

SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety



**Risk21 Quantitative
Key Events Dose-
Response Framework**

January 25, 2016



SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety

Risk21 Quantitative Key Events Dose-Response Framework

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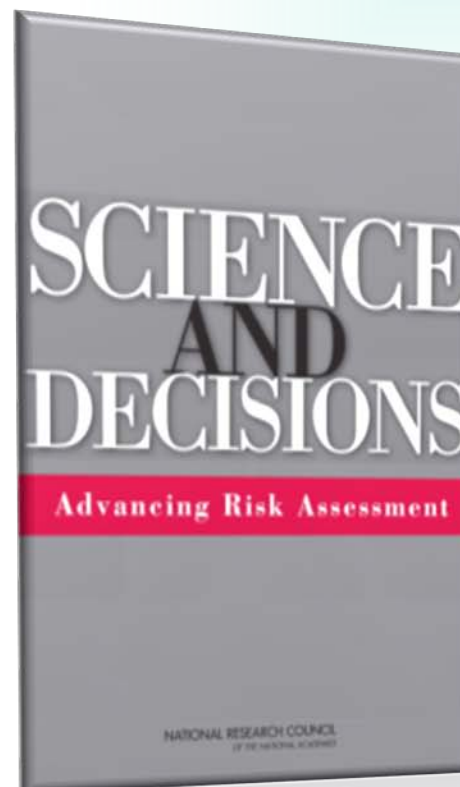
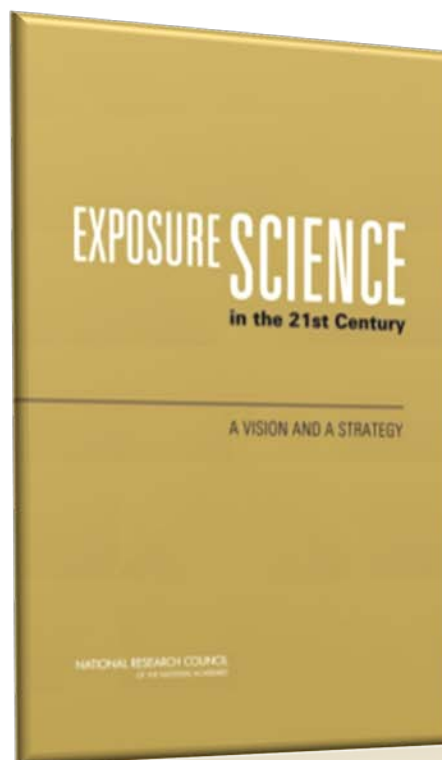
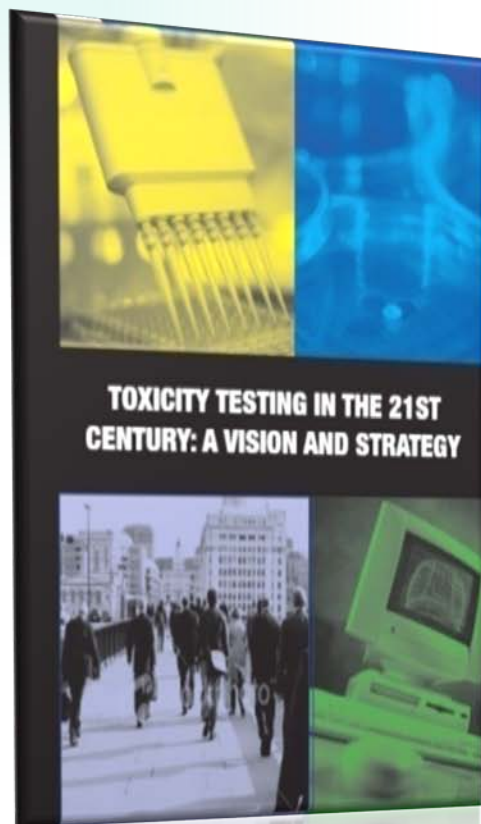
The Dow Chemical Company
Toxicology and Environmental Research &
Consulting

Outline

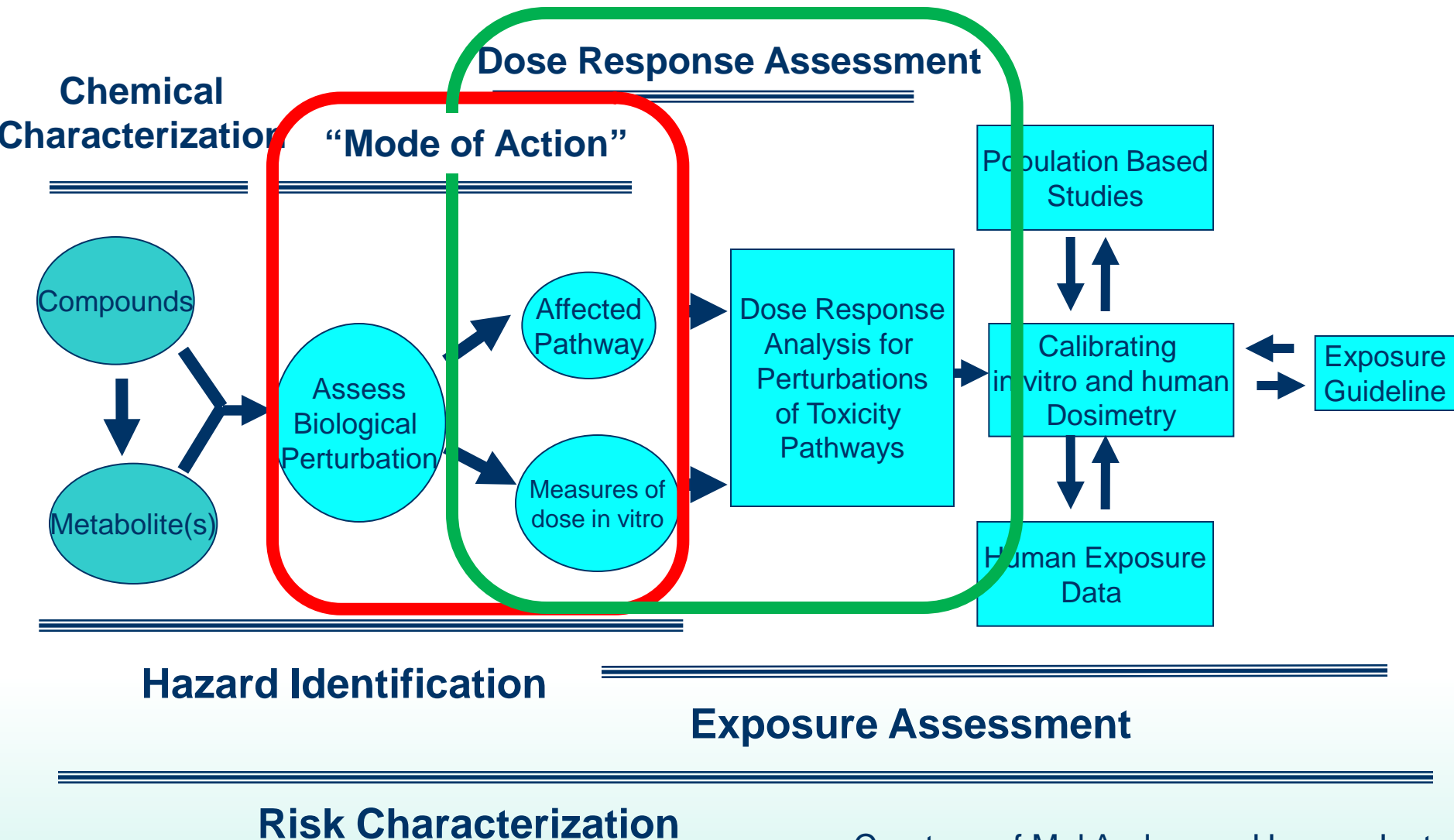
- Paradigm Shift in Risk Assessment
- HESI Risk Assessment in the 21st Century (RISK21)
- Quantitative Key Events Dose-Response Framework (Q-KEDRF)
- Case Studies



Paradigm Shift in Risk Assessment



The New 'Risk' Paradigm is Founded on Knowing the Mode of Action



HESI'S RISK21 Program

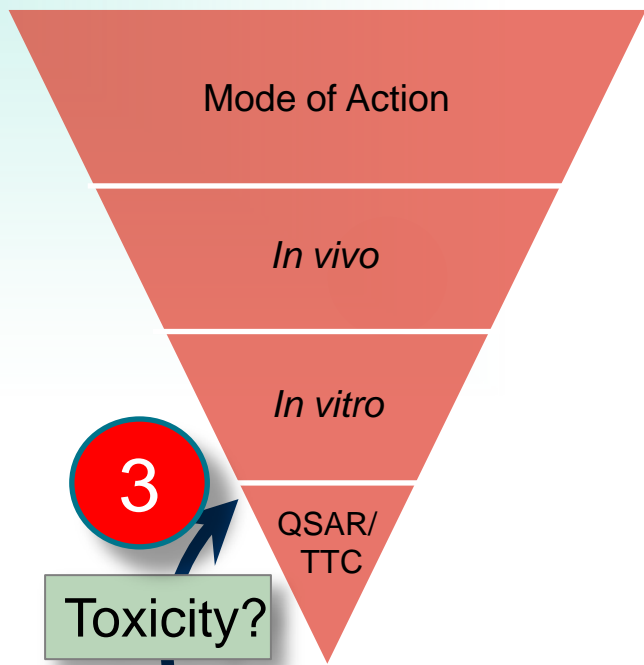


ILSI Health and
Environmental Sciences
Institute

Risk Assessment in the 21st Century (RISK21)

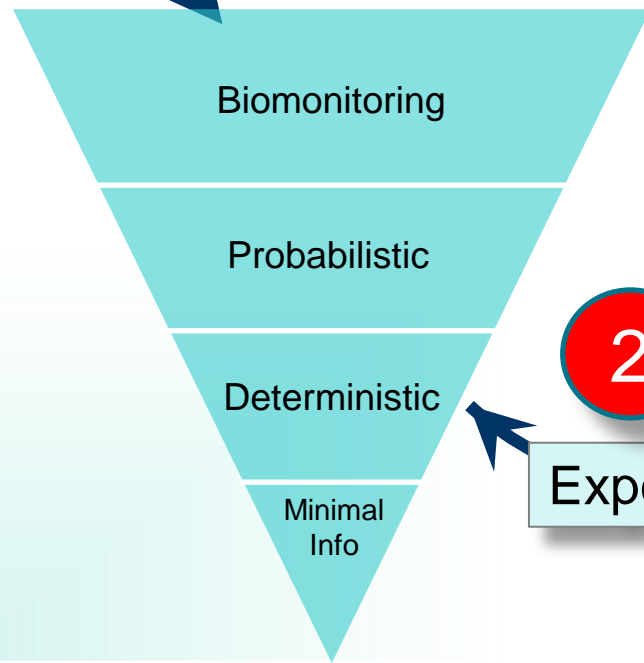
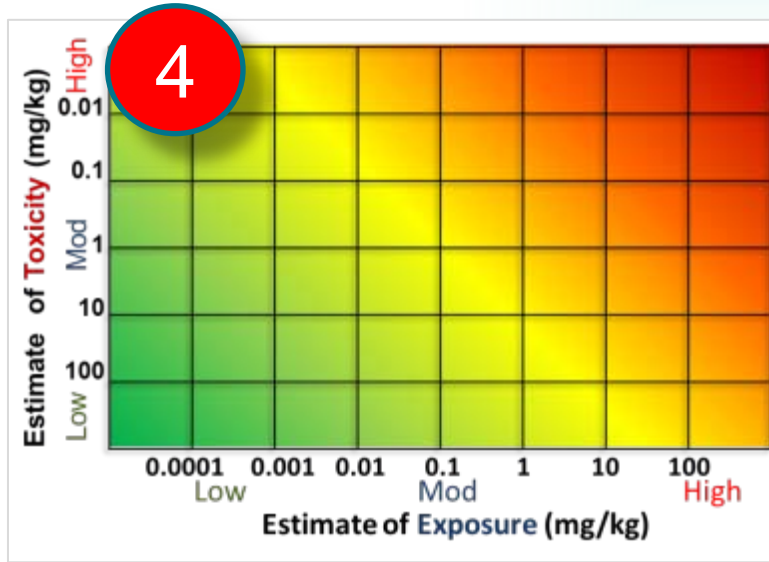
- **MISSION:** Bring applicable, accurate, and resource appropriate approaches to the evolving world of human health risk assessment
 - Convened experts from academia, industry, government and other stakeholders
 - RISK21 involved > 120 scientists from Europe and USA
 - Developed a risk assessment approach that embraces advances in scientific knowledge and methods
 - Revised current thinking about how to approach the science and art of risk assessment





3
Toxicity?

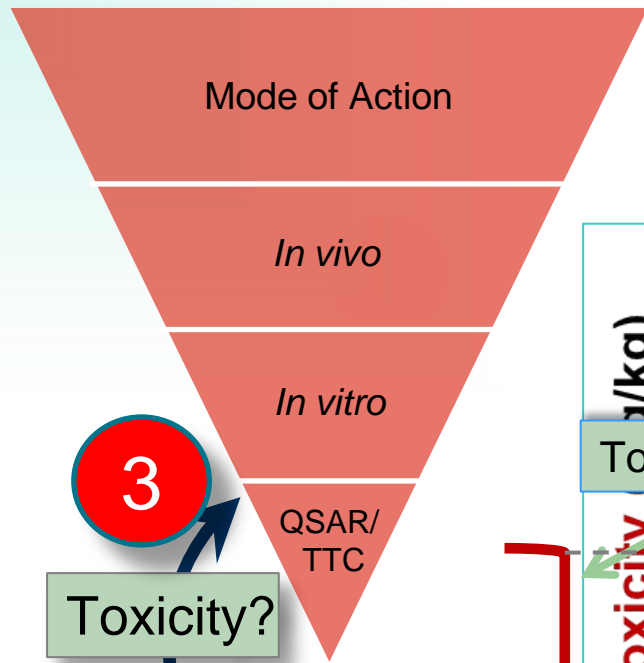
Risk? Safety?



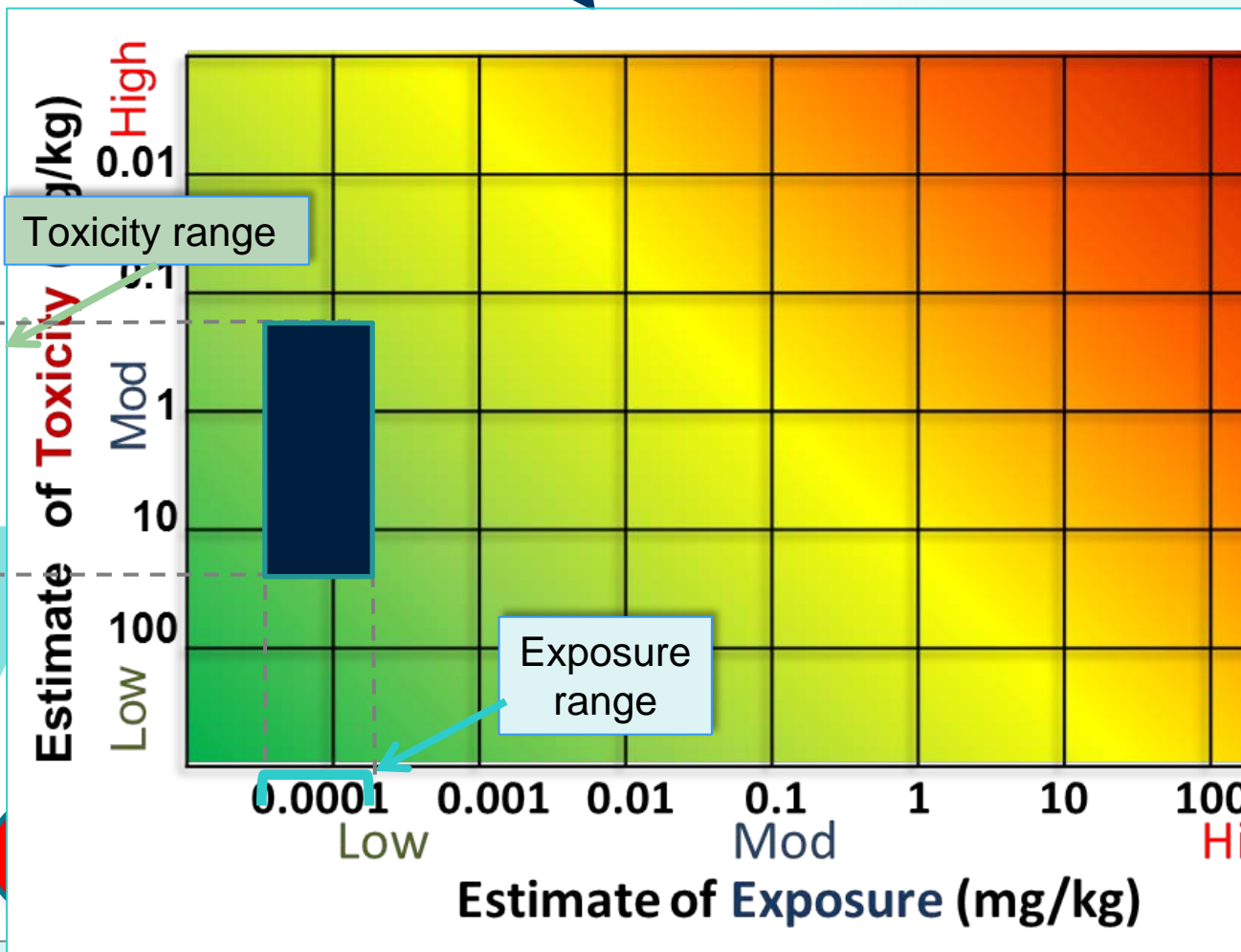
2
Exposure?

1
Problem Formulation

Conclude

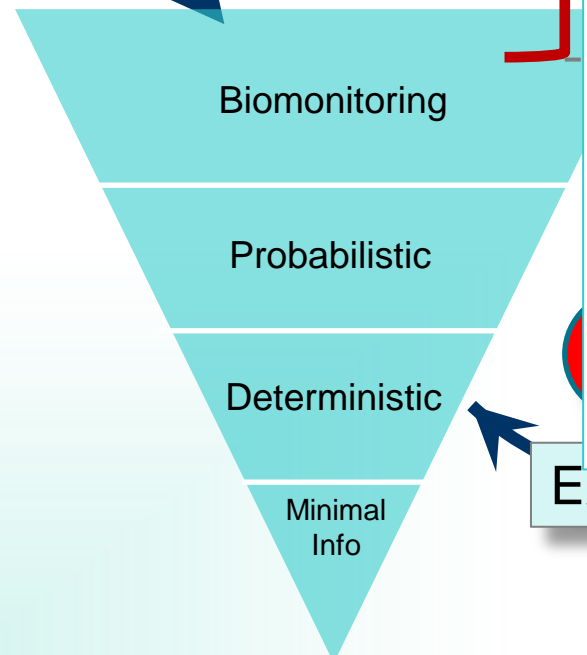


Risk? Safety?



3

Toxicity?



Exposure?

Formulation

Conclude

Problem Formulation: The Starting Point

- **Sets out:**

- Objectives

- Scope

- It

**Enough precision to
make a decision**

- **As**

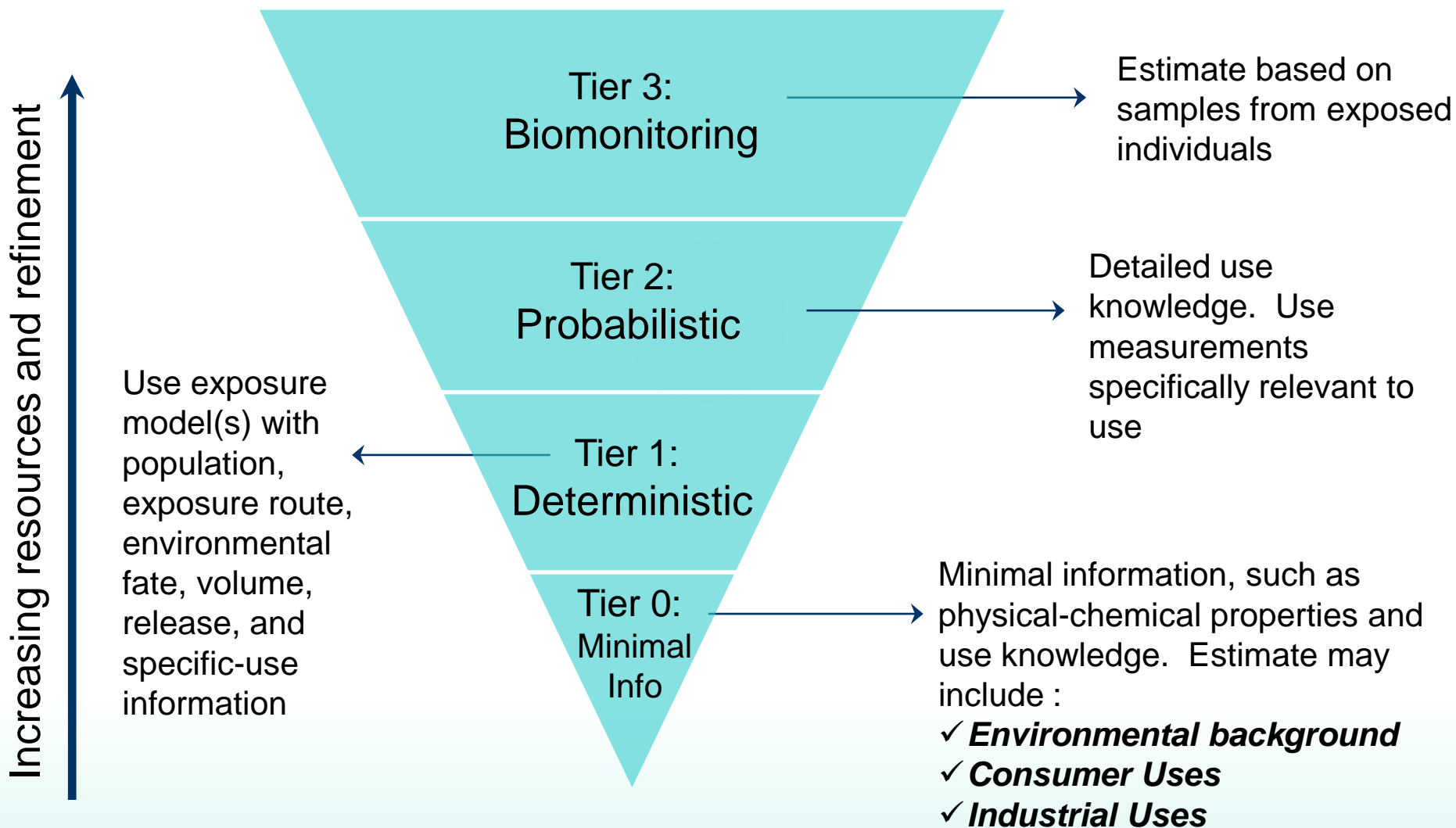
- What do you know?

- what do you need to know?

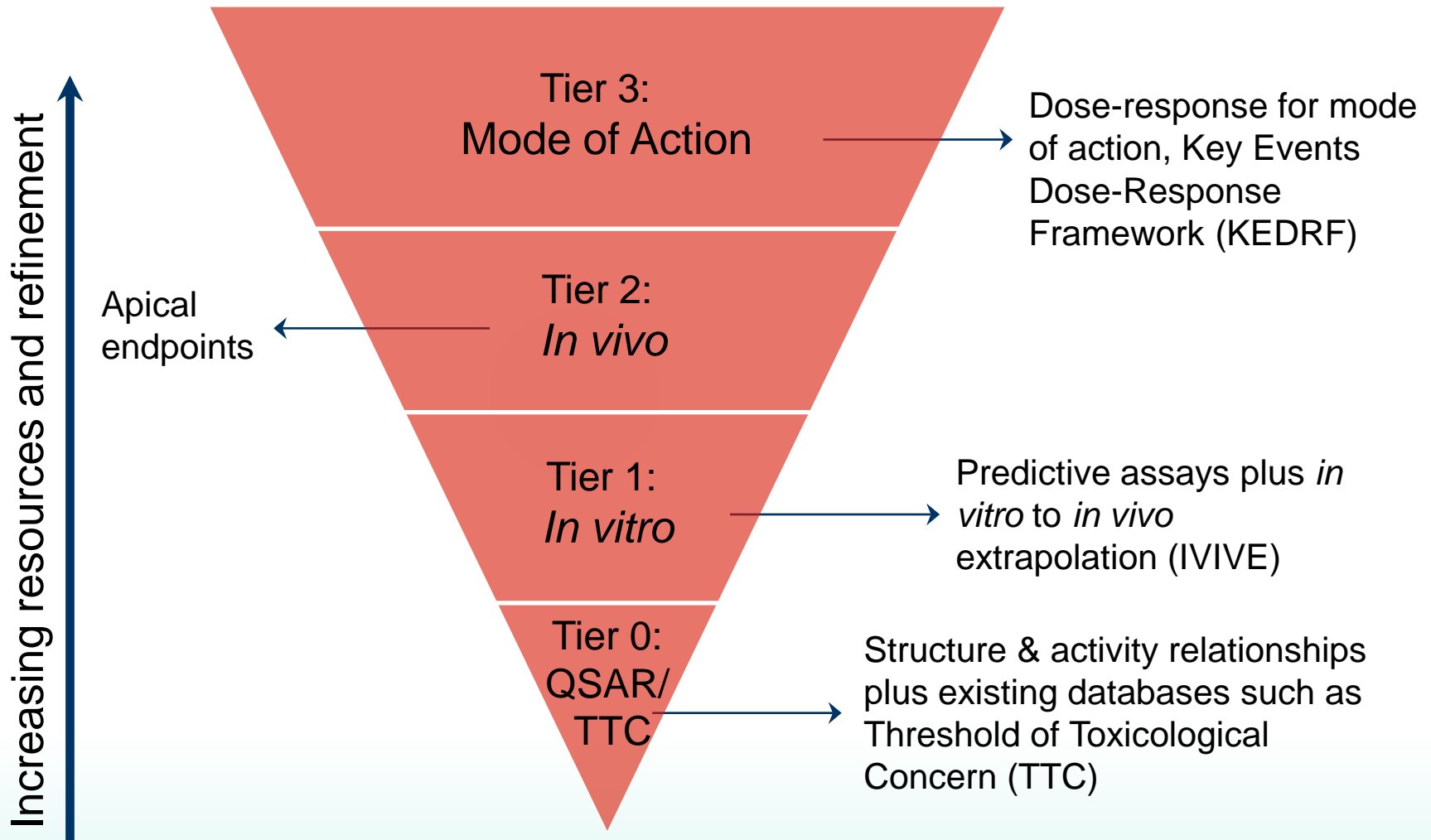
- How do you know when you're done?



Enough Precision for Exposure Estimate



Enough Precision for Toxicity Estimate



WEB-Based Tool: www.risk21.org



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http://informahealthcare.com/txc
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REVIEW ARTICLE

A 21st century roadmap for human health risk assessment

**OPEN ACCESS – links at
www.risk21.org**

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http://informahealthcare.com/txc
ISSN: 1040-8444 (print), 1547-6898 (electronic)
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REVIEW ARTICLE

Risk assessment in the 21st century: Roadmap and matrix

Timothy P. Pastoor¹, John E. Doe², Nancy G. Doerr³, Nina Heard⁴, Ronald N. Hines⁵, Anna B. Lowit⁶, Timothy Pastoor⁴, Richard D. Phillips⁷, Dana Sargent^{8a}, James H. Sherman⁹, Jennifer Young Tanir³ & Michelle R. Embry³

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REVIEW ARTICLE

The use of mode of action information in risk assessment: Quantitative key events/dose-response framework for modeling the dose-response for key events

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Critical Reviews in Toxicology Taylor & Francis
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CRITICAL REVIEWS IN TOXICOLOGY, 2016
VOL. 46, NO. 1, 43-53
http://dx.doi.org/10.3109/10408444.2015.1082973

REVIEW ARTICLE

Illustrative case using the RISK21 roadmap and matrix: prioritization for evaluation of chemicals found in drinking water

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CRITICAL REVIEWS IN TOXICOLOGY, 2016
VOL. 46, NO. 1, 54-73
http://dx.doi.org/10.3109/10408444.2015.1082974

REVIEW ARTICLE

Use of the RISK21 roadmap and matrix: human health risk assessment of the use of a pyrethroid in bed netting

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¹Syngenta Crop Protection, Ontario, Canada, ²Michigan State University, Corning Corporation, Orthopaedic Surgery, of Medicine at UCL, Michigan State University, East Lansing, MI, USA, ³Syngenta Crop Protection, Greensboro, NC, USA

John E. Doe¹, Deborah R. Lander², Nancy G. Doerr³, Nina Heard⁴, Ronald N. Hines⁵, Anna B. Lowit⁶, Timothy Pastoor⁴, Richard D. Phillips⁷, Dana Sargent^{8a}, James H. Sherman⁹, Jennifer Young Tanir³ & Michelle R. Embry³

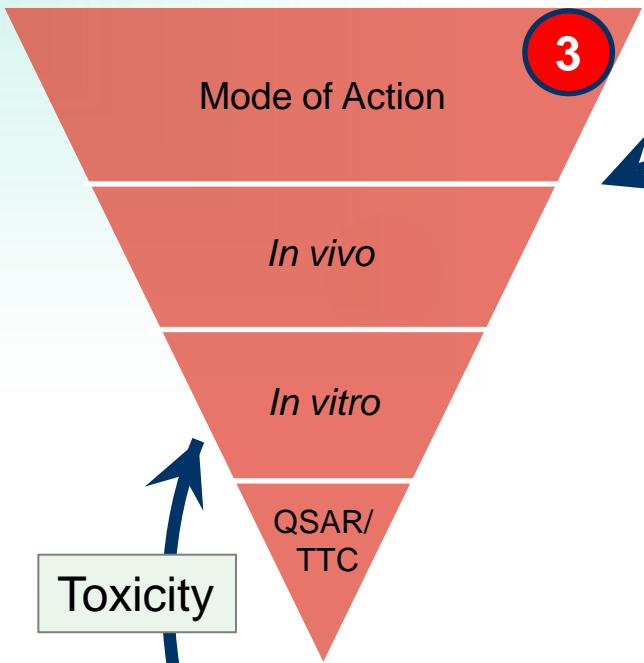
¹Parker Doe Partnership LLP, Frodsham, Cheshire, UK, ²DuPont Haskell Global Centers for Health & Environmental Sciences, Newark, DE, USA, ³ILSI Health and Environmental Sciences Institute, Washington, DC, USA, ⁴Syngenta Crop Protection LLC, Greensboro, NC, USA, ⁵US Environmental Protection Agency, NHEERL, Research Triangle Park, USA, ⁶US Environmental Protection Agency, Office of Pesticide Programs, Washington, DC, USA, ⁷ExxonMobil Biomedical Sciences, Inc., Annandale, NJ, USA, ⁸Arysta LifeScience North America, Cary, NC, USA, and ⁹Monsanto Company, Saint Louis, MO, USA

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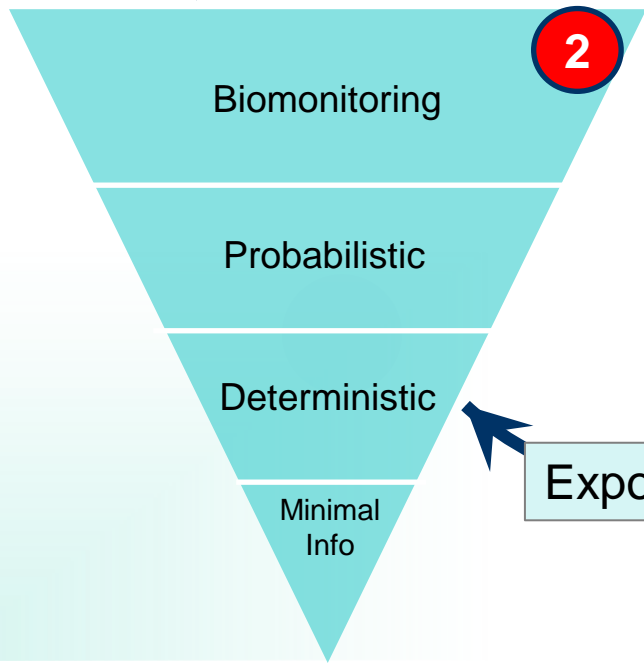
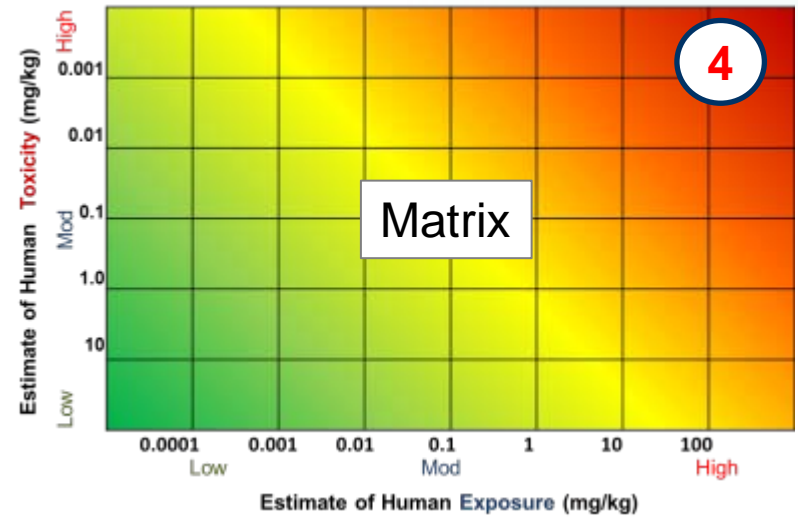
RISK21: Q-KEDRF

Case Studies:

1. Margin of Exposure
2. Dose-Response Model



Risk / Safety

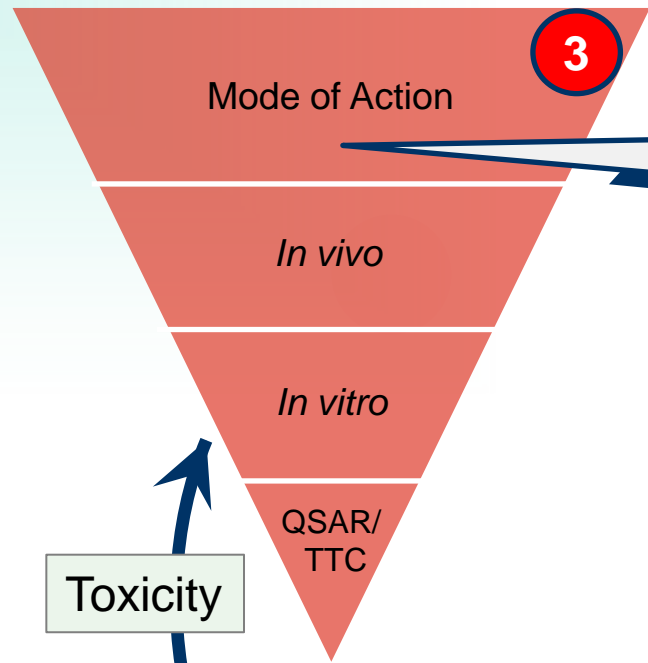


Exposure

1
Problem Formulation

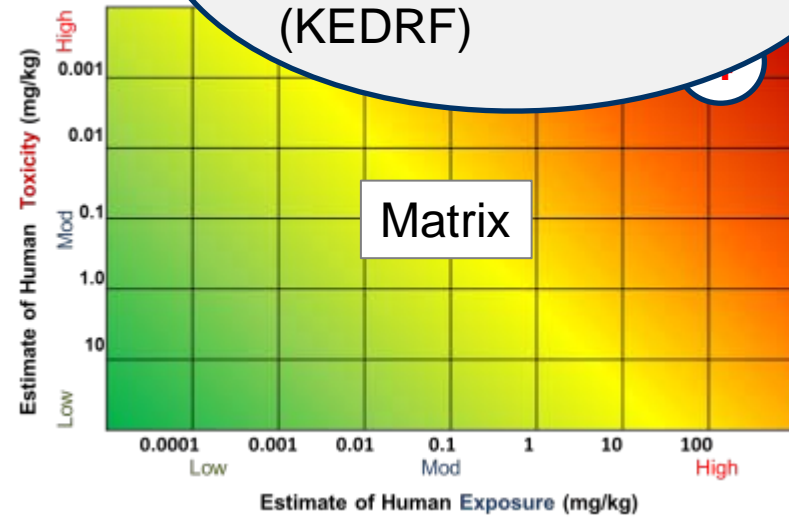
Conclude



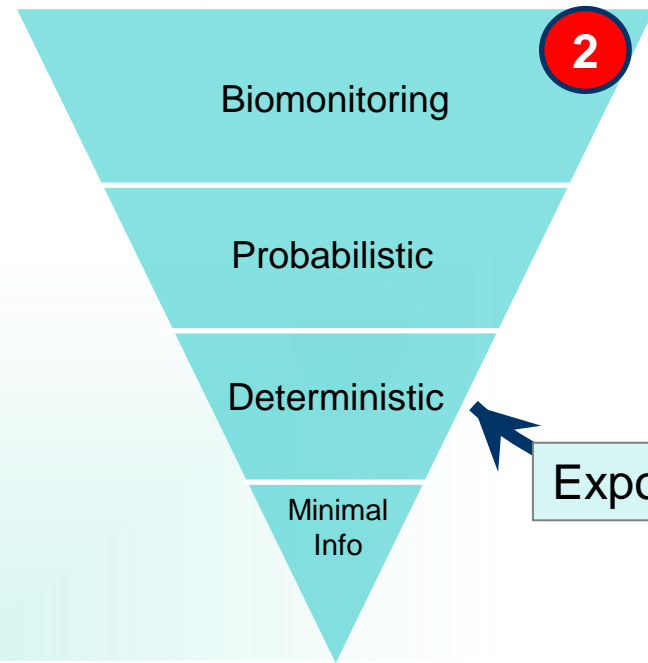


Risk / Safety

Dose-response for mode of action, Key Events Dose Response Framework (KEDRF)



Toxicity



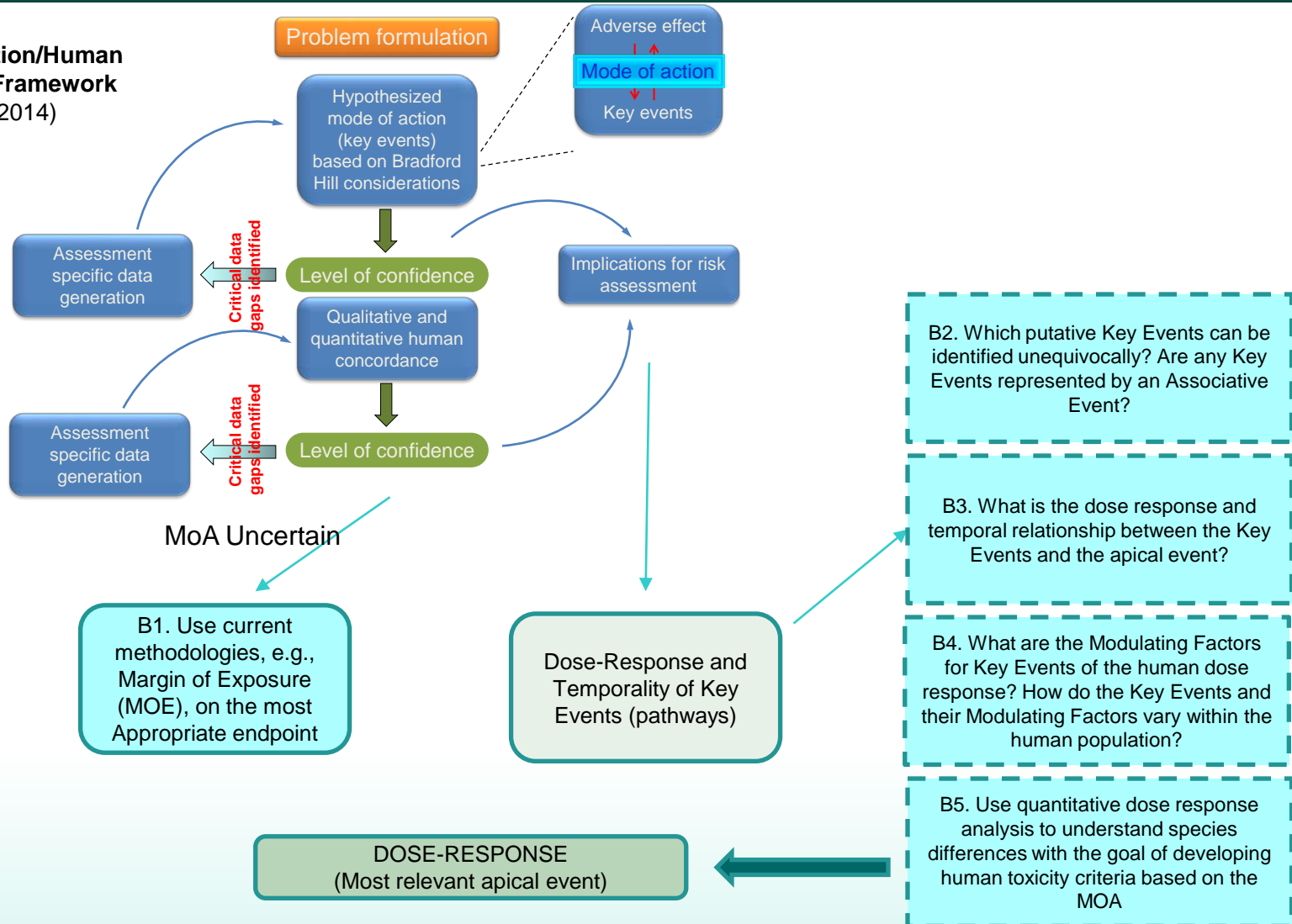
Exposure

1
Problem Formulation

Conclude

Quantitative KEDRF (Q-KEDRF)

Mode of Action/Human Relevance Framework
(Meek et al, 2014)



Risk21 Quantitative Key Events Dose-Response Framework

- A structured approach for systematic examination of KEs constituting a MOA
 - timing of KEs and the
 - quantitative aspects of dose-response
- Incorporate additional concepts for understanding MOA
 - Associative Events (AEs) and
 - Modulating Factors (ModFs).
- The Q-KEDRF provides a means to incorporate all these observations to understand
 - distributions of population sensitivity in the dose-response of the various KEs and,
 - ultimately, the adverse outcome.



Key Events vs. Modulating Factors vs. Associated Events

- **Key Event (KE):** An empirically observable causal precursor step to the adverse outcome that is itself a necessary element of the MOA.
- **Associative Events (AEs):** Biological processes that by themselves are not KEs in the hypothesized MOA but may serve as reliable indicators or biomarkers for KEs.
- **Modulating Factors (ModFs):** 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.



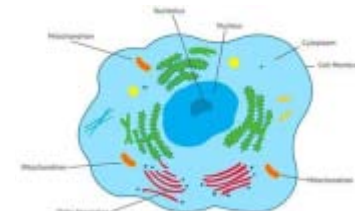
Modulating Factors (Modfs) Potentially Affecting KEs and Dose-response

Humans



- **Host factors**
 - Genetic variation (e.g. GST polymorphism)
 - Disease/ill-health (e.g. chronic kidney disease)
 - Defense mechanisms (e.g. immune responsiveness)
 - Physiology (e.g. life stage)
- **Lifestyle**
 - Diet (e.g. calorie intake)
 - Tobacco
 - Alcohol
 - Exercise
 - Medication (e.g. CYP inhibitors)
 - Illegal drugs
 - Dietary supplements (e.g. anti-oxidants)
- **Environment**
 - Co-exposures (e.g. dust, water contaminants)

Cells



- **Gene Structure**
 - Polymorphisms (e.g., SNPs)
 - Mutations
 - Deletions
 - Duplications
- **Gene Expression**
 - Transcription factors
 - Co-activators/accelerators
 - Co-repressors/decelerators
 - Co-modulators
 - Epigenetic marks
- **Post-translational modifications**
 - Acetylation
 - Methylation
 - Phosphorylation
 - Others

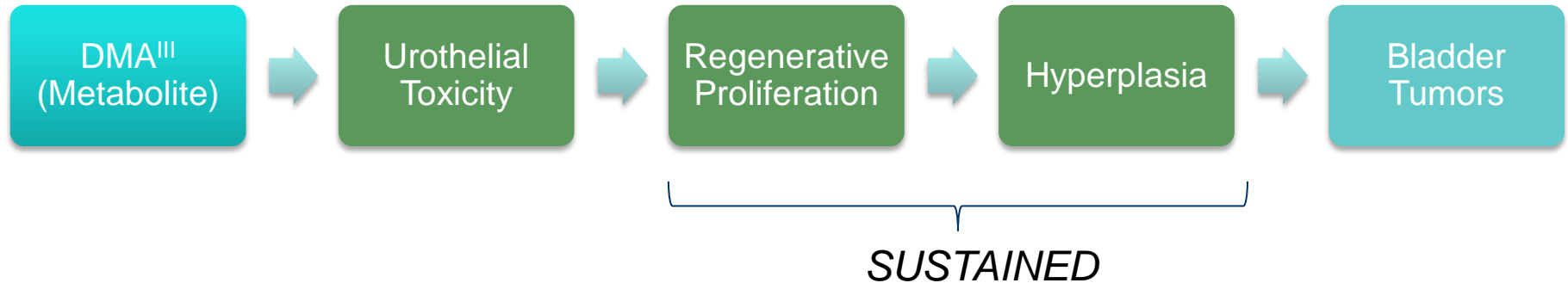
RISK21: Q-KEDRF

Margin of Exposure
Example:

Dimethylarsinic Acid
(DMA)





Example: Dimethylarsinic Acid (DMA)



- Formation of the reactive metabolite trivalent DMA (DMA^{III})
- Cytotoxicity within the superficial epithelial layer of the urinary bladder
- Regenerative proliferation
- Urothelial hyperplasia
- (Bladder tumors)

Dose-time Concordance Table For DMA^{III}

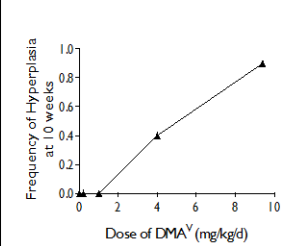
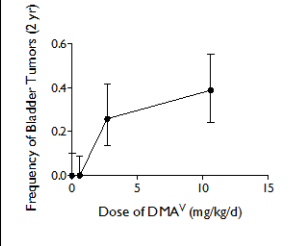
Table —Dose-Time Concordance						
Time	2 weeks	2-3 weeks	10 weeks	25 weeks	104 weeks	
Dose 	Increasing Time 					
	2	Metabolism*	Metabolism*	Metabolism* Cytotoxicity	Metabolism* Cytotoxicity*	Metabolism* Cytotoxicity*
	10	Metabolism*	Metabolism* Cytotoxicity	Metabolism* Cytotoxicity	Metabolism* Cytotoxicity*	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

EVENT OR FACTOR	QUALITATIVE CONCORDANCE			QUANTITATIVE CONCORDANCE AND QUANTITATIVE DOSE-RESPONSE		
	Animals	Humans	Concordance	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

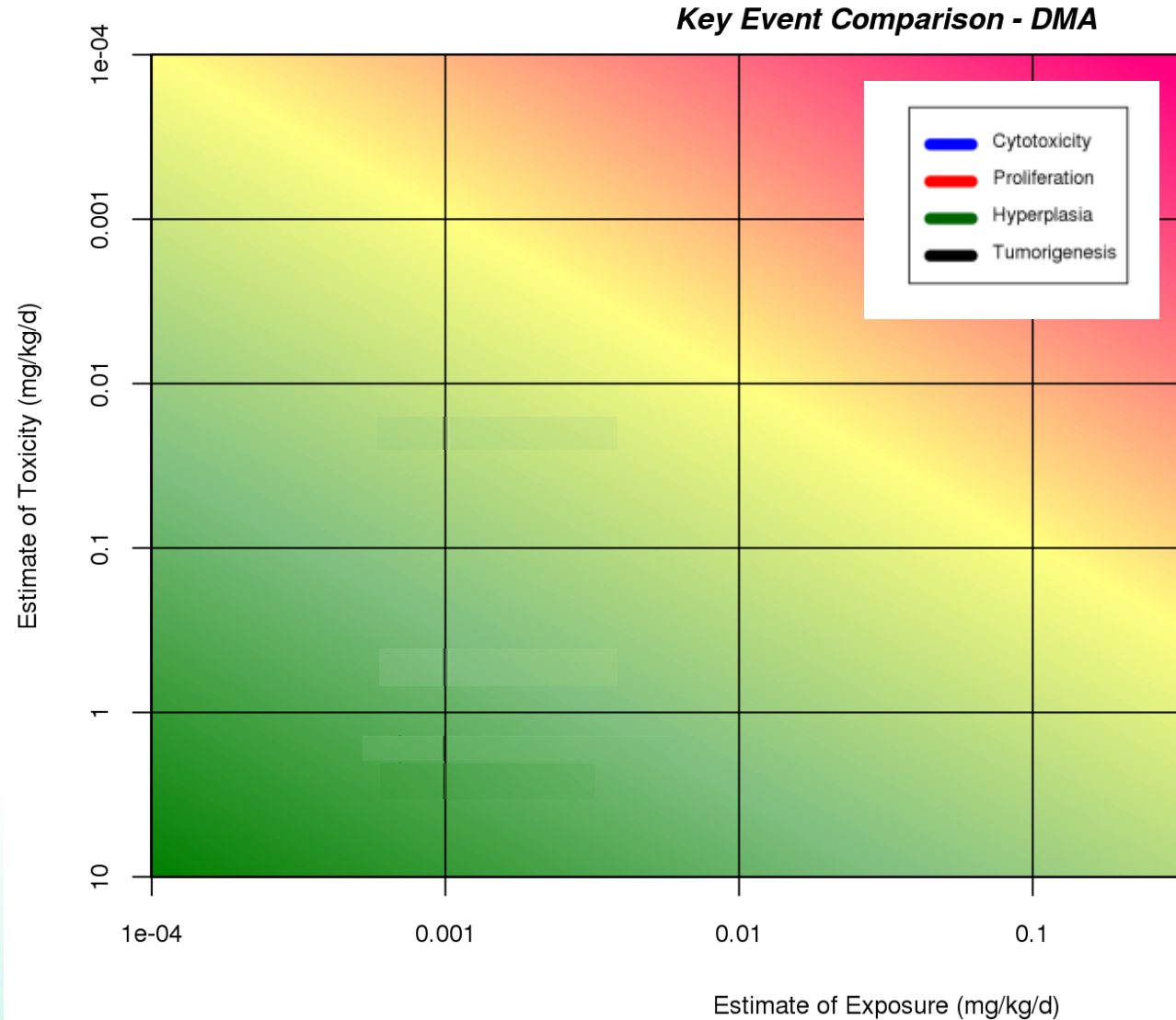
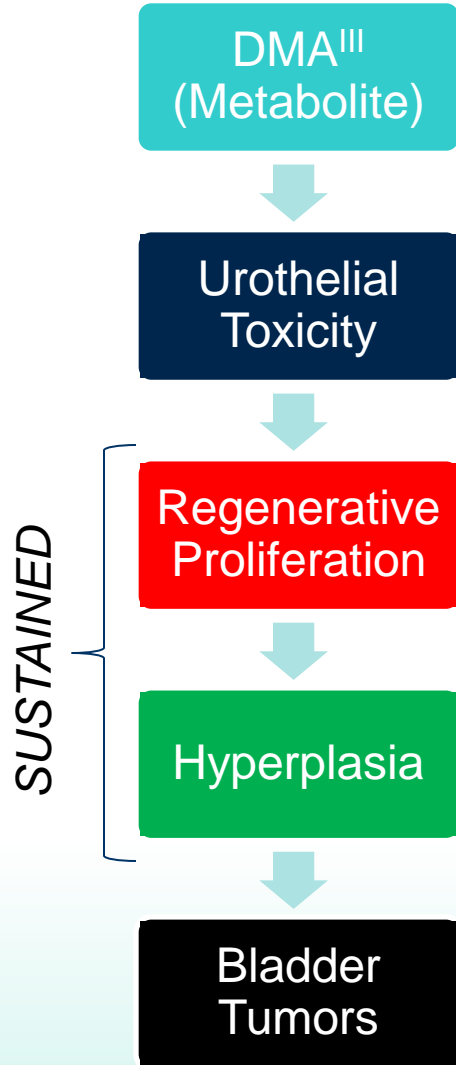
*Str. = strength

Dose-response Species Concordance

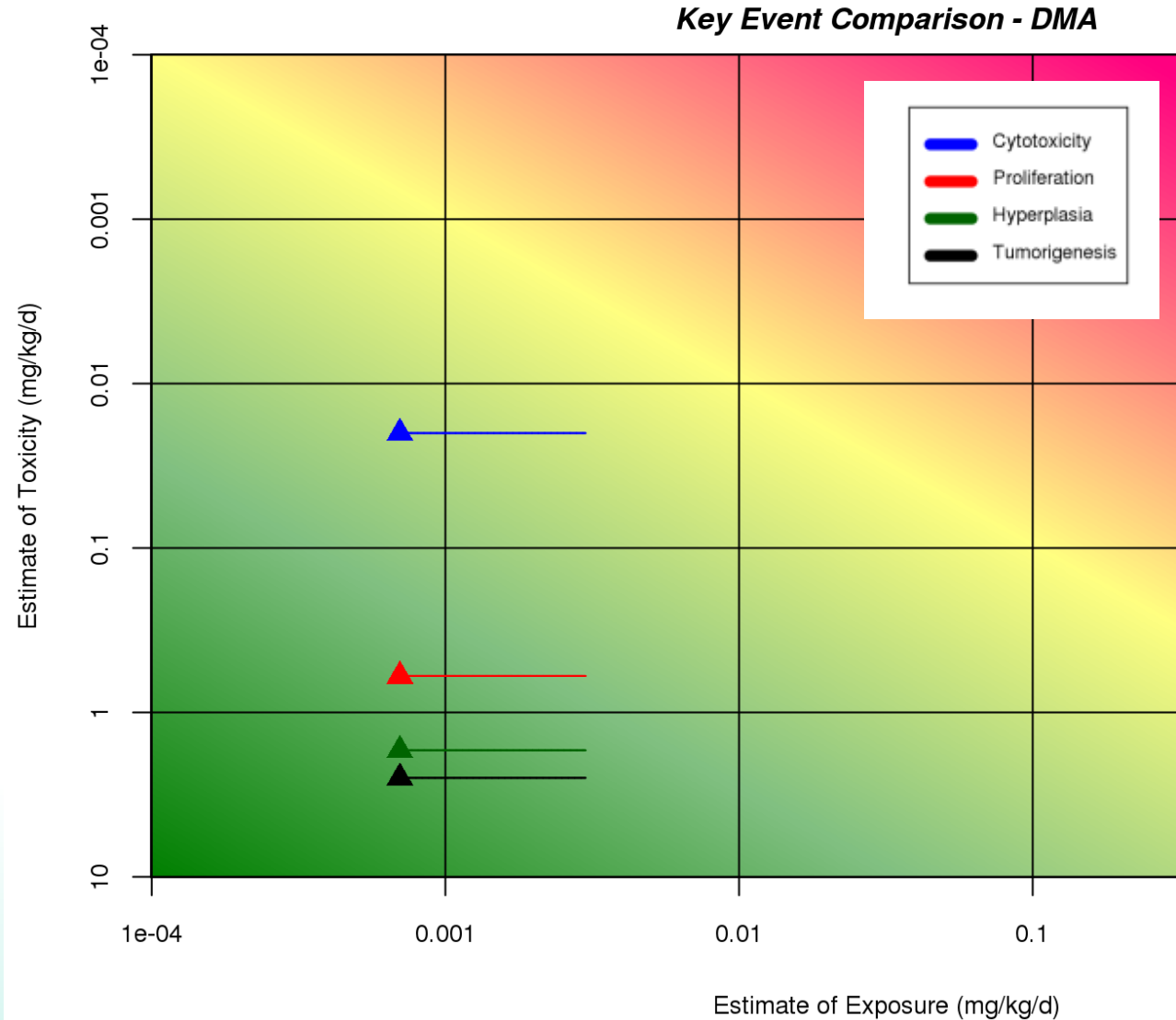
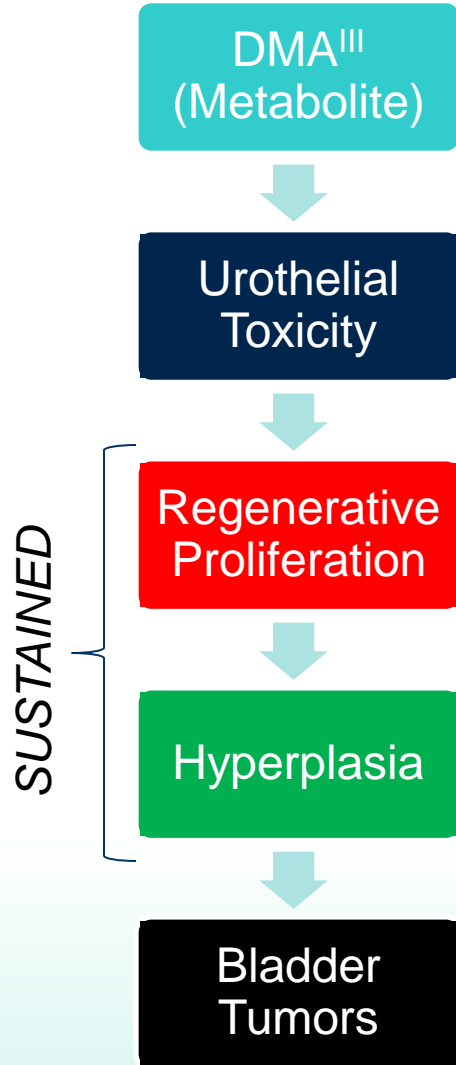
EVENT OR FACTOR	QUALITATIVE CONCORDANCE			QUANTITATIVE CONCORDANCE AND QUANTITATIVE DOSE-RESPONSE		
	Animals	Humans	Concordance	Str.*	Animals	Humans
KEY EVENTS						
Key Event #4 Hyperplasia	observed at 2 mg/kg/d or 0.3 to 2 μ mol DMA ^{III} in urine	Potential to occur in humans but unknown if sufficient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

*Str. = strength

Q-KEDRF and RISK21 Matrix



Q-KEDRF and RISK21 Matrix



Conclusions on MOA Example

- 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



RISK21: Q-KEDRF

Dose-Response Model
Example:

AHR Rat Liver Tumor
Promotion MOA



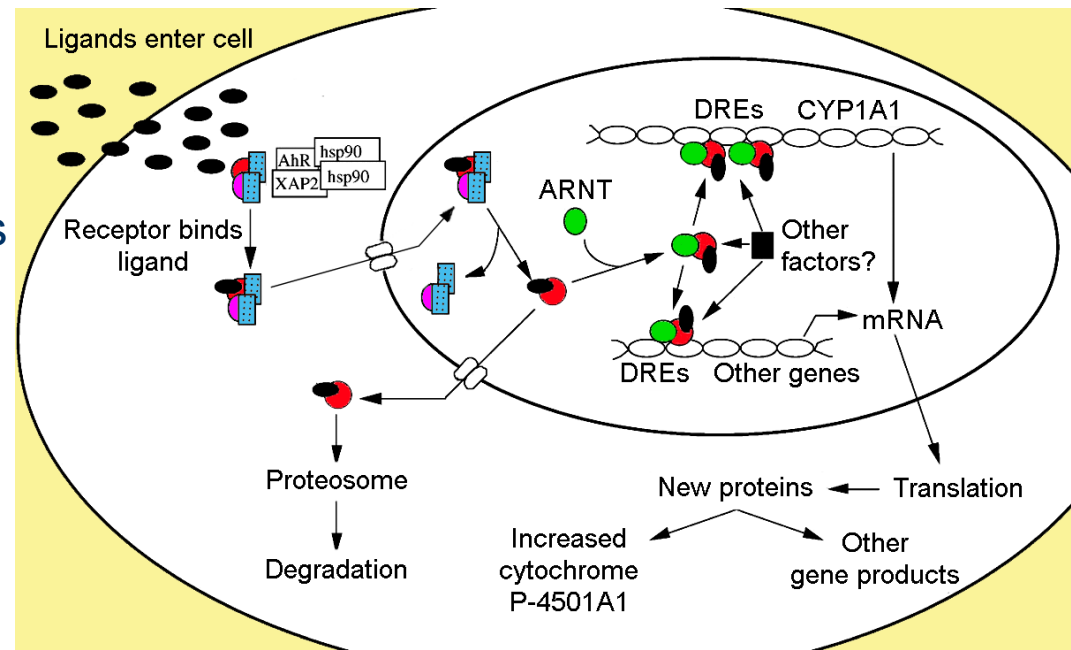
Q-KEDRF Case Study: Aryl Hydrocarbon Receptor (AHR)-Mediated Rodent Liver Tumor Promotion

- AHR mediated rodent liver tumor promotion extensively studied - consensus MOA proposed (Budinsky et al., 2014)
- Highlights concepts impacting dose-response of
 - **KEs**: AHR activation, a ligand-activated nuclear receptor and transcription factor.
 - **ModFs**: Cellular and physiological factors can modulate the dose-response for AHR activation, subsequent KEs
 - **AEs**: AHR-induced gene expression and enzyme activity as surrogates for AHR activation
- Informs the dose-response model for the tumor response

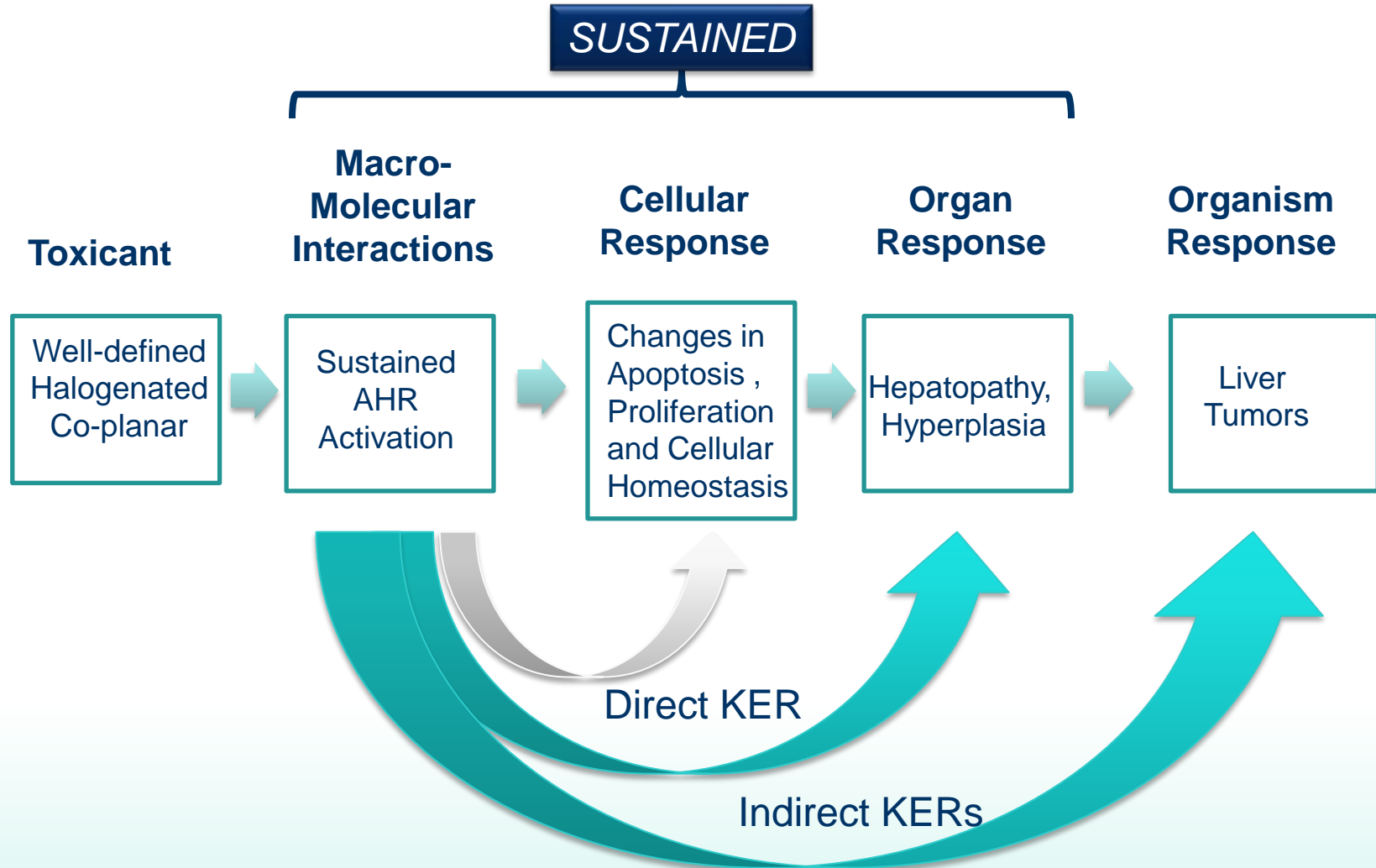


The Aryl Hydrocarbon Receptor (AHR)

- The AHR is a ligand-activated transcription factor in 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 plant flavionoids, polyphenolics, and indoles
 - ▣ Multiple endogenous ligands proposed, but not known
- Regulates a diverse array of genes
 - ▣ Phase I&II metabolic enzymes (e.g., Cyp1a1, Cyp1a2, Ugt1a2, Gsta1)
 - ▣ Others (e.g., Tiparp, p27Kip1, Bach2)





AHR Rat Liver Tumor Promotion MOA



AHR Rat Liver Tumor Promotion MOA: Dose-Temporality Concordance

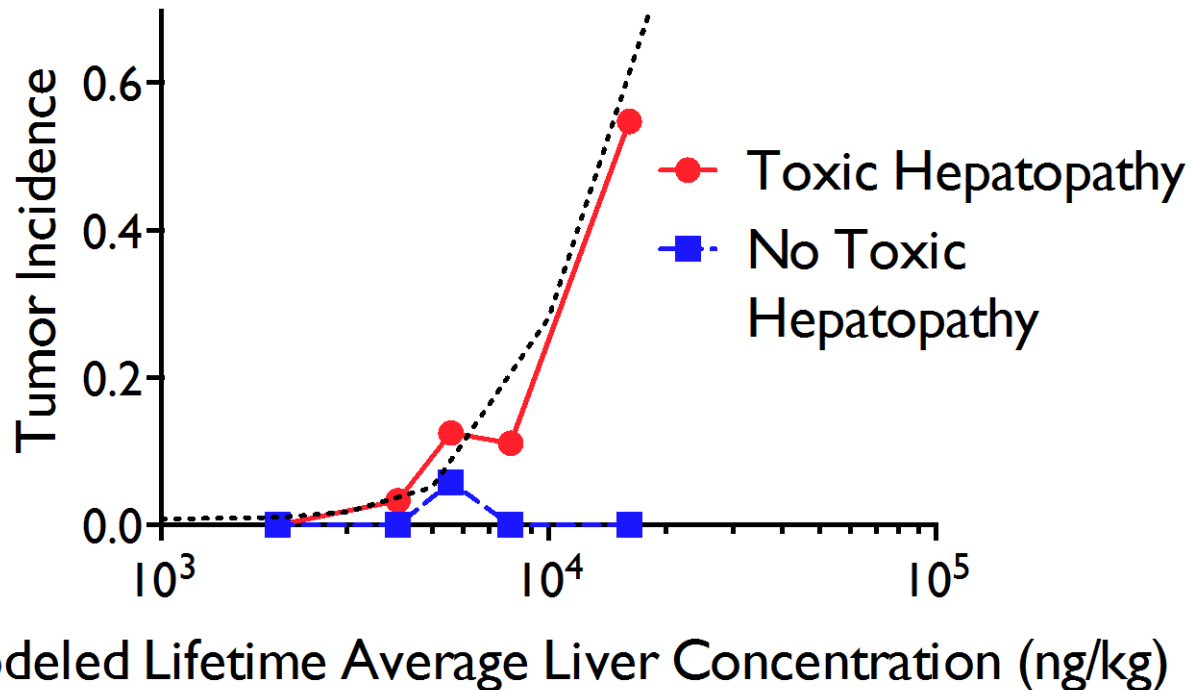
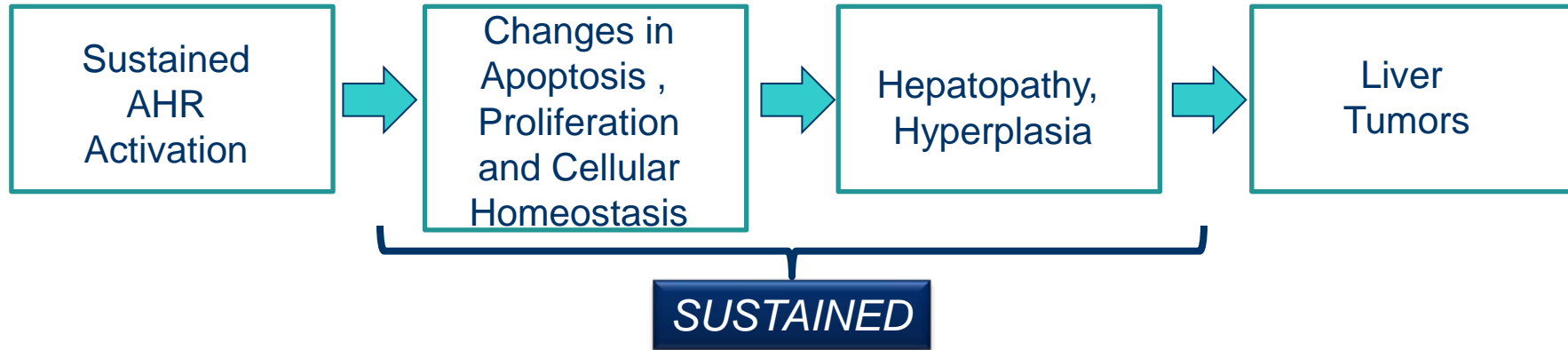
Table 3—Dose-Time Concordance—Page 1

Increasing Dose as average liver conc. in ng/kg	Increasing Time 				
	Immediate	Days to weeks	Months	1 year	2 years
<100	IME	<ul style="list-style-type: none"> Apoptosis decrease 			
100 - 1000	IME	<ul style="list-style-type: none"> Apoptosis decrease 	<ul style="list-style-type: none"> Apoptosis decrease Proliferation / AHF volume increase Proliferation / BrdU labeling 		
1000 - 2000	IME	<ul style="list-style-type: none"> Apoptosis decrease 	<ul style="list-style-type: none"> Apoptosis decrease Proliferation / AHF volume increase Proliferation / BrdU labeling Bile duct hyperplasia 		
2000 - 5000	IME	<ul style="list-style-type: none"> Apoptosis decrease 	<ul style="list-style-type: none"> Apoptosis decrease Increased proliferation (BrdU labeling) Bile duct hyperplasia 	<ul style="list-style-type: none"> Bile duct hyperplasia 	<ul style="list-style-type: none"> cholangiocarcinomas
5000 - 10000	IME		<ul style="list-style-type: none"> Apoptosis decrease Proliferation / AHF volume increase Proliferation / BrdU labeling Bile duct hyperplasia Multinucleated hepatocytes 	<ul style="list-style-type: none"> Proliferation / BrdU labeling Bile duct hyperplasia Multinucleated hepatocytes Diffuse fatty change 	<ul style="list-style-type: none"> Hepatic adenomas, cholangiomas, chloangiocarcinomas
> 10000	IME	<ul style="list-style-type: none"> Apoptosis decrease 	<ul style="list-style-type: none"> Apoptosis decrease Proliferation / AHF volume increase Proliferation / BrdU labeling Bile duct hyperplasia Oval cell hyperplasia Multinucleated hepatocyte Diffuse fatty change 	<ul style="list-style-type: none"> Proliferation / BrdU labeling Bile duct hyperplasia Multinucleated hepatocytes Diffuse fatty change 	<ul style="list-style-type: none"> Hepatic adenomas, cholangiomas, chloangiocarcinomas



(Simon et al. 2014, Crit Rev Toxicol 44 Supp 3:17)

KEs are Necessary but not Sufficient Alone: Ex. Hepatotoxicity and Tumor Formation



Q-KEDRF Informs Apical Dose-Response Model

Macro-Molecular Interactions

Sustained
AHR
Activation



Cellular Response

Changes in
Apoptosis,
Proliferation
and Cellular
Homeostasis



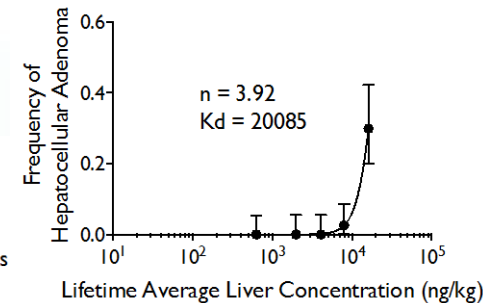
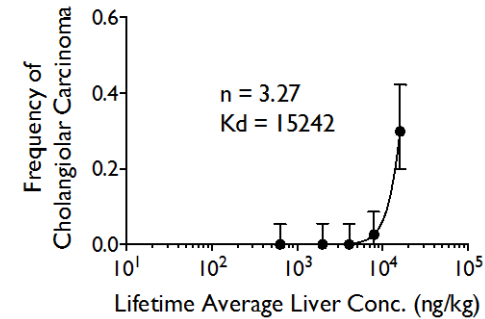
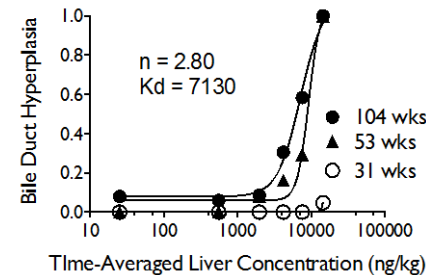
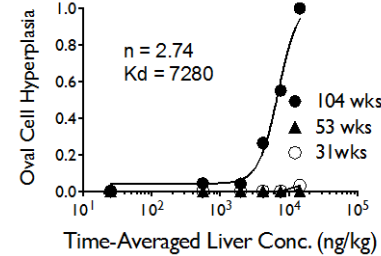
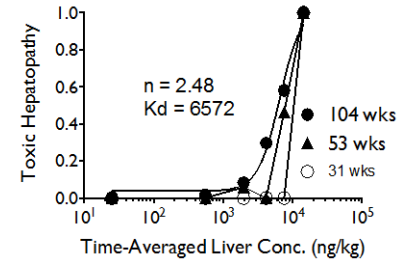
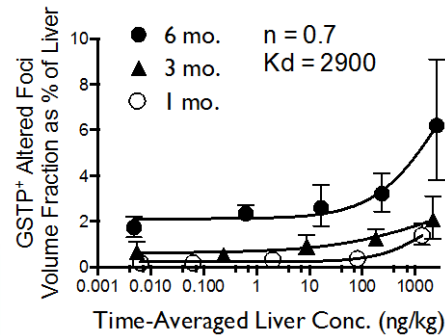
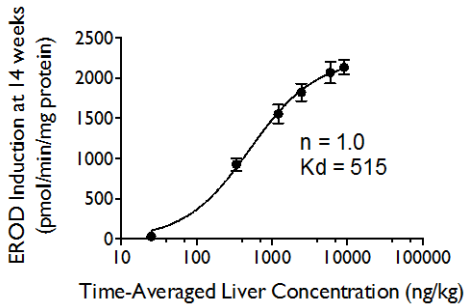
Organ Response

Hepatopathy,
Hyperplasia



Organism Response

Liver
Tumors



Lessons Learned: “Associative Events” and “Modulating Factors”

- Modulating Factors (ModFs) and Associative Events (AEs) are useful in describing the nuances of a particular MOA.
 - A ModF is a factor that can alter the nature or occurrence of one or more KEs and thus the AO whereas
 - An AE is a reliable indicator for a KE but not in itself causal or necessary for the AO. (an AE may serve as a marker for a KE)
- AHR Rat Liver Tumor Promotion MOA:
 - Induction of xenobiotic metabolizing enzymes (XME) reflects acute transcriptional and proteomic changes (IMEs) - these measurements can be considered as an AE for AHR activation.
 - These XME gene changes, and or changes in cellular response genes, may also be Modulating Factors in that they act to prevent cellular changes that would otherwise support tumor promotion.



Q-KEDRF Summary

- The MOA/HRF along with the Q-KEDRF provides
 - a strong foundation for using the information gathered as a means of reducing uncertainty in risk assessments.
 - provides additional tools to relate KEs to each other and to the adverse outcome/apical event in a quantitative way in both the dose- and time-dimensions.
- The dose-responses for the KEs can be used to inform the shape of the dose-response for the apical effect of concern
 - The ability to calculate possible threshold or transition dose values from quantitative dose-response modeling provides a means to determine whether linear or nonlinear extrapolation is appropriate



RISK21 and Cumulative Risk Assessment

- The RISK21 approach is feasible and transparent:
 - Problem formulation-based
 - Exposure-driven
 - Iterative
 - Introduces modulating factors stepwise to address non-chemical stressors
 - Provides transparent and visually “simple” documentation of the process at each step
 - Resource efficient



China SOT –October 2015



Taiwan-2015



Brazil-2016

US FDA-May 2015



China- October 2015

HESI'S RISK21 Program

For more information:

Visit www.risk21.org

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