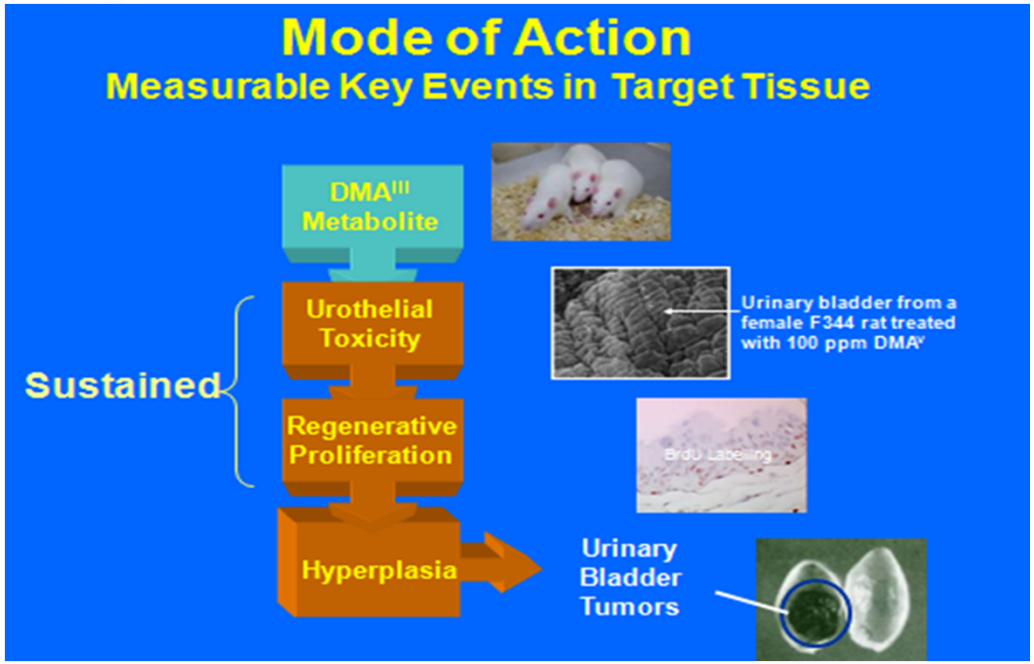
RISK 21 Matrix



Q-KEDRF is part of the tiered toxicity assessment.

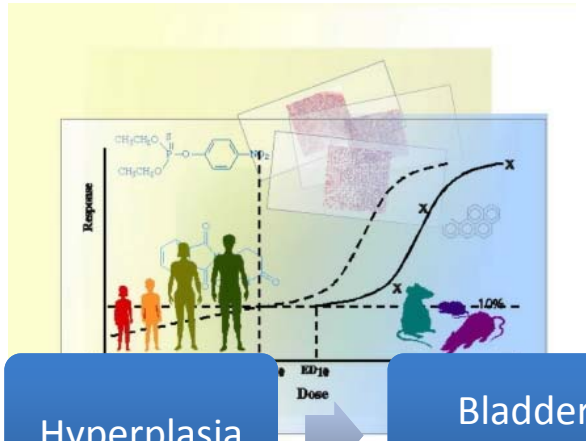


Ex. #1—Cacodylic Acid



Science Issue Paper:
Mode of Carcinogenic Action for Cacodylic Acid (Dimethylarsinic Acid, DMA^V) and Recommendations for Dose Response Extrapolation

July 26, 2005

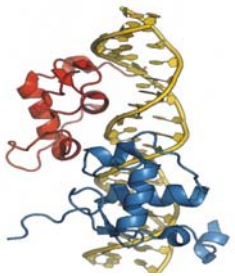


- OPP concluded the MOA was plausible in humans despite quantitative species
- More recent in vitro studies suggest quantitative Species Extrapolation based on levels of DMA^{III} metabolite in urine

DME^{III} (Metabolite) → Urothelial Toxicity → Regenerative Proliferation → Hyperplasia → Bladder Tumors

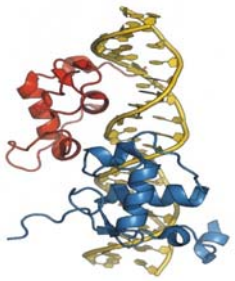
Prepared by:

Health Effects Division
Office of Pesticide Programs
US Environmental Protection Agency

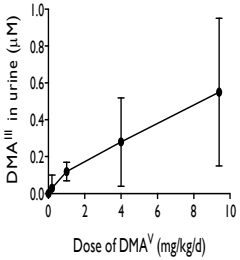
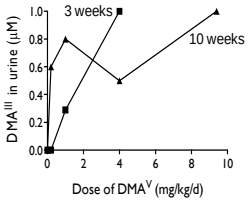
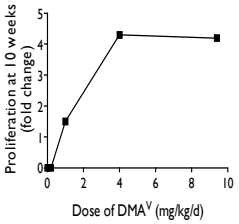


Dose-Time Concordance Table for DMA^{III}

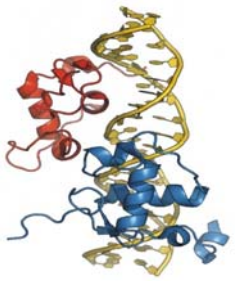
Table —Dose-Time Concordance						
Time		**2 weeks**	**2-3 weeks**	**10 weeks**	**25 weeks**	**104 weeks**
Dose	**Increasing Time →**					
↓	2	Metabolism*	Metabolism*	Metabolism* Cytotoxicity	Metabolism* Cytotoxicity*	Metabolism* Cytotoxicity*
	10	Metabolism*	Metabolism* Cytotoxicity	Metabolism* Cytotoxicity	Metabolism* Cytotoxicity*	Metabolism* Cytotoxicity*
	40	Metabolism*	Metabolism* Cytotoxicity	Metabolism* Cytotoxicity Proliferation Hyperplasia	Metabolism* Cytotoxicity* Proliferation* Hyperplasia	Metabolism* Cytotoxicity* Proliferation* Hyperplasia **Carcinomas**
	100	Metabolism*	Metabolism Cytotoxicity Proliferation Hyperplasia	Metabolism Cytotoxicity Proliferation Hyperplasia	Metabolism Cytotoxicity* Proliferation Hyperplasia	Metabolism* Cytotoxicity* Proliferation* Hyperplasia **Carcinomas**



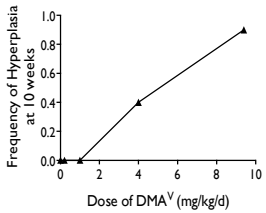
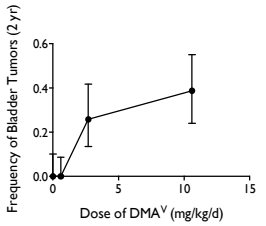
Dose-Response Species Concordance - 1

EVENT OR FACTOR	QUALITATIVE CONCORDANCE			QUANTITATIVE CONCORDANCE AND QUANTITATIVE DOSE-RESPONSE		
	Animals	Humans	Concord-ance	Str.*	Animals	Humans
KEY EVENTS						
Key Event #1 Metabolism to DMA^{III}	DMA^{III} detected in urine following 26 weeks treatment with 100 ppm DMA^{V}	Evidence following DMA^{V} exposure too limited to draw conclusions, but DMA^{III} shown to be present following human exposure to iAs	Plausible	+/-		NA
Key Event #2 Urothelial Cytotoxicity	Urothelial toxicity observed in vivo in rats at 2 ppm but not enough for successive key events	Potential to occur in humans but unknown if sufficient DMA^{III} formed	Plausible	+/-		NA
Key Event #3 Urothelial Proliferation	observed at 0.5 mg/kg/d DMA^{V}	Potential to occur in humans but unknown if sufficient DMA^{III} formed	Plausible	+/-		NA

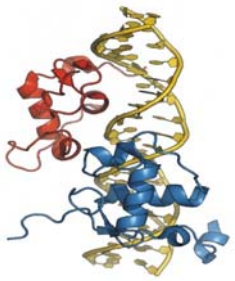
*Str. = strength



Dose-Response Species Concordance Table - 2

EVENT OR FACTOR	QUALITATIVE CONCORDANCE			QUANTITATIVE CONCORDANCE AND QUANTITATIVE DOSE-RESPONSE		
	Animals	Humans	Concord-ance	Str.*	Animals	Humans
KEY EVENTS						
Key Event #4 Hyperplasia	observed at 2 mg/kg/d or 0.3 to 2 µmol DMA^{III} in urine	Potential to occur in humans but unknown if sufficient DMA^{III} formed	Plausible	+/-		NA
Apical Event Tumors	observed at 5 mg/kg/d DMA^V or 0.8 to 5.05 µmol DMA^{III} in urine	No data in humans	Concordance cannot be made because there is no human data	-		NA

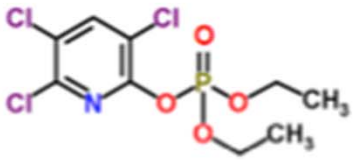
*Str. = strength



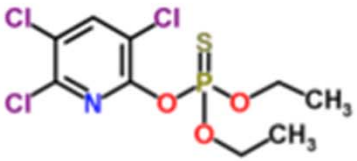
Ex. #2—MOA for Chlorpyrifos

AChE inhibited *in vitro* at *in vivo* concentrations

Inhibtion of neuronal of AChE



P450 (CYP2B6)



Chlorpyrifos oxon

Chlorpyrifos

PON1

Inactive metabolites

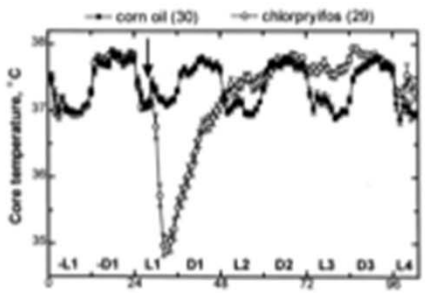
Enzyme polymorphism is a Modulating Factor

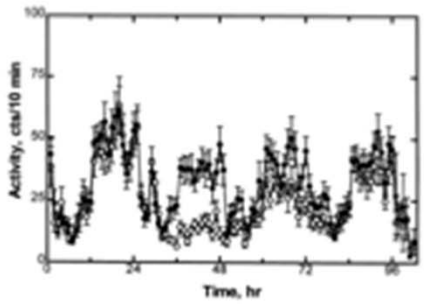
AChE levels increased in brain and periphery

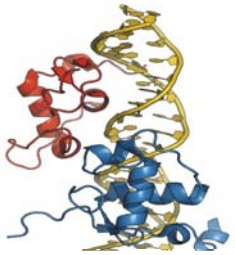
Increased ACh in synaptic cleft

Over-stimulation of ACh receptors

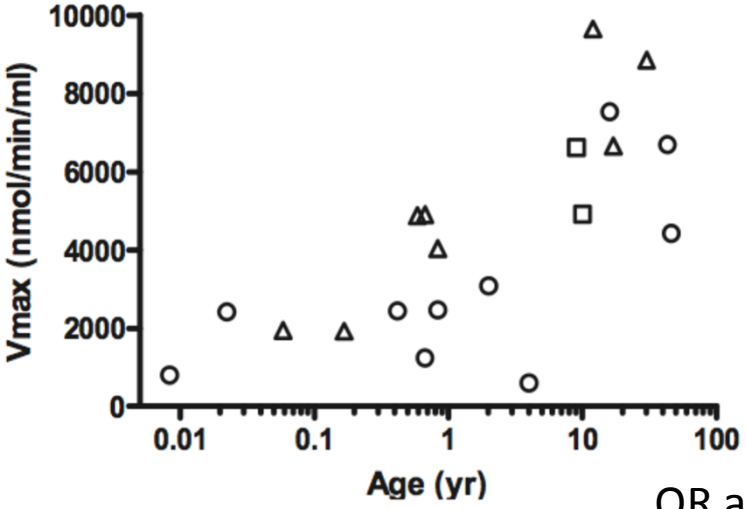
Clinical signs

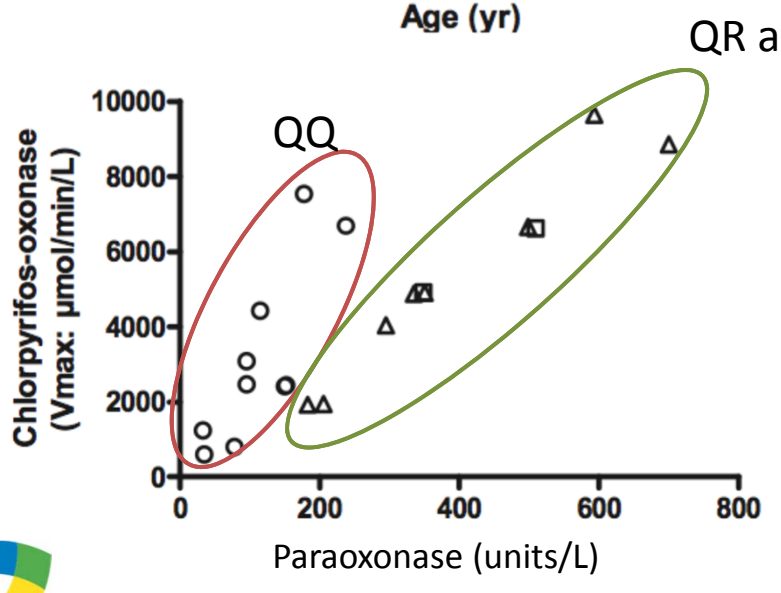




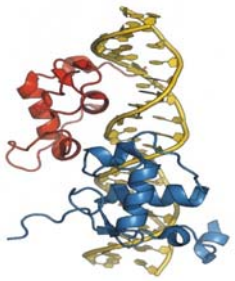


Modulating Factors— Age and Polymorphisms



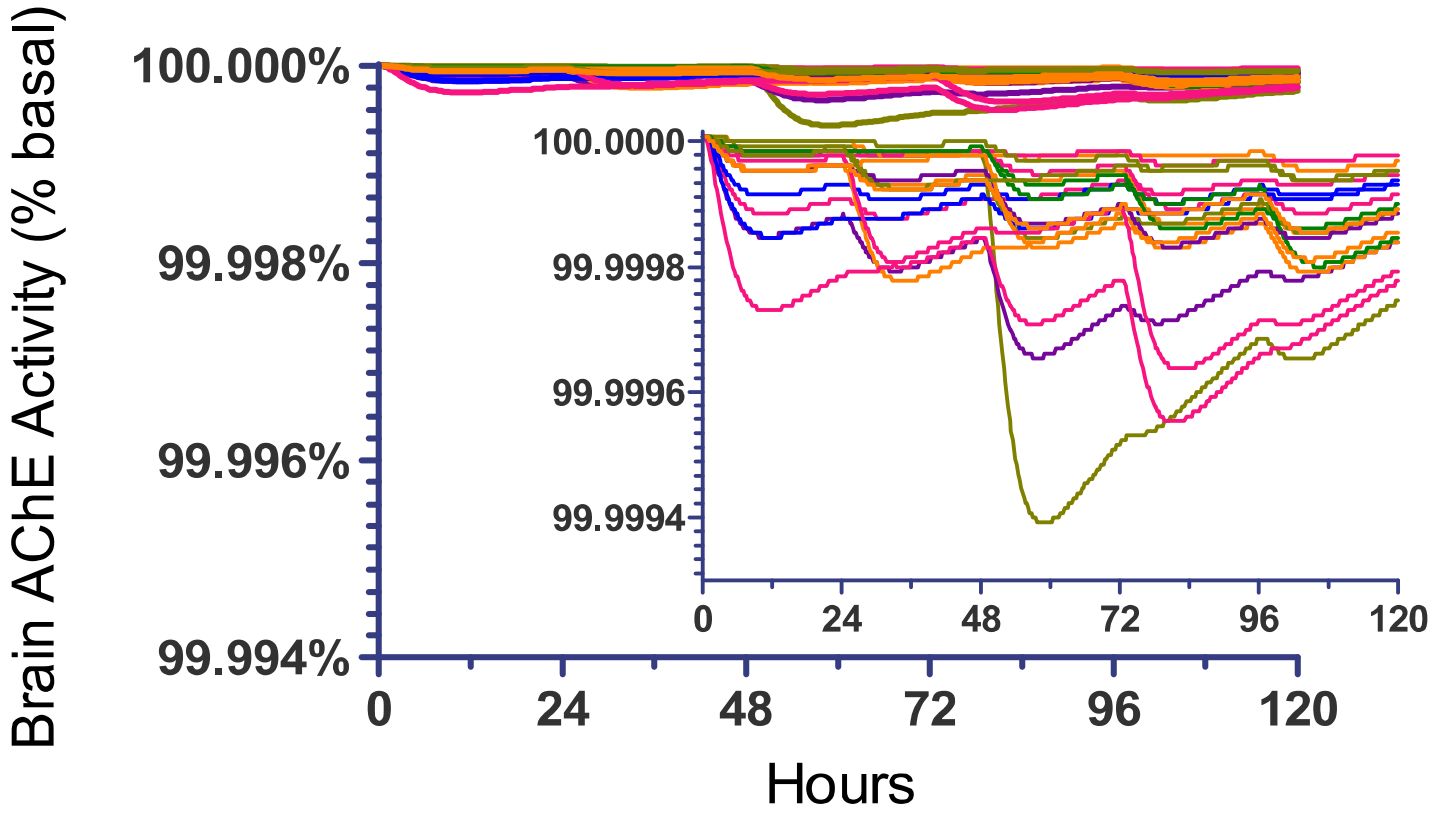


- PON1 GLN:192 (Q allele)
- PON1 ARG:192 (R allele)
- RR metabolizes paraoxonase fastest
- RR > QR > QQ
- PON1 activity affected by diet, alcohol use, and statins

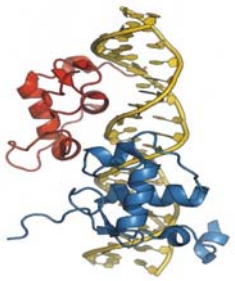


Both Exposure/Dose and MFs need to be considered

Plot (C)

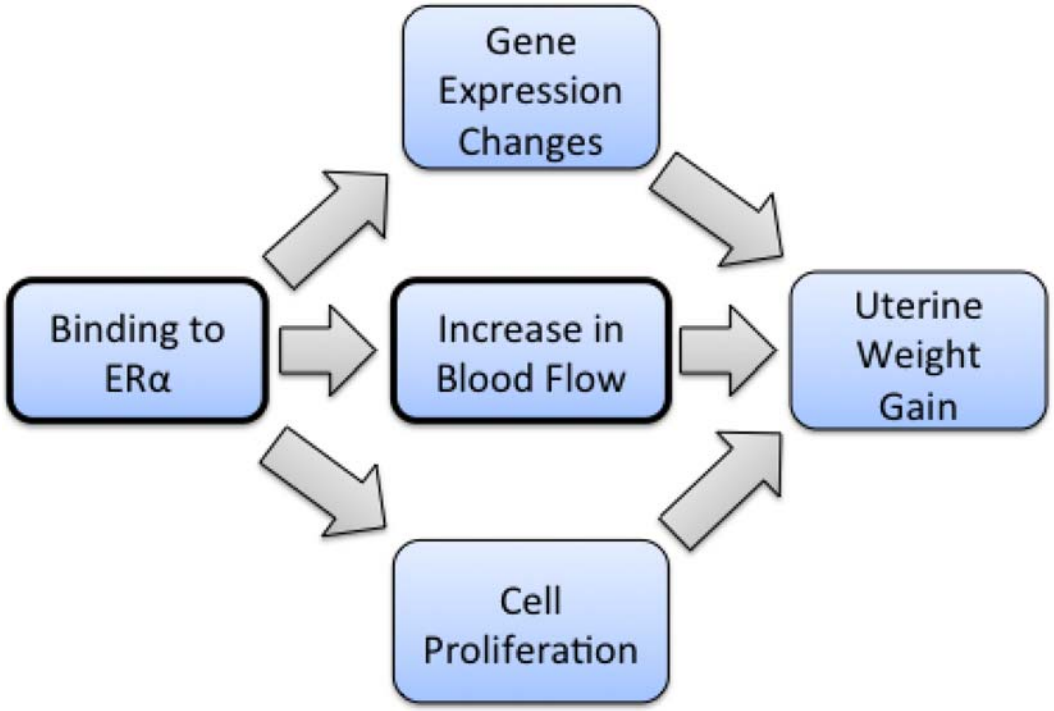


- Current dietary exposures are low enough that the polymorphism doesn't make a difference!

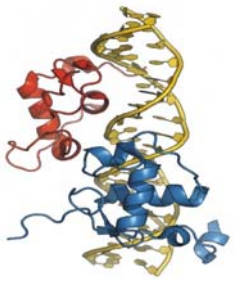


Ex. #4—Uterotrophy as a Model System

- Counterfactual identification of KEs



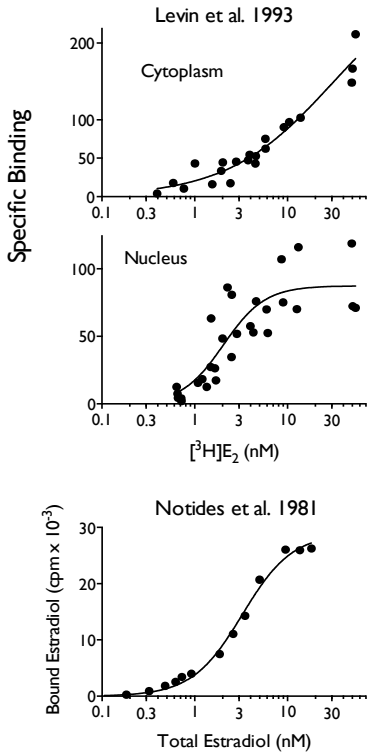
- ERα binding—ERKO mice do not show uterotrophy
- Blood flow—LNAME blocks NO synthase and also prevents uterotrophy



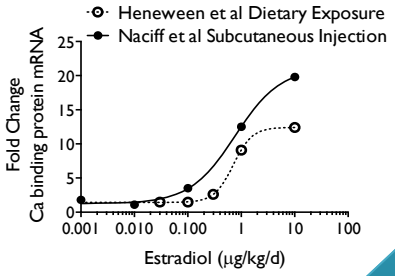
Dose Response Modeling of Uterotrophy

Uterine Peroxidase is a biomarker or AE for increase in uterine blood flow

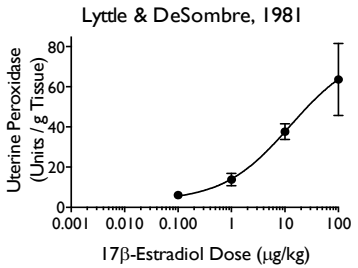
Binding to ERα



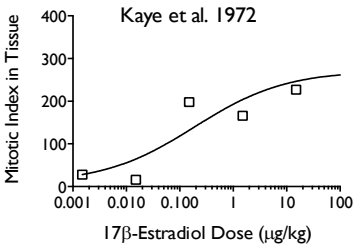
Gene Expression Changes



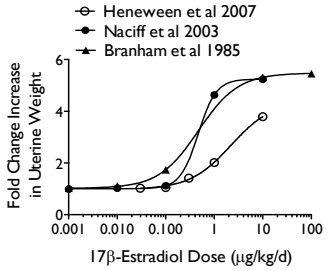
Increase in Blood Flow



Cell Proliferation

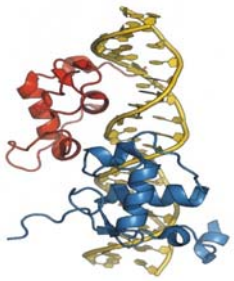


Uterine Weight Gain



Variation in Quantitative DR

- Heneweer et al. 2007
  - Kd = 2.22 µg/kg/d, n = 1.02
- Naciff et al. 2003
  - Kd = 0.47 µg/kg/d, n = 2.33



Quantitative D-R Analysis of the Hill Model for Threshold Analysis

$$\Pr(Response) = \frac{V_{\max}\, dose^{n}}{dose^{n} + K^{n}}$$

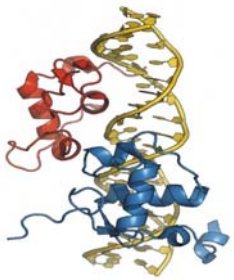
$$BMR_{05} = 5\% = \frac{BMD_{05}^{n}}{BMD_{05}^{n} + K^{n}}$$

1) Solve implicitly for BMD05;

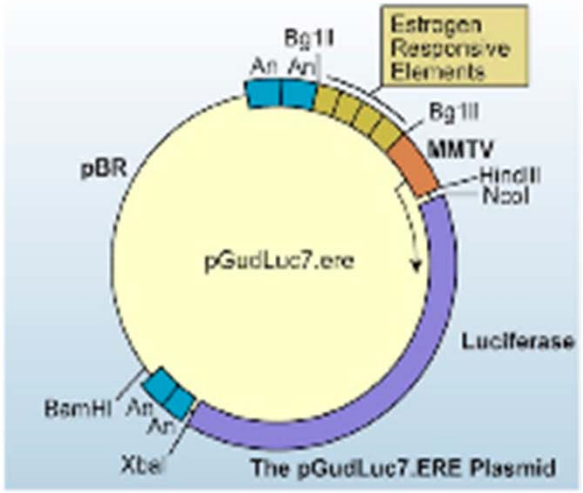
2) use that value to calculate the slope at the point (BMD05, BMR05)

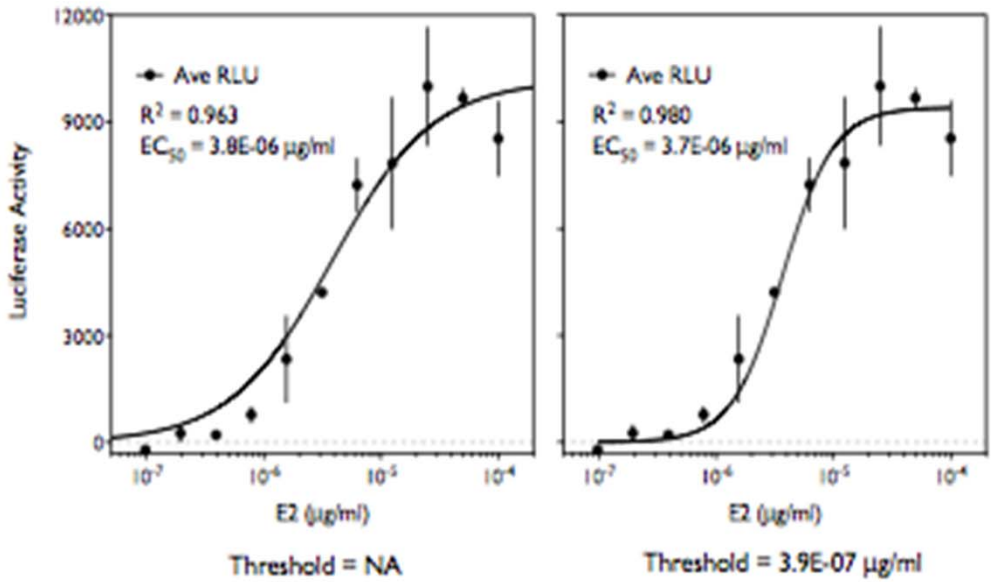
$$\frac{d\left[\Pr(Response)\right]}{d(dose)} = \frac{dose^{n-1}n}{dose^{n} + K^{n}} - \frac{dose^{n}\, dose^{n-1}n}{\left(dose^{n} + K^{n}\right)^{2}}$$

$$Threshold = BMD_{05} - \frac{BMR_{05}}{Slope\, at\left(BMD_{05},\, BMR_{05}\right)}$$



Calculating Thresholds



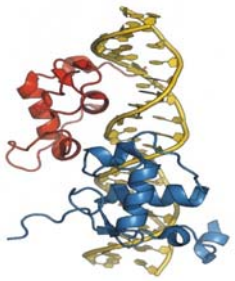


Assume 1st order Hill, i.e. n = 1, Kd = 3.8 pg/ml

Assume 2nd order Hill, i.e. n = 2, Kd = 3.7 pg/ml

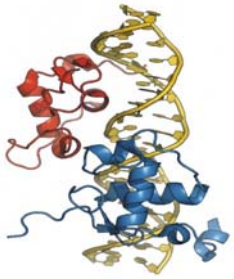
1st order: BMD_{05} = 0.2 pg/ml; Slope = 0.24 per pg/ml; Threshold < 0

2nd order: BMD_{05} = 0.85 pg/ml; Slope = 0.11 per pg/ml; Threshold = 0.4 pg/ml

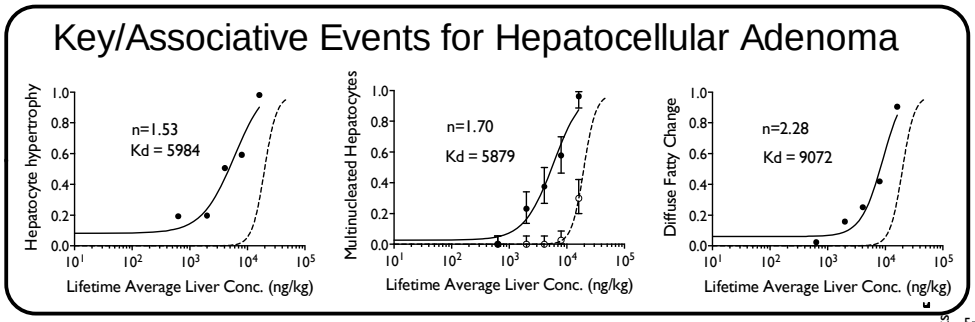


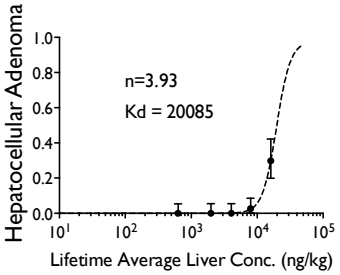
Slopes for Other Models

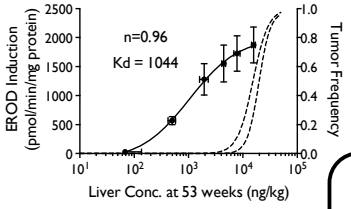
Model	Equation	Derivative
Logistic	$\Pr(response) = \gamma + \frac{1-\gamma}{1+e^{-(\alpha+\beta\, dose)}}$	$\frac{d[\Pr(response)]}{d(dose)} = \frac{\beta e^{-(\alpha+\beta\, dose)}(\gamma-1)}{\left(e^{-(\alpha+\beta\, dose)}+1\right)^2}$
Log-Logistic	$\Pr(response) = \gamma + \frac{1-\gamma}{1+e^{-(\alpha+\beta\ln(dose))}}$	$\frac{d[\Pr(response)]}{d(dose)} = \frac{\beta e^{-(\alpha+\beta\ln(dose))}(\gamma-1)}{\left(e^{-(\alpha+\beta\ln(dose))}+1\right)^2}$
Multistage (2nd order)	$\Pr(response) = \gamma + (1-\gamma)\left(1-e^{-\beta_1 dose-\beta_2 dose^2}\right)$	$\frac{d[\Pr(response)]}{d(dose)} = -e^{-\beta_1 dose-\beta_2 dose^2}(\gamma-1)(\beta_1+2\beta_2\, dose)$
Weibull	$\Pr(response) = \gamma + (1-\gamma)\left(1-e^{-\beta\, dose^{\alpha}}\right)$	$\frac{d[\Pr(response)]}{d(dose)} = -\alpha\,\beta\, dose^{\alpha-1}e^{-\beta\, dose^{\alpha}}(\gamma-1)$
Dichotomous Hill	$\Pr(response) = v\,g + \frac{v-v\,g}{1+e^{-\alpha-\beta\ln(dose)}}$	$\frac{d[\Pr(response)]}{d(dose)} = \frac{-\beta(v-vg)e^{-\alpha-\beta\ln(dose)}}{dose\left(e^{-\alpha-\beta\ln(dose)}-1\right)^2}$
Linear	$\Pr(response) = \beta_0 + \beta_1\, dose + \beta_2\, dose + \beta_3\, dose$	$\frac{d[\Pr(response)]}{d(dose)} = 3\beta_3\, dose^2 + 2\beta_2\, dose + \beta_1$
Power	$\Pr(Response) = \gamma + \beta\, dose^{\delta}$	$\frac{d[\Pr(Response)]}{d(dose)} = \beta\,\delta\, dose^{\delta-1}$

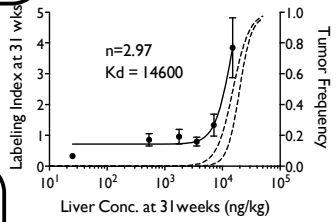


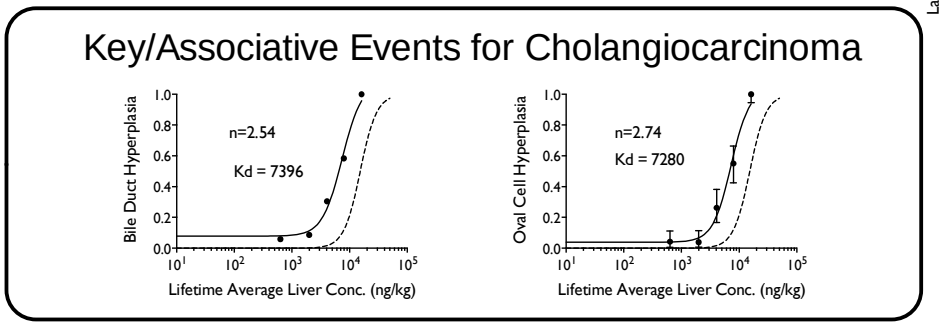
Analysis of TCDD Liver Tumorigenesis Provides a Model for the Q-KEDRF

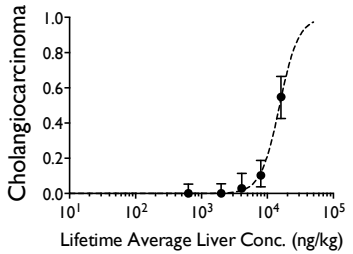










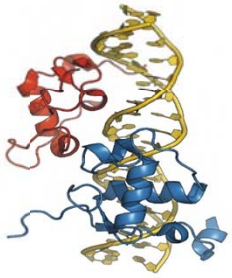


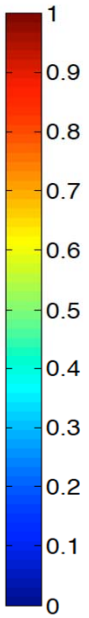
XME Induction
n < 1.5
Kd = 1000-2000

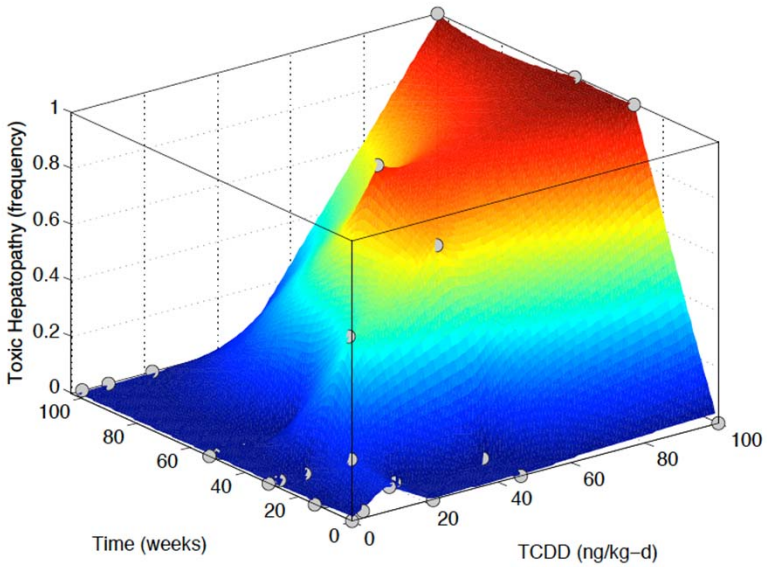
Cellular change and Initial Hyperplastic Effects
1.5 < n < 3
Kd = 5000-10000

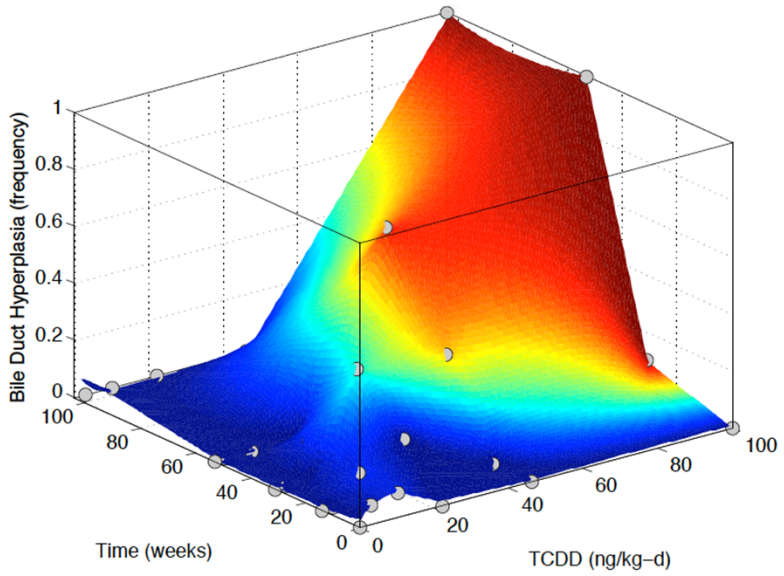
Proliferation
n ≅ 3
Kd > 10000

Apical Effects
n > 3
Kd > 15000



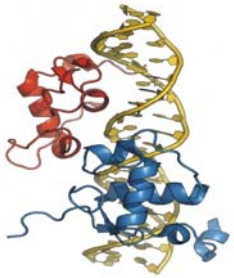






Plotting Key Events along the Dose-Time Continuum

- Inflection points change with dose and time
- AUC or average tissue conc. over time may be a better dose metric than administered dose



Conclusions

- High quality D-R data for both KEs and the apical event are needed
- Which KEs can be unequivocally identified as such?
- Both the position and steepness of the D-R should be considered
- MFs need to be taken into account relative to dose levels of interest
- Quantitative DR of KEs can provide much information about the MOA