Toxicity Pathways to Assessment Endpoints

P. Schmieder, S. Bradbury, G. Veith, J. McKim

Toxicity Pathway

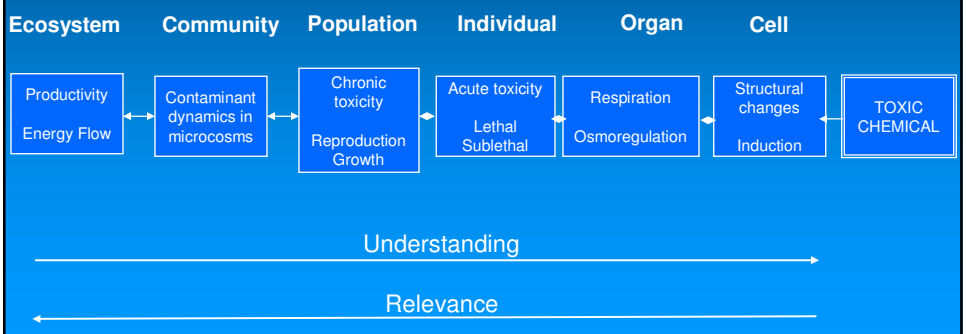
WHAT:

- A concept; a way of depicting a chain of events starting with a molecular initiating event (site of chemical –biological interaction) and ending with an adverse effect manifested in an individual, or higher level – population, community, ecosystem
- May include a biochemical/signaling pathway, but goes beyond, to at least hypothesize how something observed at one level of biological organization is linked to response manifested at another level.

WHY:

- Chemical similarity is defined in the context of biological similarity
  - "Similar" chemicals, by definition, invoke the same toxicity pathway (within a specified biological model)
  - QSARs are developed for "similar" chemicals from a known or hypothesized "mode/mechanism" of action; hypothesis is tested to refine the models
- QSAR requires a well-defined biological system

Effects of toxicants occur at different levels of biological organization. Toxic effects are best known and understood at the cell and organ level, while the ecosystem and community level are least understood although most relevant. (Haux and Forlin, 1988)



Toxicity Pathway Uses

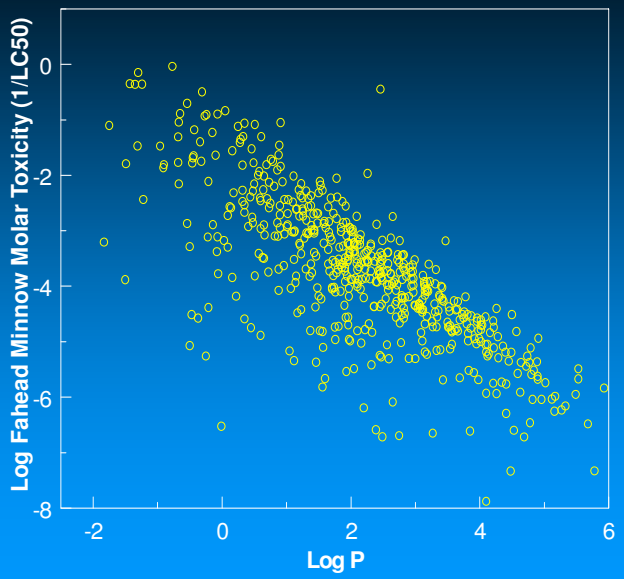
- Assess knowledge gaps - what we know and what we don't know about a chemical's toxicity (toxicodynamics)
- Assess the plausibility that a series of events are linked, i.e., degree of connectedness;
  - degree of specificity/certainty needed depends upon intended use
    - prioritization for further testing – correlation; "good" hypothesis?
    - quantitative RA - confirm cause and effect?
- Pinpoint molecular initiating event for chemical extrapolation
  - QSAR – can be based on *in vivo* endpt if system is simple enough, e.g., fish acute/chronic for narcotic chemicals where applied chem conc is directly related to chemical activity in blood and further to the whole organism effect
  - Measurements closer to molecular initiating event will be more definitive for QSAR but some degree of relevance should be established (Linkage across levels of biological organization)
- Basis for species extrapolation
- Shifting RA paradigm - predict most likely tox pathways for a chemical to pinpoint most appropriate testing

Well-Defined Biological System

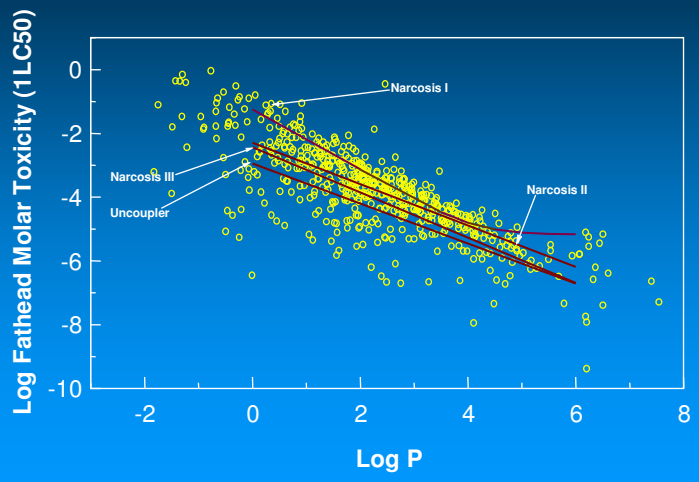
(Know what you know and what you don't know)

- Metabolism
  - Is the system used for collection of empirical data capable of xenobiotic metabolism?
  - Is what you're measuring due to parent chemical or a metabolite?
- Kinetics
  - What do you understand about the chemical kinetics within the system?
  - Is the chemical in solution
    - Bound and unavailable
    - Loss to hydrolysis

Measure chemical form and concentration in your system



Fathead Minnow Acute Toxicity Database

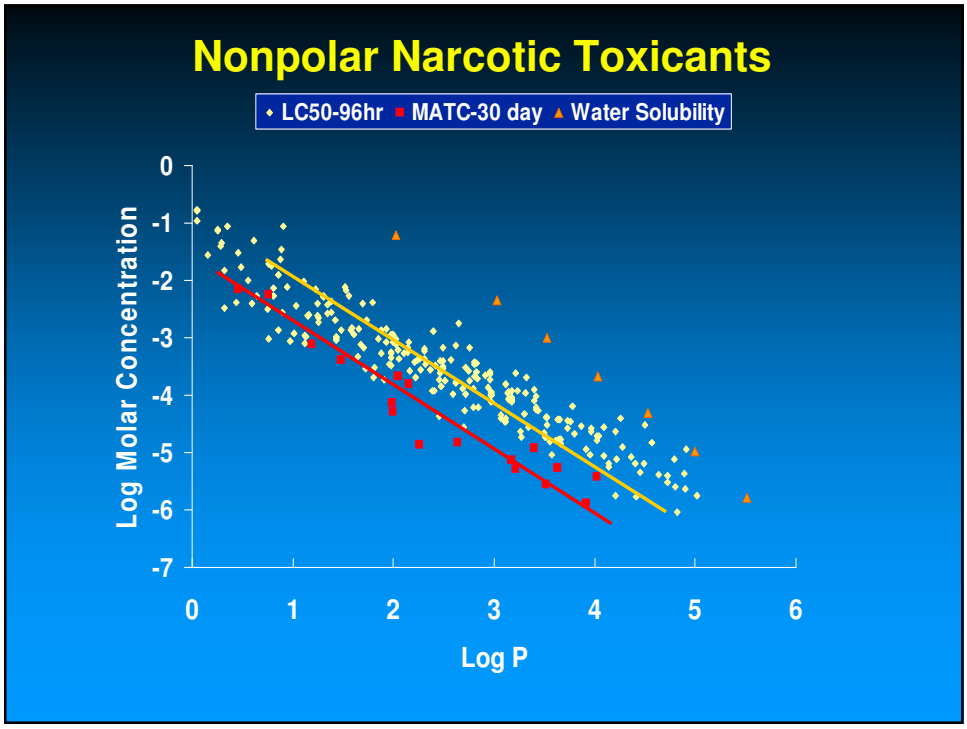


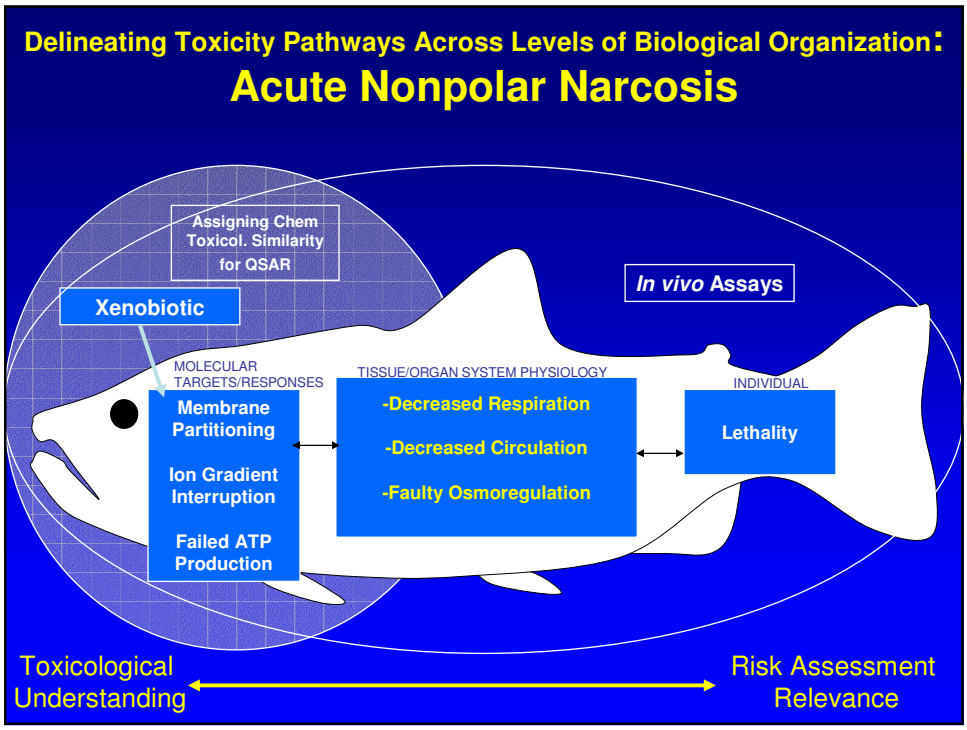
Sorting Modes of Action (Toxicity Pathways)

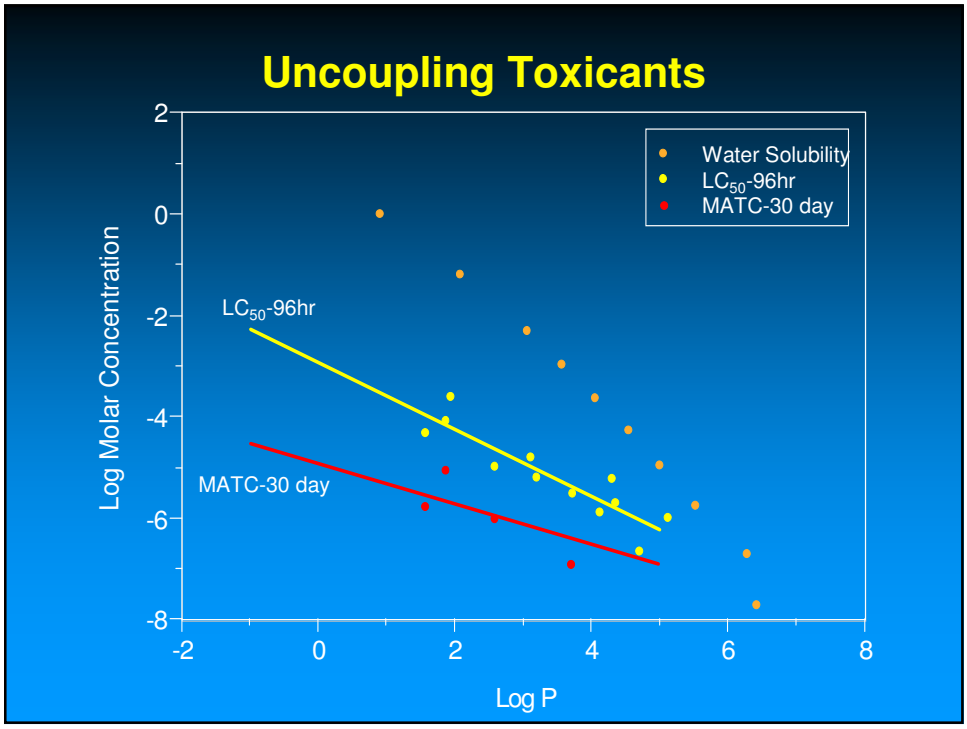
Fish Acute Toxicity Syndromes
- respiratory/cardiovascular responses (RBT)

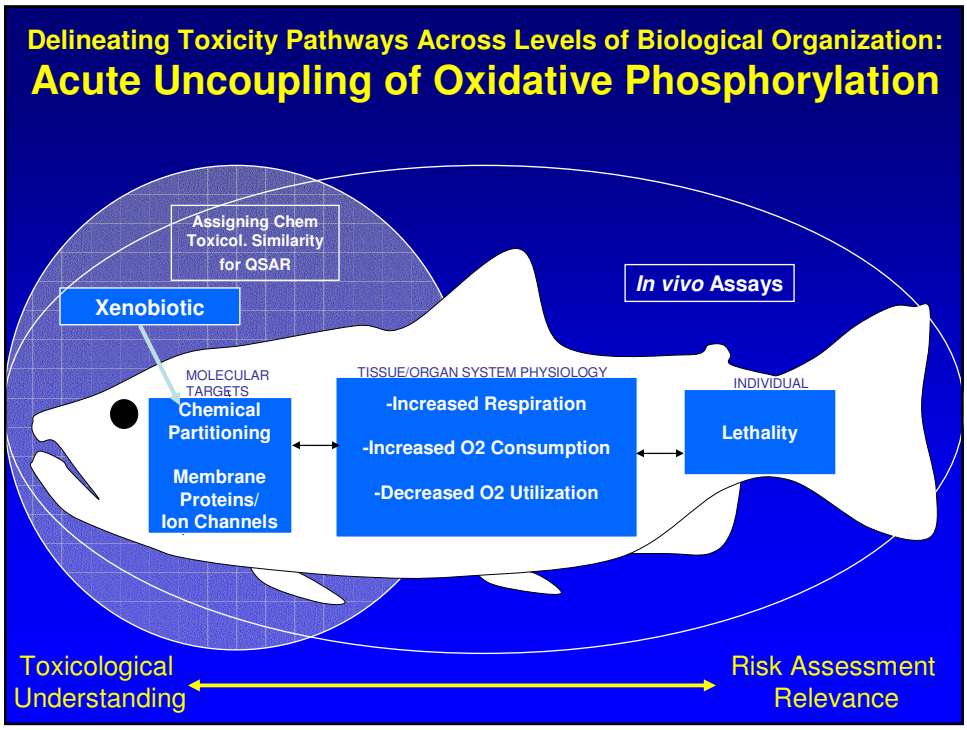
Behavioral observations (FHM)

Mixture studies (FHM)

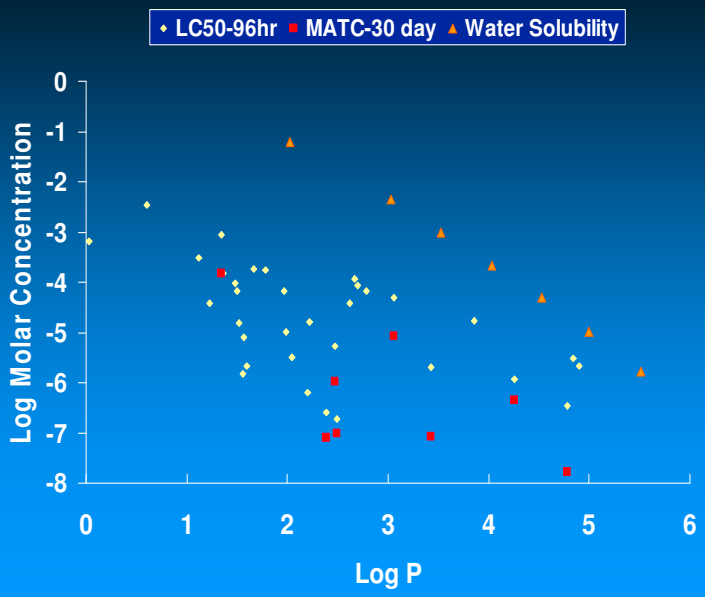

Nonpolar Narcotic Toxicants
LC50-96hr
MATC-30 day
Water Solubility
Log Molar Concentration
0
-1
-2
-3
-4
-5
-6
-7
0
1
2
3
4
5
6
Log P


Delineating Toxicity Pathways Across Levels of Biological Organization:
Acute Nonpolar Narcosis
Assigning Chem Toxicol. Similarity for QSAR
Xenobiotic
In vivo Assays
MOLECULAR TARGETS/RESPONSES
Membrane Partitioning
Ion Gradient Interruption
Failed ATP Production
TISSUE/ORGAN SYSTEM PHYSIOLOGY
-Decreased Respiration
-Decreased Circulation
-Faulty Osmoregulation
INDIVIDUAL
Lethality
Toxicological Understanding
Risk Assessment Relevance


Uncoupling Toxicants
Water Solubility
LC50-96hr
MATC-30 day
LC50-96hr
MATC-30 day
Log Molar Concentration
Log P
2
0
-2
-4
-6
-8
-2
0
2
4
6
8


Delineating Toxicity Pathways Across Levels of Biological Organization:
Acute Uncoupling of Oxidative Phosphorylation
Assigning Chem Toxicol. Similarity for QSAR
Xenobiotic
In vivo Assays
MOLECULAR TARGETS
Chemical Partitioning
Membrane Proteins/ Ion Channels
TISSUE/ORGAN SYSTEM PHYSIOLOGY
-Increased Respiration
-Increased O2 Consumption
-Decreased O2 Utilization
INDIVIDUAL
Lethality
Toxicological Understanding
Risk Assessment Relevance

Reactive Toxicants



Sorting Modes of Action (Toxicity Pathways)

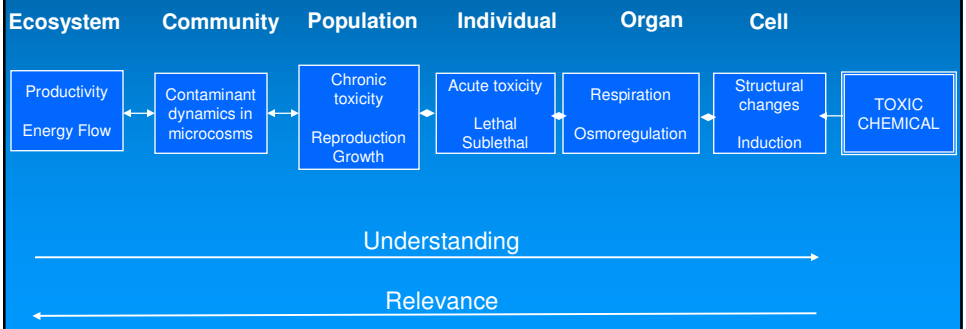
Fish Acute Toxicity Syndromes
- respiratory/cardiovascular responses (RBT)

Behavioral observations (FHM)

Mixture studies (FHM)

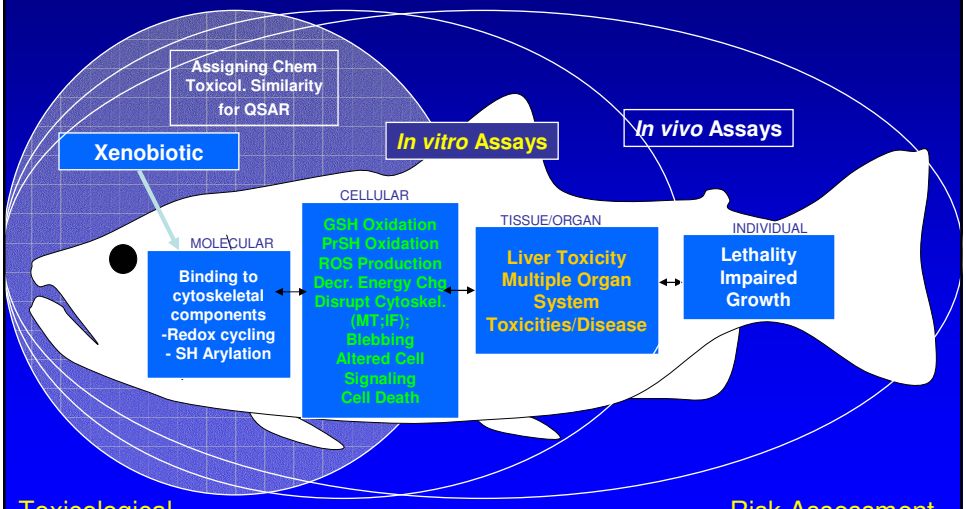
Biochemical responses – in vitro

Effects of toxicants occur at different levels of biological organization. Toxic effects are best known and understood at the cell and organ level, while the ecosystem and community level are least understood although most relevant. (Haux and Forlin, 1988)

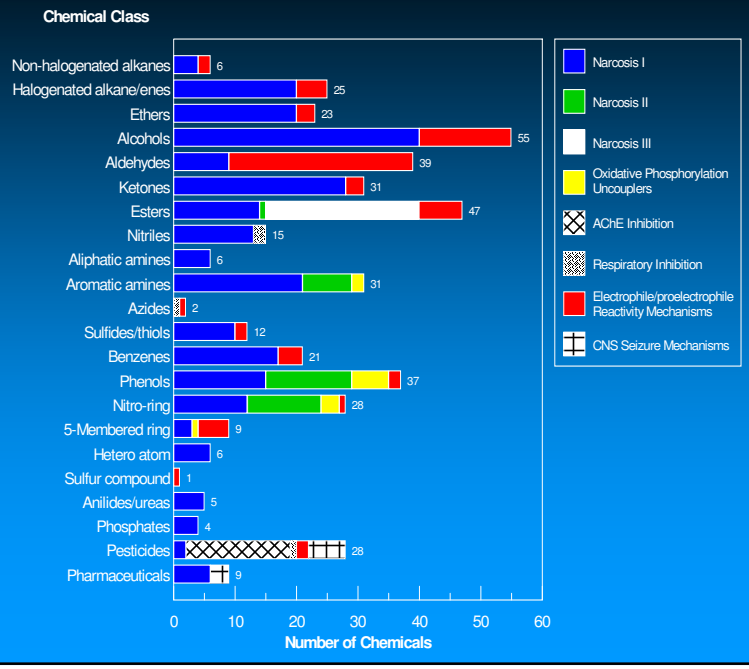


Defining Toxicity Pathways Across Levels of Biological Organization:

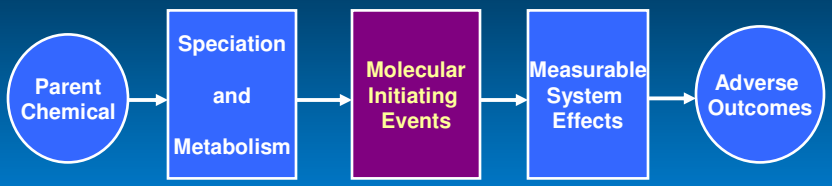
Redox cycling_Arylation



Chemical Class is not MOA for Industrial Chemical Acute Tox

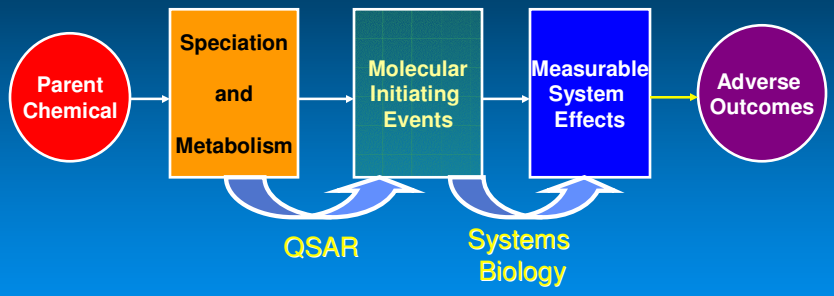


Knoxville Workshop Framework for Predicting Reactive Toxicity



Rather than developing statistical models of complex endpoints, molecular initiating events are identified as well-defined QSAR endpoints…..and used to estimate the probabilities for important downstream biological effects based on transparent assumptions

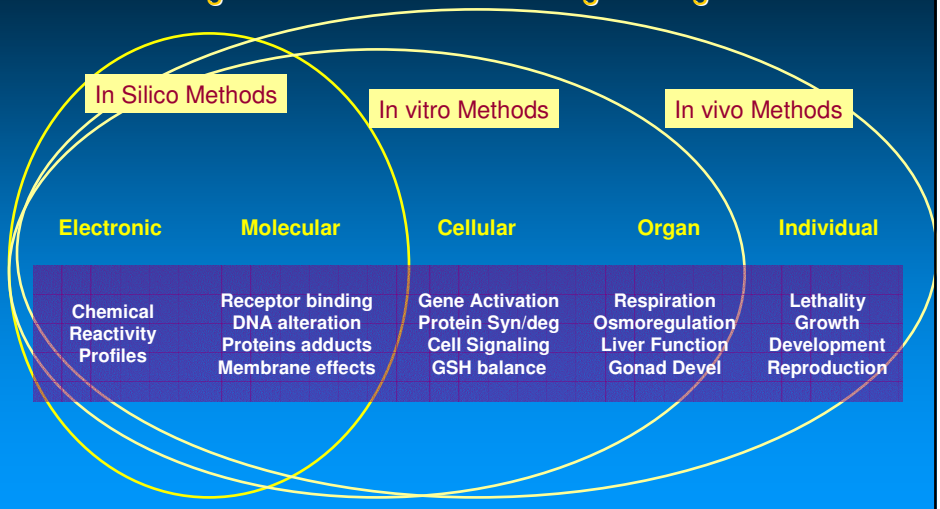
Steps to the Development of QSAR for Reactive Toxicants



1. Establish Plausible Molecular Initiating Events
2. Design Database for Abiotic Binding Affinity/Rates
3. Explore Correlations/Pathways to Downstream Effects
4. Explore QSARs to Predict Initiating Event from Structure

Delineation of Toxicity Pathways

Linkages Across Levels of Biological Organization

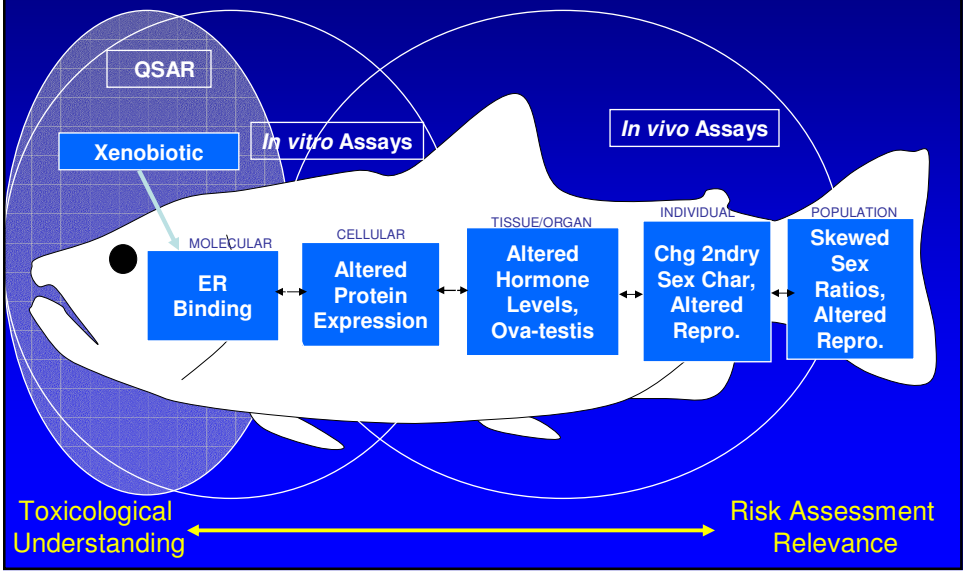


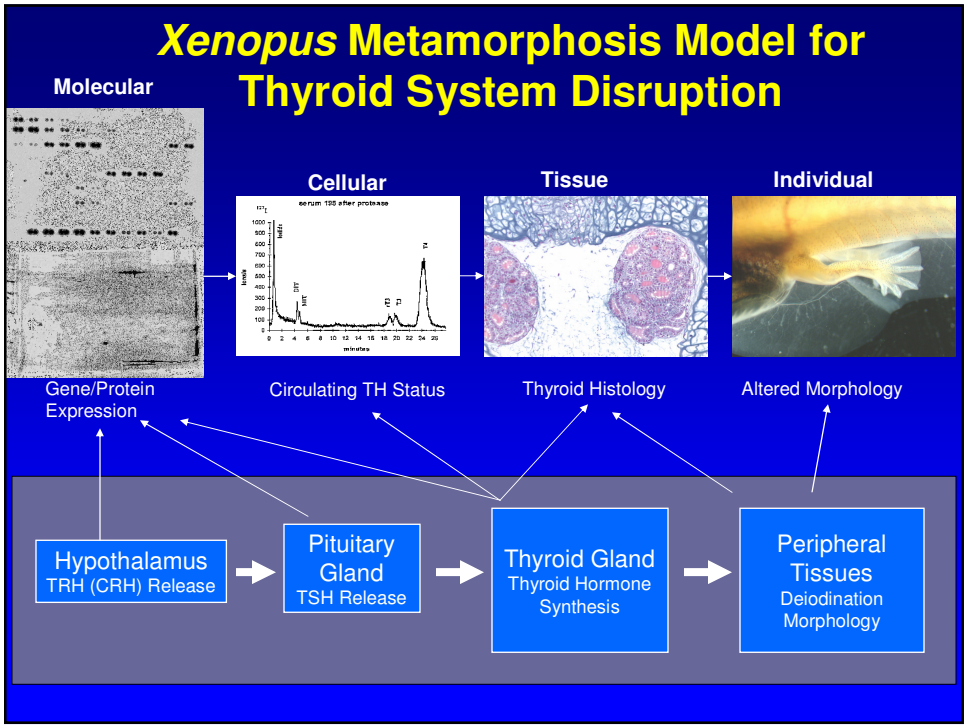
Understanding "Specific" Toxicities

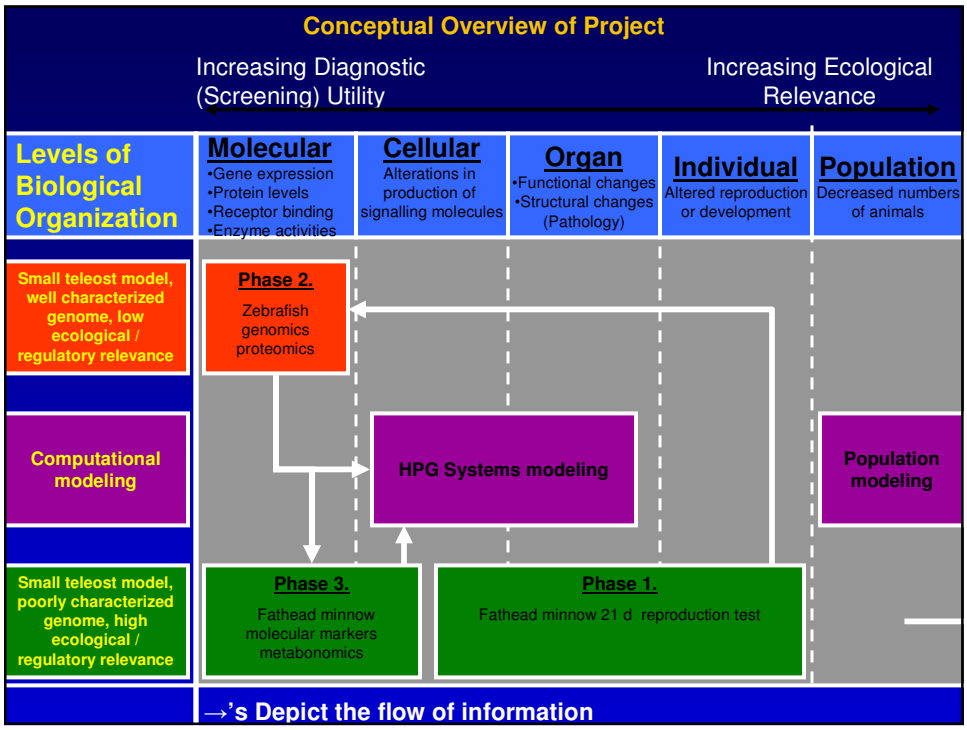
Endocrine Disruptors:

- -Receptor-Mediated Toxicity Pathways
  - ER, AR, TR?
- -Enzyme Inhibition (aromatase)
- -Steroidogenesis (altered steroid metab)

Delineating Toxicity Pathways Across Levels of Biological Organization:
Direct Chemical Binding to ER



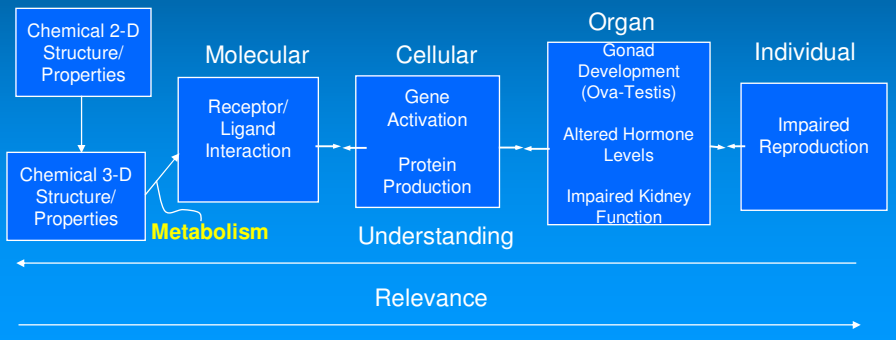

Xenopus Metamorphosis Model for Thyroid System Disruption
Molecular
Cellular
Tissue
Individual
Gene/Protein Expression
Circulating TH Status
Thyroid Histology
Altered Morphology
Hypothalamus
TRH (CRH) Release
Pituitary Gland
TSH Release
Thyroid Gland
Thyroid Hormone Synthesis
Peripheral Tissues
Deiodination
Morphology


Conceptual Overview of Project
Increasing Diagnostic (Screening) Utility
Increasing Ecological Relevance
Levels of Biological Organization
Molecular
•Gene expression
•Protein levels
•Receptor binding
•Enzyme activities
Cellular
Alterations in production of signalling molecules
Organ
•Functional changes
•Structural changes (Pathology)
Individual
Altered reproduction or development
Population
Decreased numbers of animals
Small teleost model, well characterized genome, low ecological / regulatory relevance
Phase 2.
Zebrafish genomics proteomics
Computational modeling
HPG Systems modeling
Population modeling
Small teleost model, poorly characterized genome, high ecological / regulatory relevance
Phase 3.
Fathead minnow molecular markers metabonomics
Phase 1.
Fathead minnow 21 d reproduction test
→'s Depict the flow of information

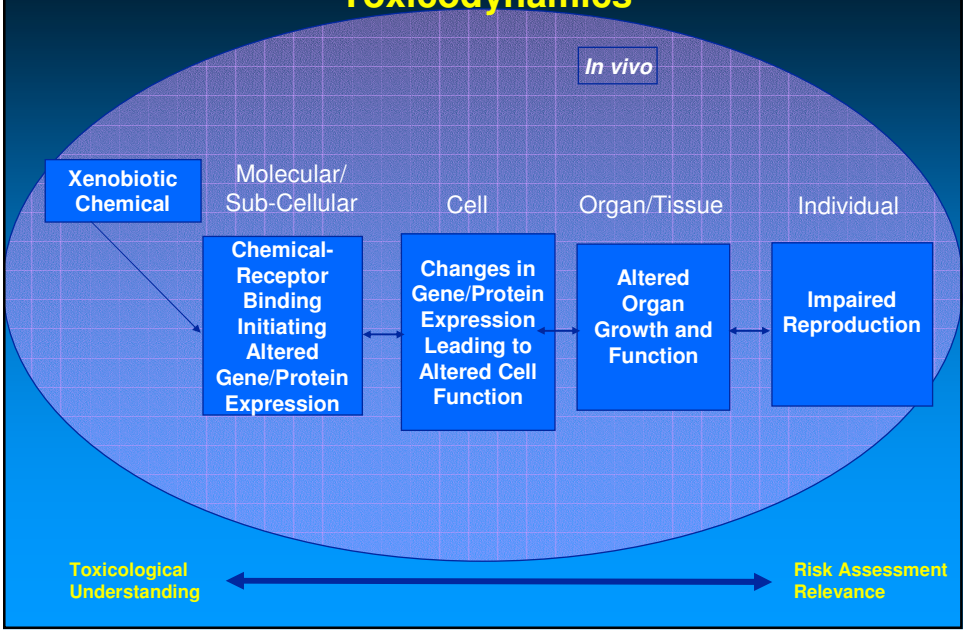
Chemical Risk Assessments

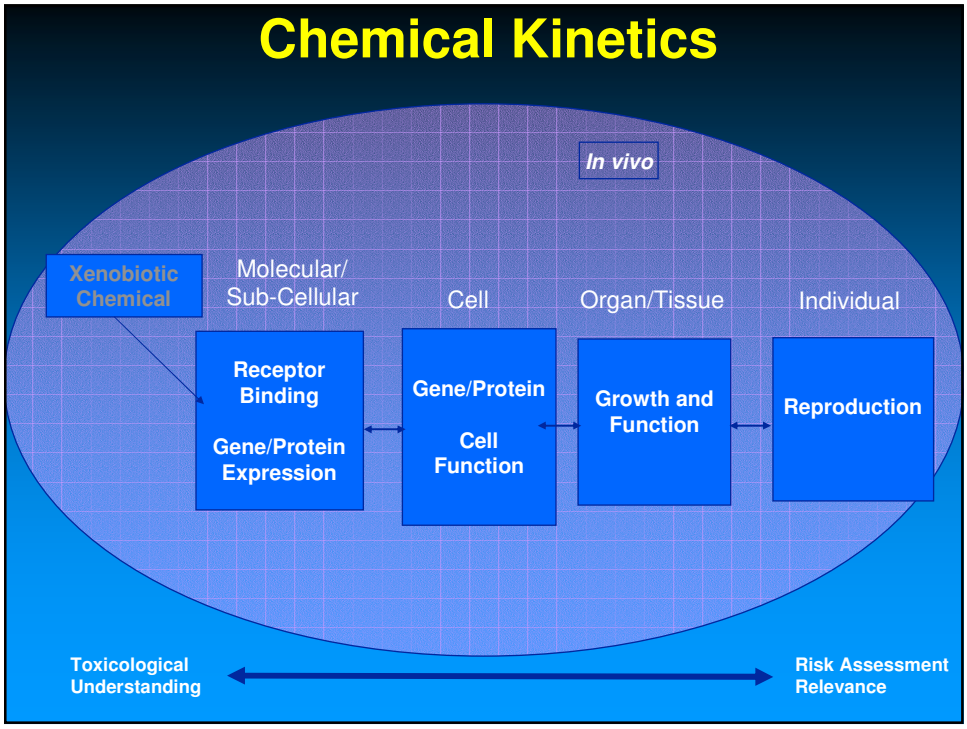
Linkages Across Levels of Biological Organization

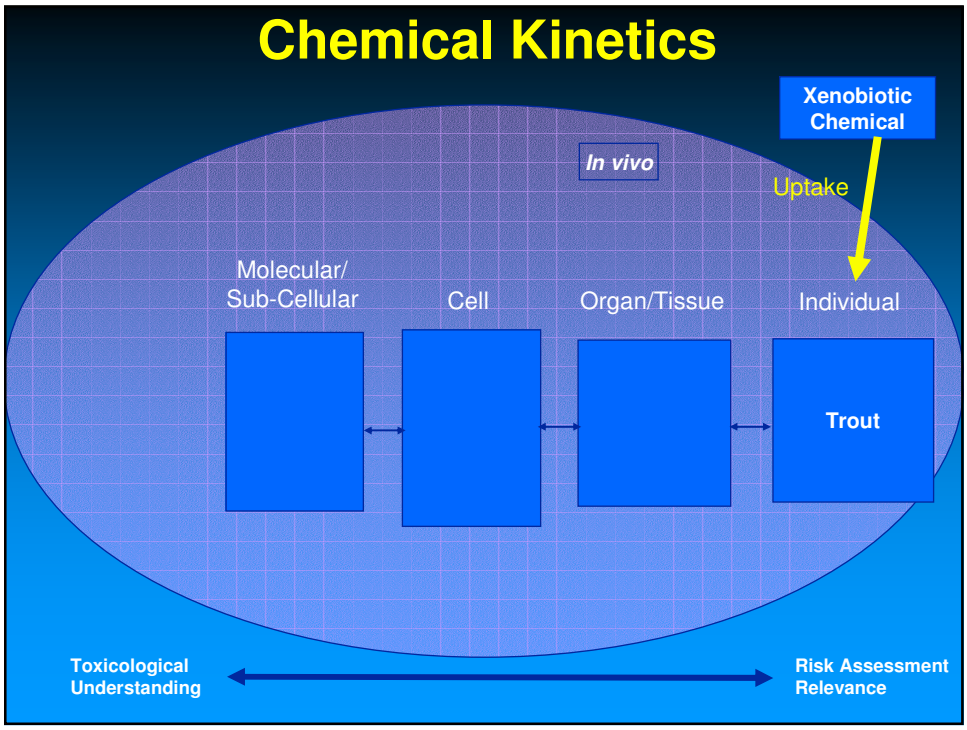
Receptor-Mediated Pathways

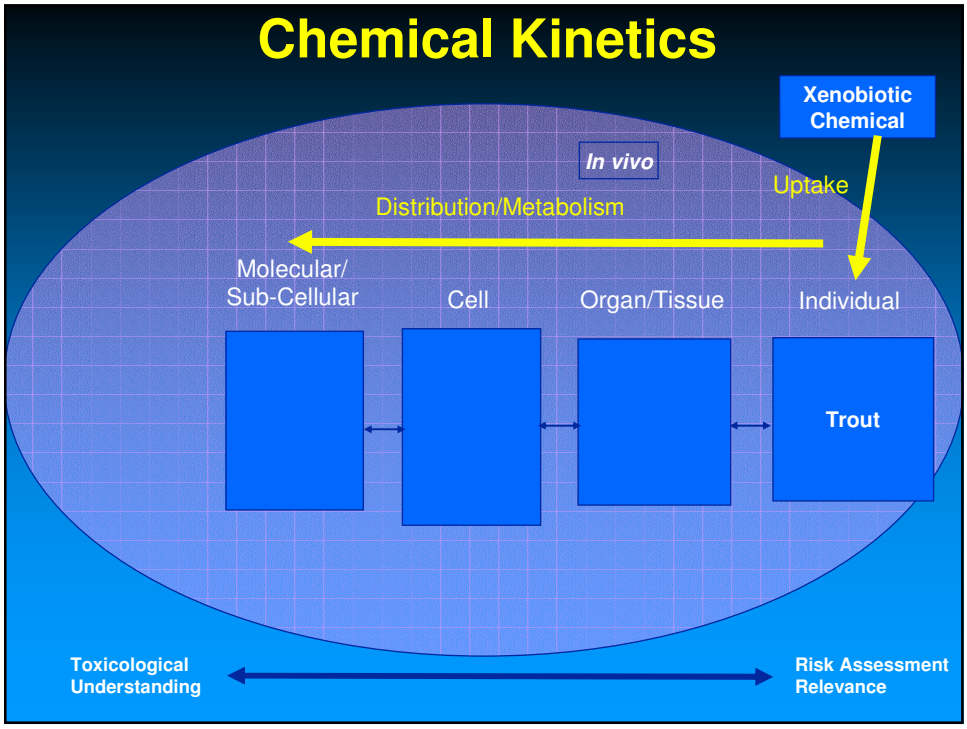


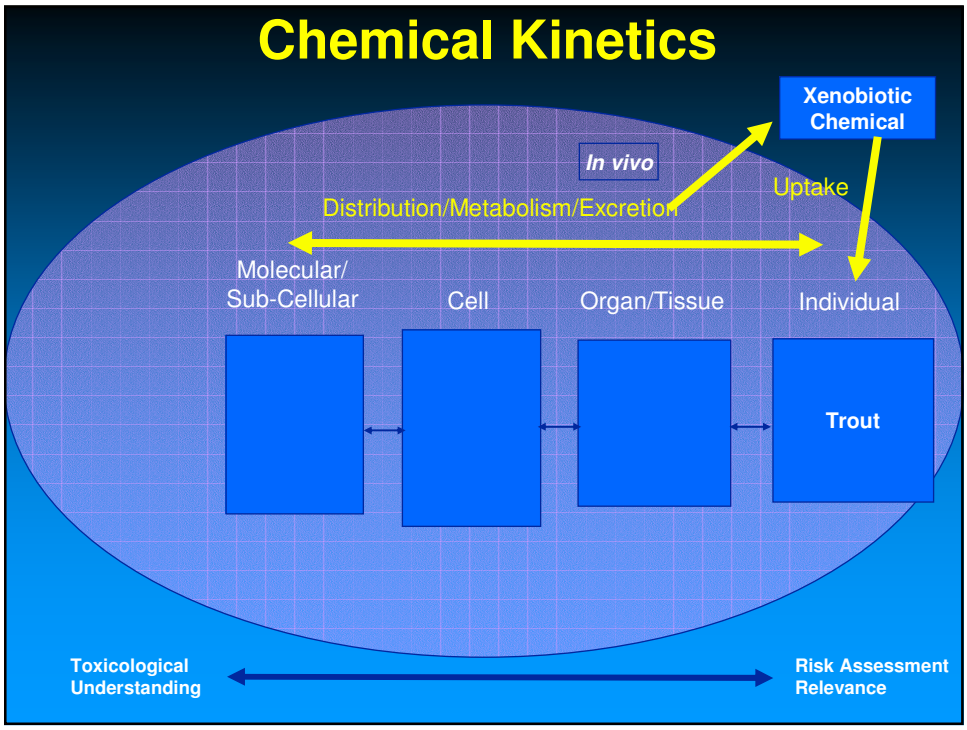
Toxicokinetics Toxicodynamics

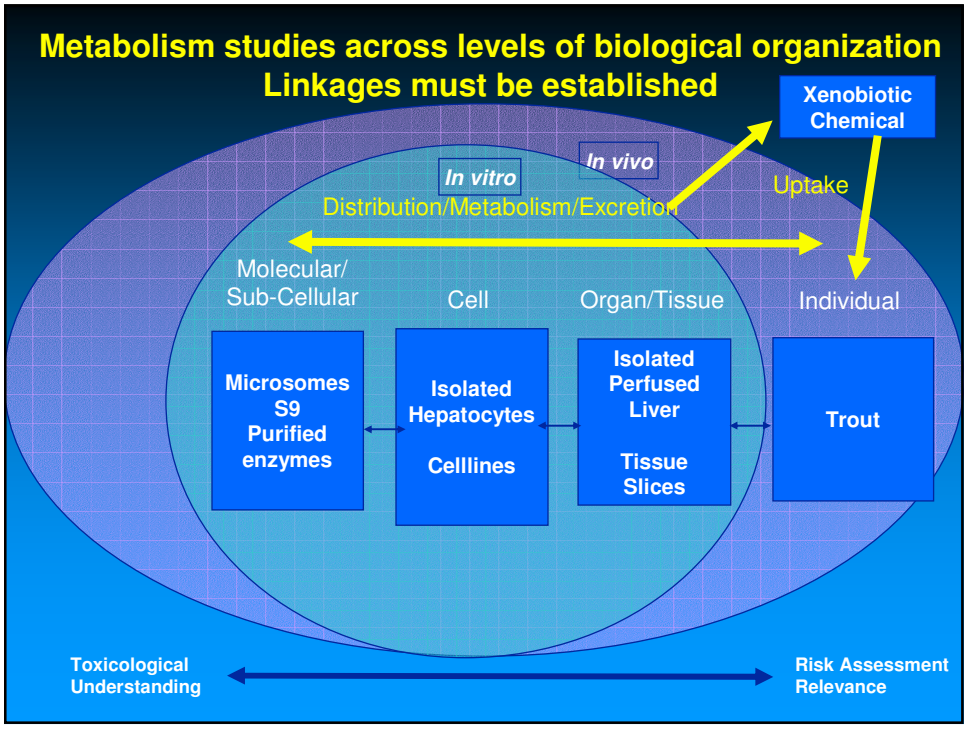


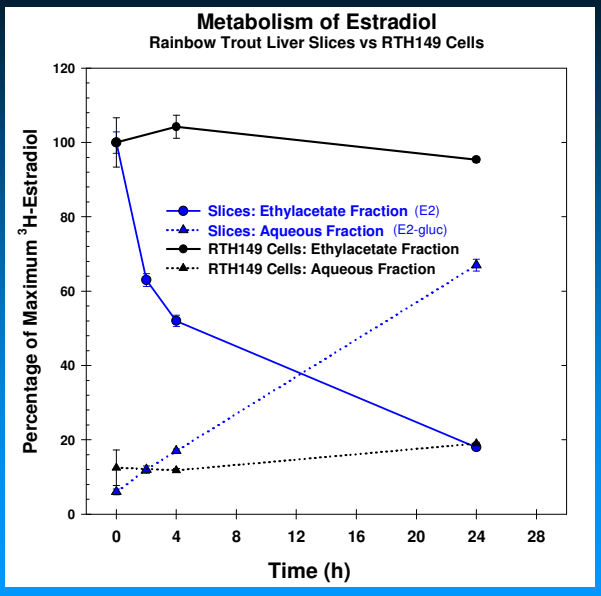

Chemical Kinetics
In vivo
Xenobiotic Chemical
Molecular/ Sub-Cellular
Cell
Organ/Tissue
Individual
Receptor Binding
Gene/Protein Expression
Gene/Protein
Cell Function
Growth and Function
Reproduction
Toxicological Understanding
Risk Assessment Relevance

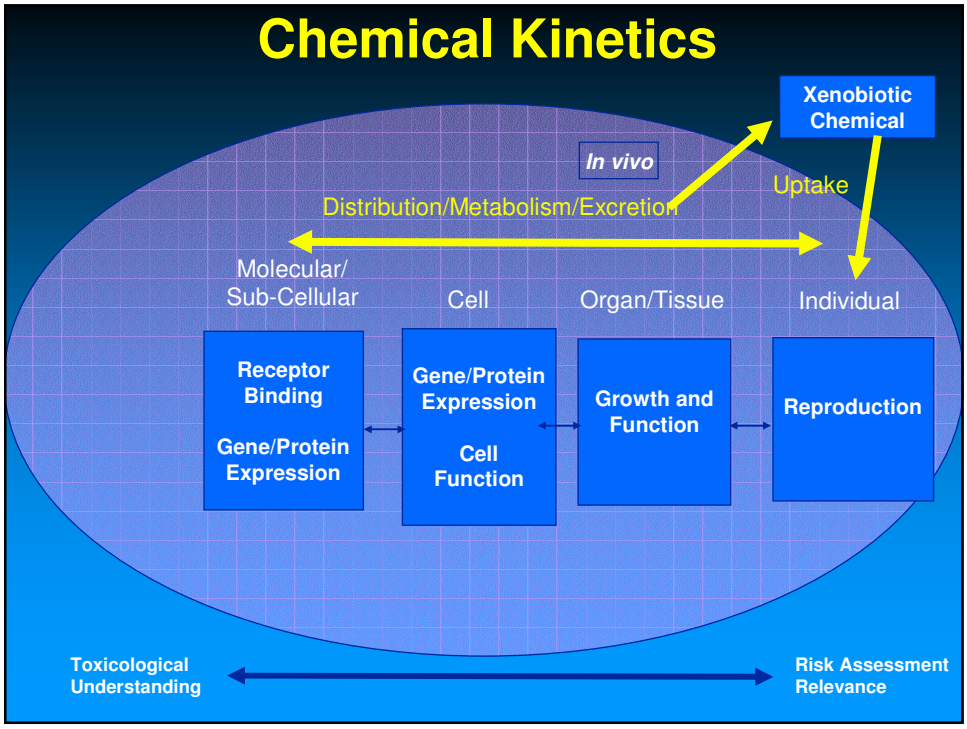

Chemical Kinetics
Xenobiotic Chemical
In vivo
Uptake
Molecular/ Sub-Cellular
Cell
Organ/Tissue
Individual
Trout
Toxicological Understanding
Risk Assessment Relevance

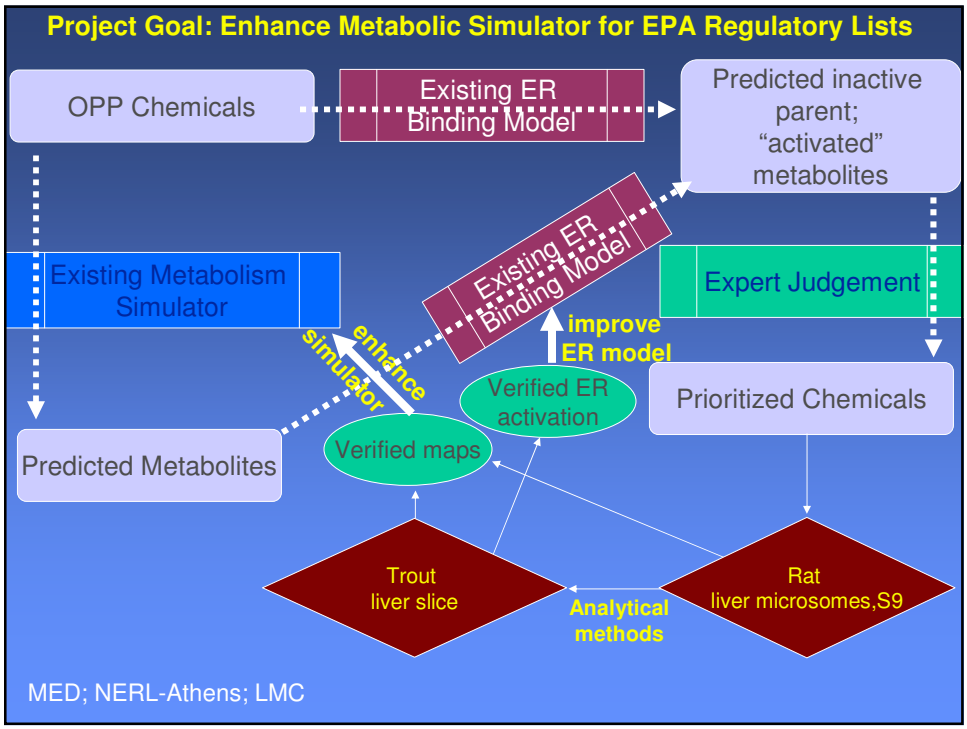

Chemical Kinetics
Xenobiotic Chemical
In vivo
Uptake
Distribution/Metabolism
Molecular/ Sub-Cellular
Cell
Organ/Tissue
Individual
Trout
Toxicological Understanding
Risk Assessment Relevance


Chemical Kinetics
Xenobiotic Chemical
In vivo
Uptake
Distribution/Metabolism/Excretion
Molecular/ Sub-Cellular
Cell
Organ/Tissue
Individual
Trout
Toxicological Understanding
Risk Assessment Relevance


Metabolism studies across levels of biological organization
Linkages must be established
Xenobiotic Chemical
In vitro
In vivo
Uptake
Distribution/Metabolism/Excretion
Molecular/ Sub-Cellular
Cell
Organ/Tissue
Individual
Microsomes S9 Purified enzymes
Isolated Hepatocytes
Celllines
Isolated Perfused Liver
Tissue Slices
Trout
Toxicological Understanding
Risk Assessment Relevance


Metabolism of Estradiol
Rainbow Trout Liver Slices vs RTH149 Cells
Percentage of Maximum ^{3}H-Estradiol
Time (h)
Slices: Ethylacetate Fraction (E2)
Slices: Aqueous Fraction (E2-gluc)
RTH149 Cells: Ethylacetate Fraction
RTH149 Cells: Aqueous Fraction
0
20
40
60
80
100
120
0
4
8
12
16
20
24
28


Chemical Kinetics
Xenobiotic Chemical
In vivo
Uptake
Distribution/Metabolism/Excretion
Molecular/ Sub-Cellular
Cell
Organ/Tissue
Individual
Receptor Binding
Gene/Protein Expression
Gene/Protein Expression
Cell Function
Growth and Function
Reproduction
Toxicological Understanding
Risk Assessment Relevance


Project Goal: Enhance Metabolic Simulator for EPA Regulatory Lists
OPP Chemicals
Existing ER Binding Model
Predicted inactive parent; "activated" metabolites
Existing Metabolism Simulator
Existing ER Binding Model
Expert Judgement
improve ER model
enhance simulator
Verified ER activation
Prioritized Chemicals
Predicted Metabolites
Verified maps
Trout liver slice
Analytical methods
Rat liver microsomes,S9
MED; NERL-Athens; LMC

Toxicity Pathways

A useful concept for organizing toxicity data across levels of biological organization

-Linking toxicological understanding to risk assessment relevance

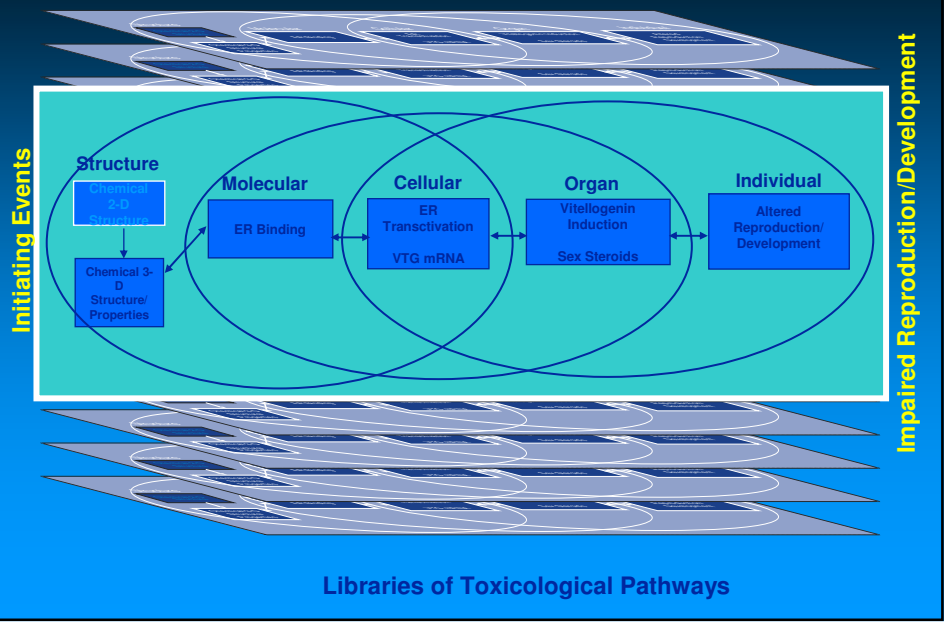
A conceptual framework for:

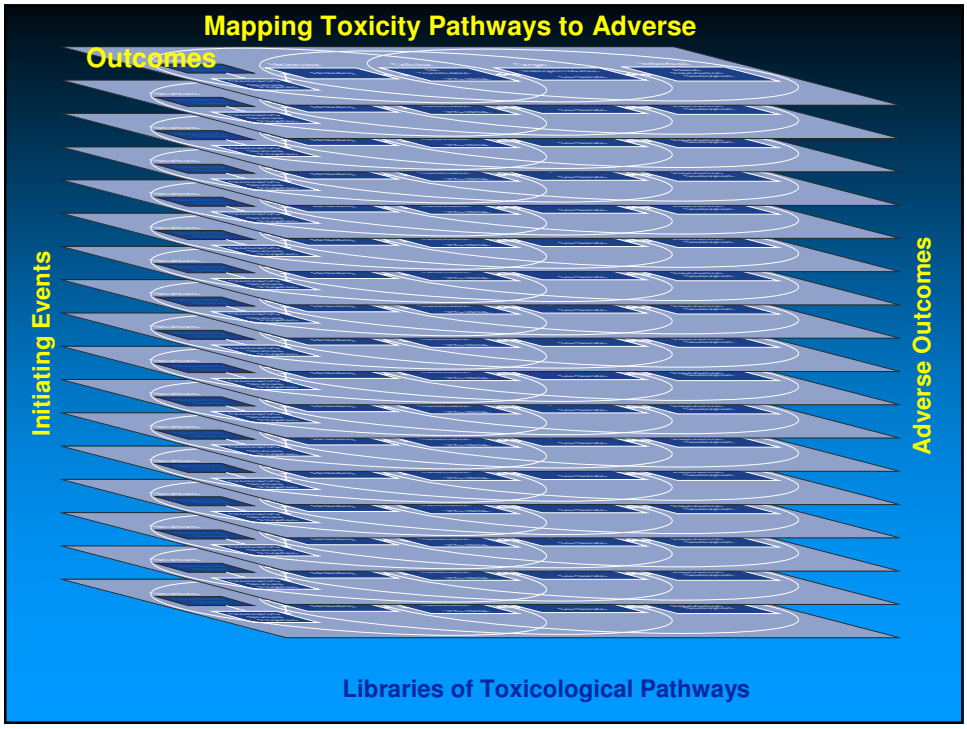
- chemical extrapolation
  - molecular initiating events are the key to linking chemical reactivity continuum to biological response continuum
- species extrapolation

A useful concept in Predictive Toxicology

- Predict most likely tox pathway for a chemical to pinpoint most appropriate testing

Mapping Toxicity Pathways to Adverse Outcomes




Mapping Toxicity Pathways to Adverse Outcomes
Initiating Events
Adverse Outcomes
Libraries of Toxicological Pathways