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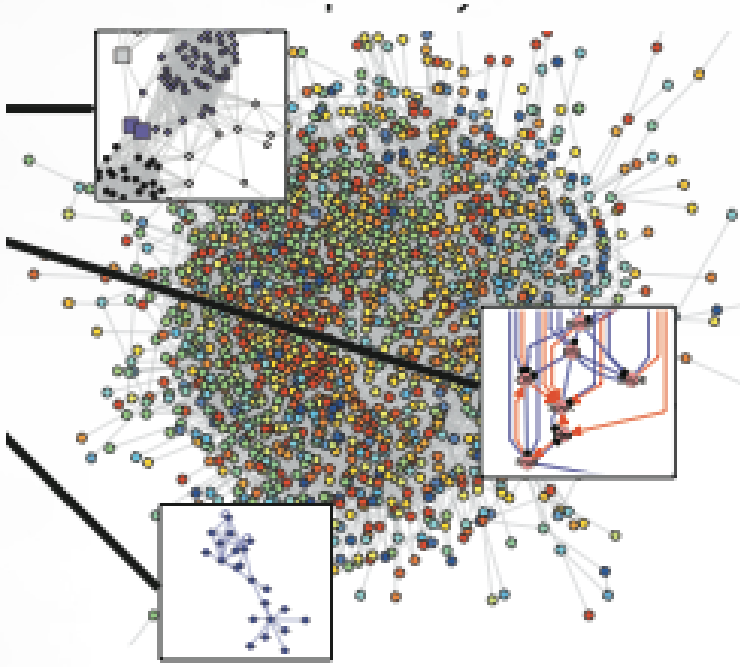
Dosimetry-Anchored Systems Models of Toxicity for Derivation of No Effect Levels



Katrina Waters and Justin Teeguarden

October 24th, 2012

Why use ‘Omics Data and Systems Models to Quantify Hazard and Influence Risk?

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- Biological systems are defined by multiple redundant and interdependent signaling networks and metabolic pathways
- ‘Omics data are useful to broadly assess biological response and identify phenotype-associated pathways
- Conserved features of *in vivo* and *in vitro* systems can be identified
- Quantitative relationships between dose, response and outcome can be derived
- Integration of dosimetry between experimental systems and human exposure can provide realistic extrapolation of risk
- Predictive models can be used to deconvolute the interactions of mixtures mechanistically and quantitatively

Current Approaches and Challenges Using 'Omics Data for Dose-Response Assessment

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- Most 'omics technologies provide relative quantification, not absolute, so comparisons across studies are difficult
- Biological systems are frequently nonlinear; what do you do with multiple pathways that fit different regression models?
- Bench Mark Dose approaches use an average of all genes within a "pathway" or ontology group; this reduces sensitivity and relies on the accuracy of the ontology categories
- Pattern and ontology-based approaches do not account for regulatory mechanisms or cross-talk between pathways
- Rarely is time-dependent behavior considered in the experiment
- *In vivo* outcomes are not mirrored *in vitro,* and "concentration" *in vitro* is not the same thing as "dose" or "exposure" *in vivo*

Example: Macrophage Response to Silica Nanoparticles is Dose and Time Dependent

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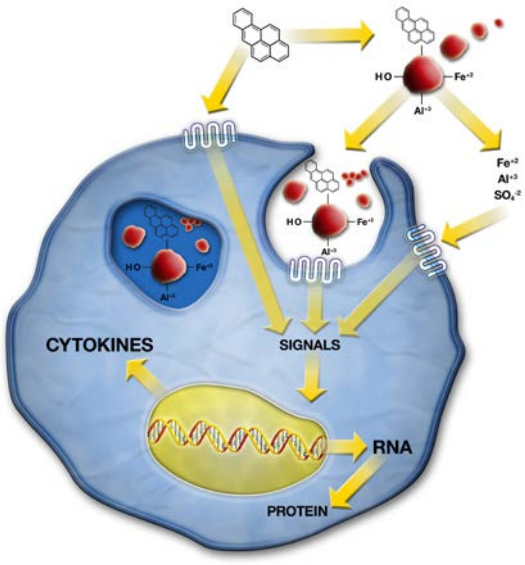

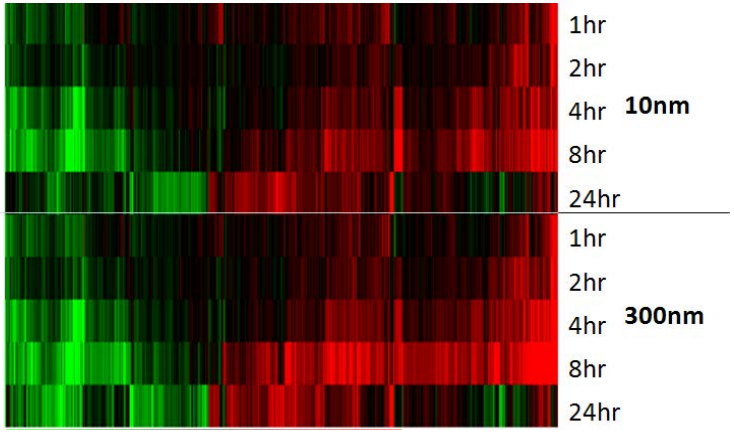

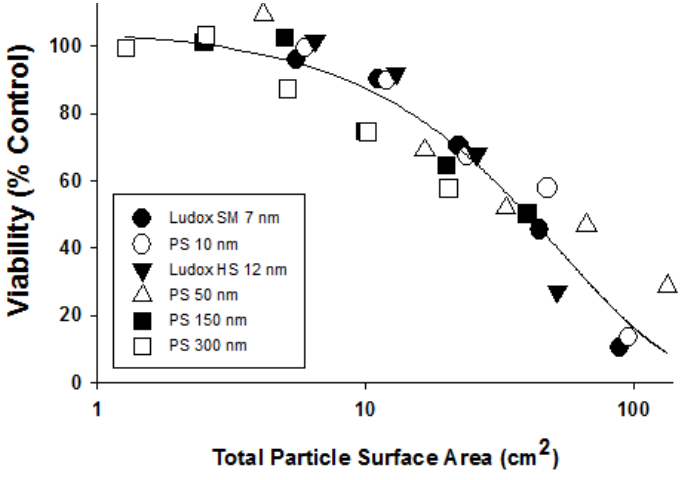

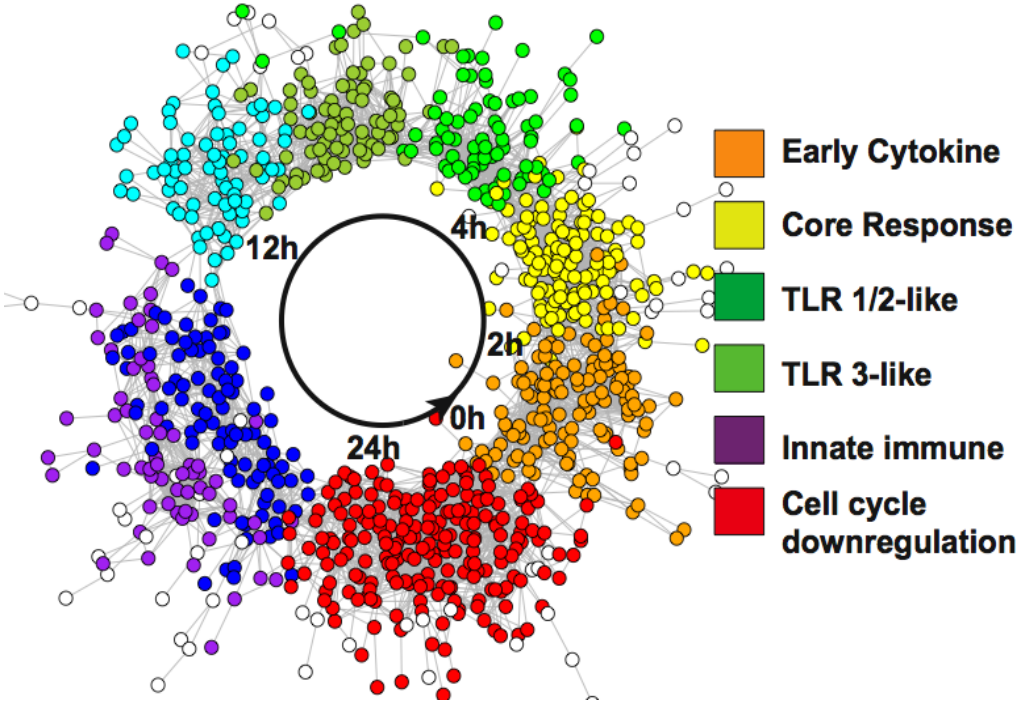

Hybrid pathway/regression approach

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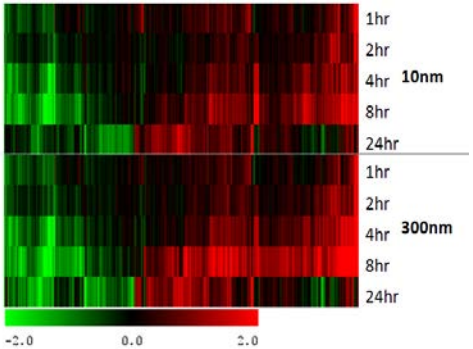
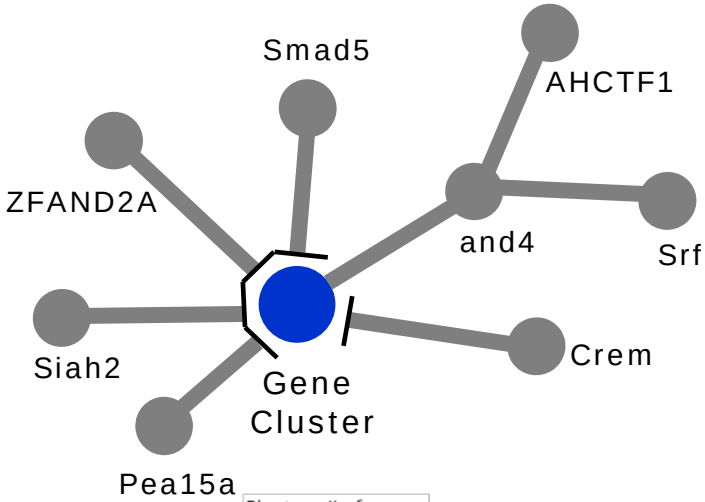
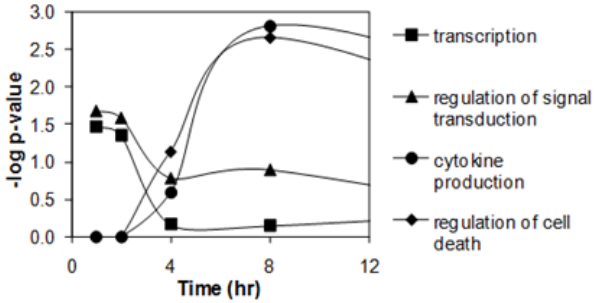
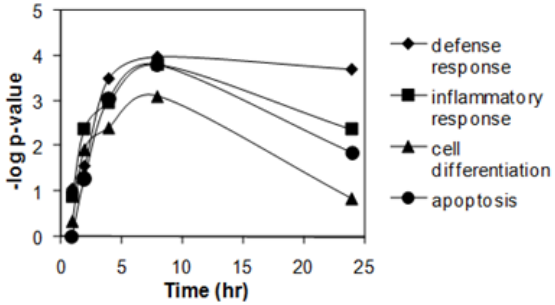
Gene Expression Profile





Exposure, Time, Silica Particle Size





Cluster	# of genes
1	244
2	145
3	85
4	303
5	180
6	318
7	103
8	170
9	83
10	156
11	70
12	97
13	34
14	110
15	93
16	48
17	124
18	56
19	13
20	105

Hybrid pathway/regression approach

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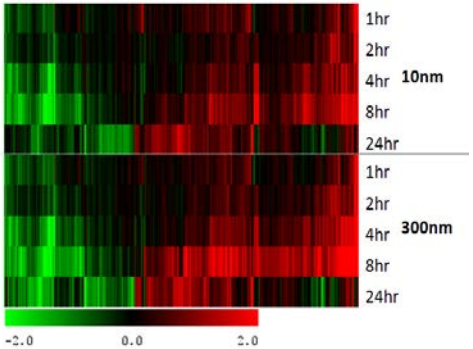
Gene Expression Profile



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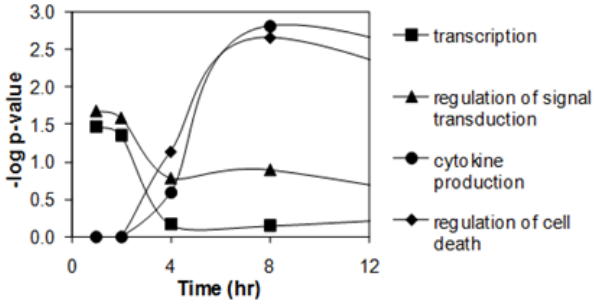

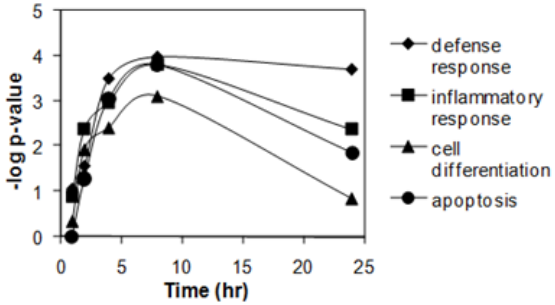

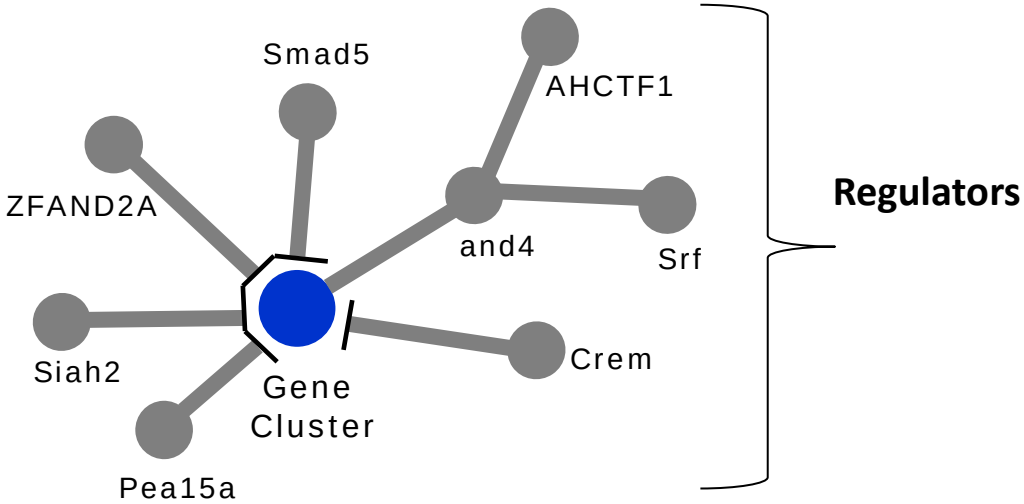

Descriptive Toxicity Pathway Models

Predictive Models of Gene Expression

Testable Hypotheses about Pathway Structure

Model Predicts the Evolution of Gene Regulation after Exposure to NP and Identifies Key Regulator

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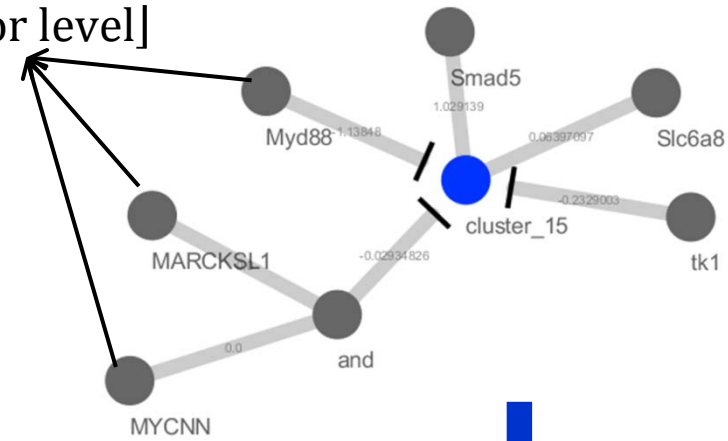
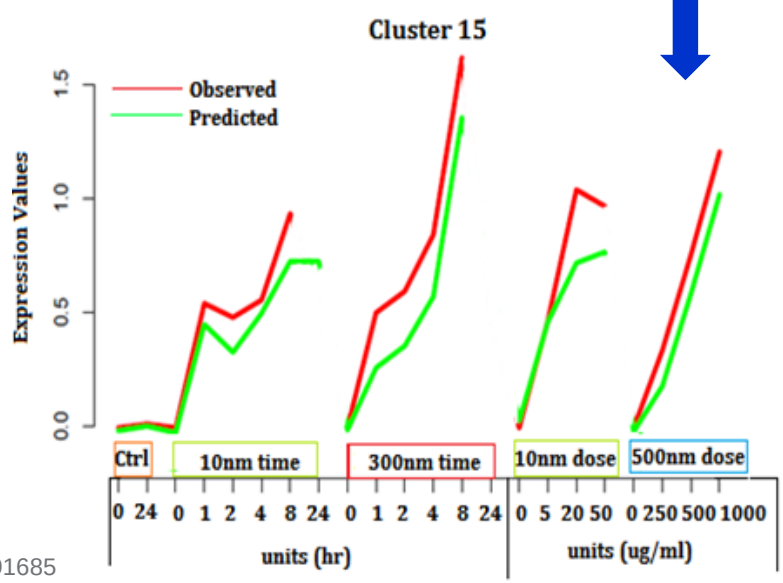
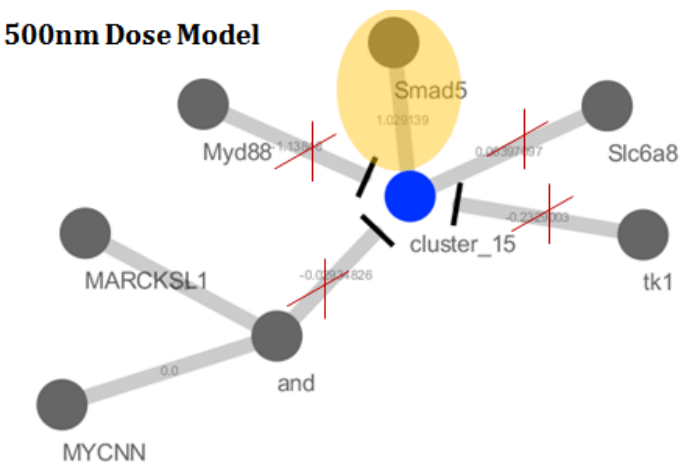
$$\tau \frac{dy}{dt}(expression) = \sum_{\text{regulator } 1}^{i} \text{Influence} * [\text{regulator level}]$$

Regulatory Model Of Cluster 15 Transcription







Estimating Dose For a No Observable Effect Level and Extrapolate to relevant Human Exposure

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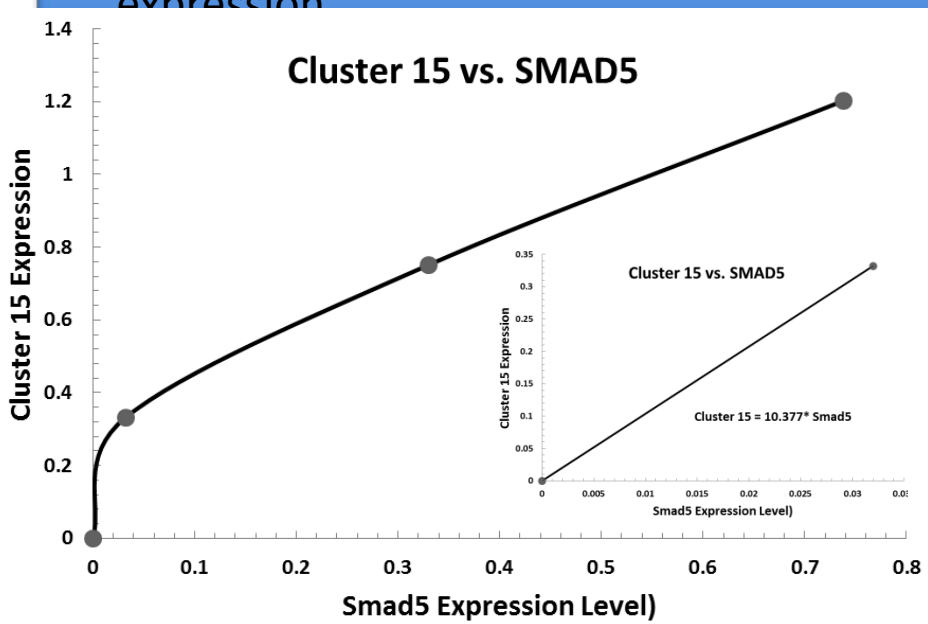
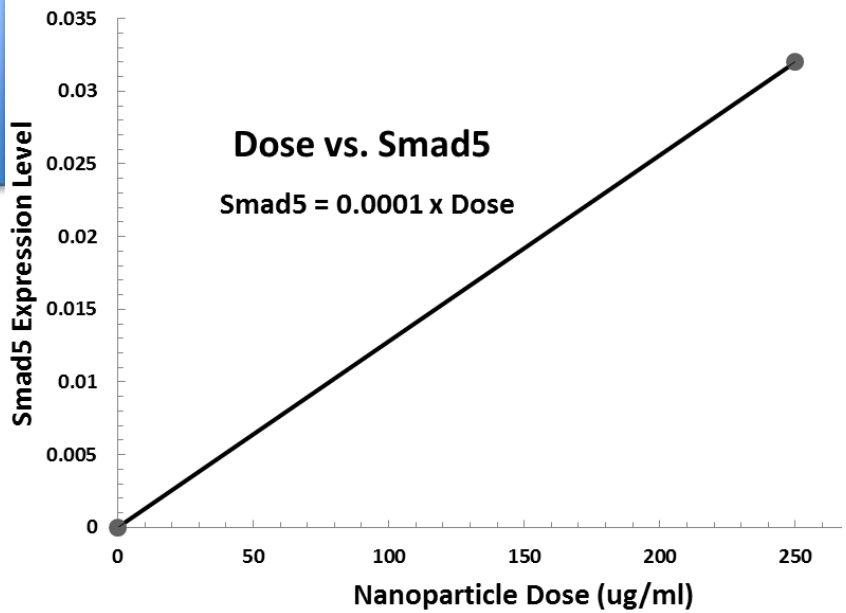
- Find Smad5 expression for a 10% increase in Cluster 15 expression
- Find the in vitro dose for the Smad5 level of expression





The dose for a 10% increase in Cluster 15 expression = 96 µg/ml

MPPD Model → Particle # or Surface Area/Cell ← ISDD Model

Equivalent Human Exposure (#/Macrophage) = 3.9 mg/m^3
Equivalent Human Exposure (#/Pulmonary surface area) = 171 mg/m^3

Predicted Regulatory Targets Validated in KO Mouse Models of Viral Pathogenesis

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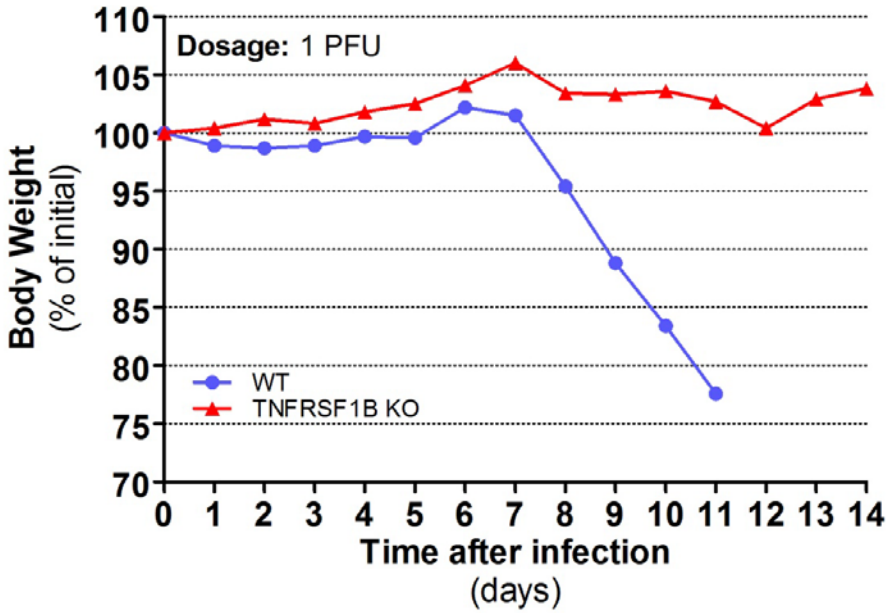
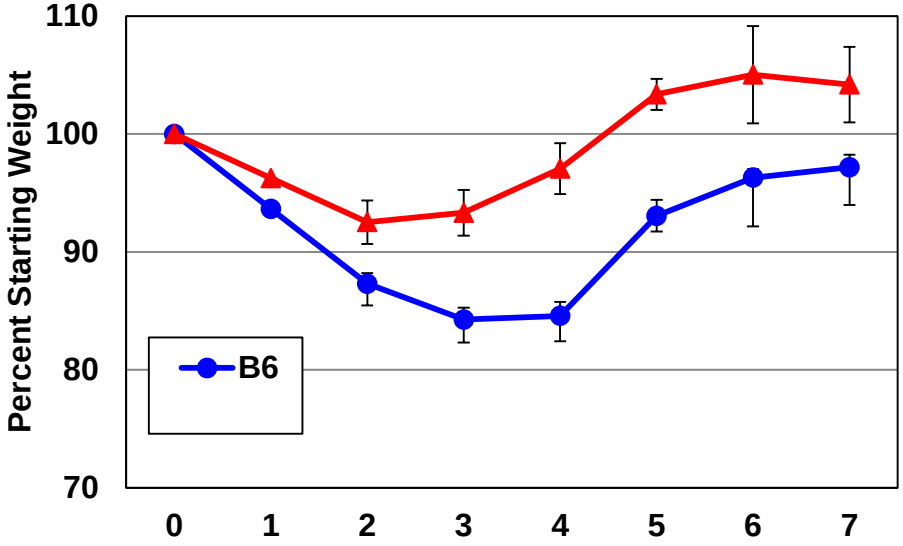
- TNFRSF1B KO mice are resistant to Avian Influenza and SARS pathogenesis



H5N1 Avian Influenza infection



SARS-CoV infection

How to Translate Approaches to Mixtures? We Need a LOT More Data

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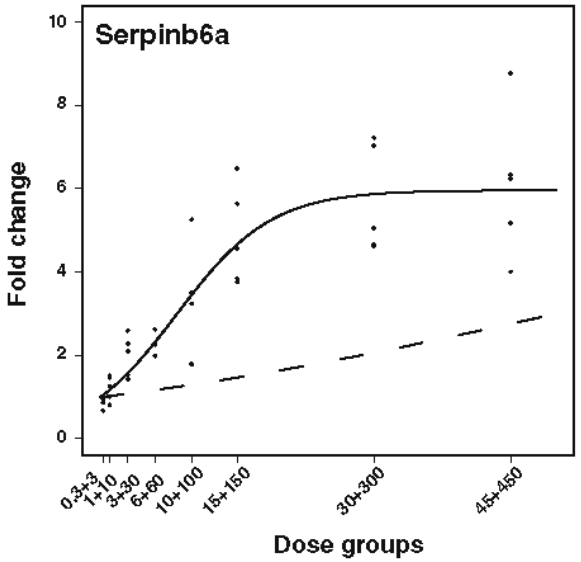
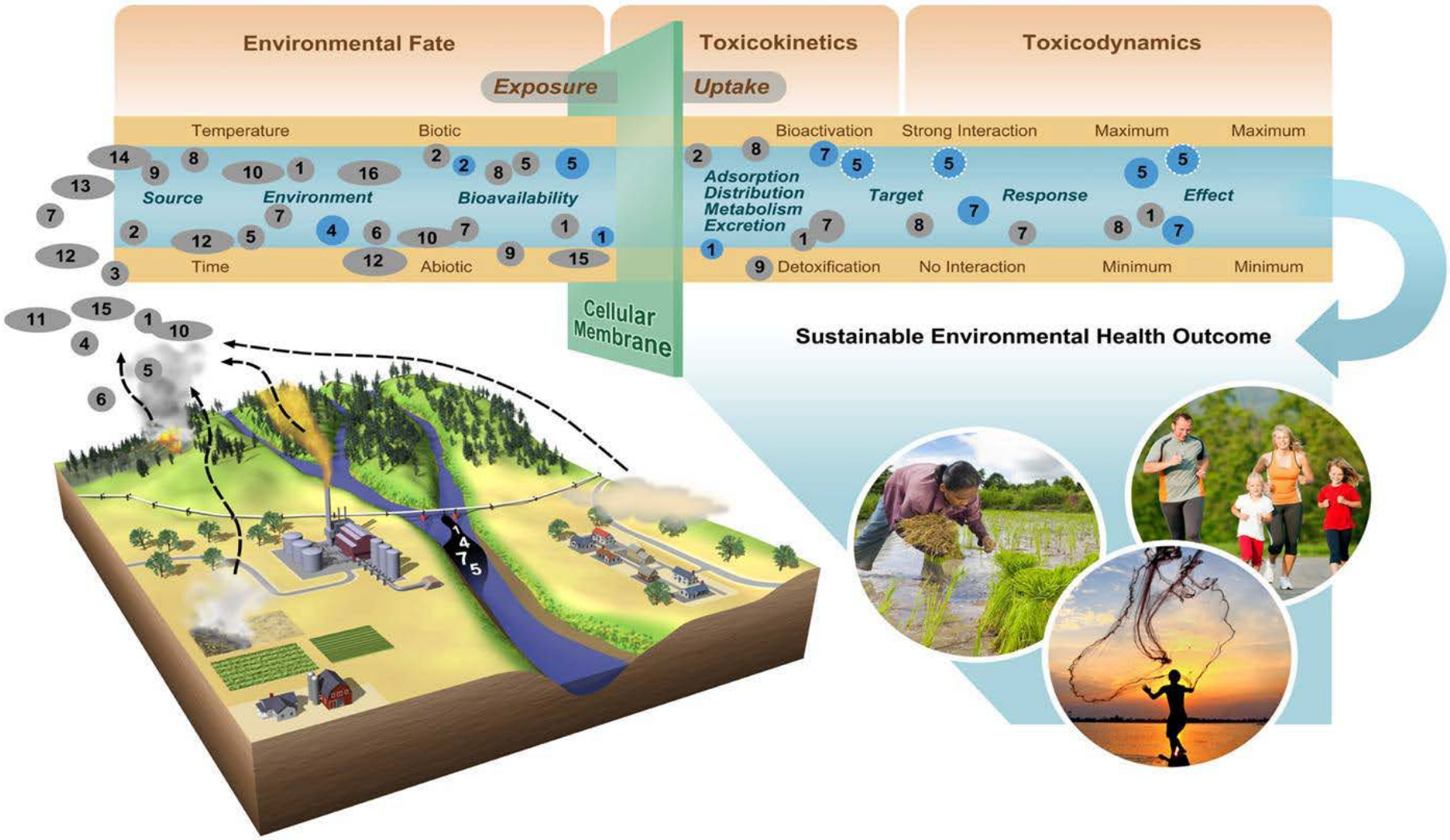
- Example Studies from Kopec
  - TCDD, PCB and mixture
  - Time course (5) and dose response (8 doses) microarrays
  - Integrated histopathology and functional outcomes (lipids)
  - Chemical concentrations measured in tissues



Kopec, AK, et al. 2010. ToxSci, 118:286-297.
Kopec, AK, et al. 2011. TAAP, 256:154-167.

- Modeling results
  - Genes identified as temporal & dose-responsive using non-linear models
  - Used qPCR to measure quantitative dose-response relationships for pure chemicals and mixtures
  - Statistical models used to determine non-additive effects of gene response, consistent with histopath and lipid accumulation

Environmental Mixtures are Composed of Diverse Chemical Constituents



OSU/PNNL Superfund Program is Focused on Emerging Health Risks associated with PAHs

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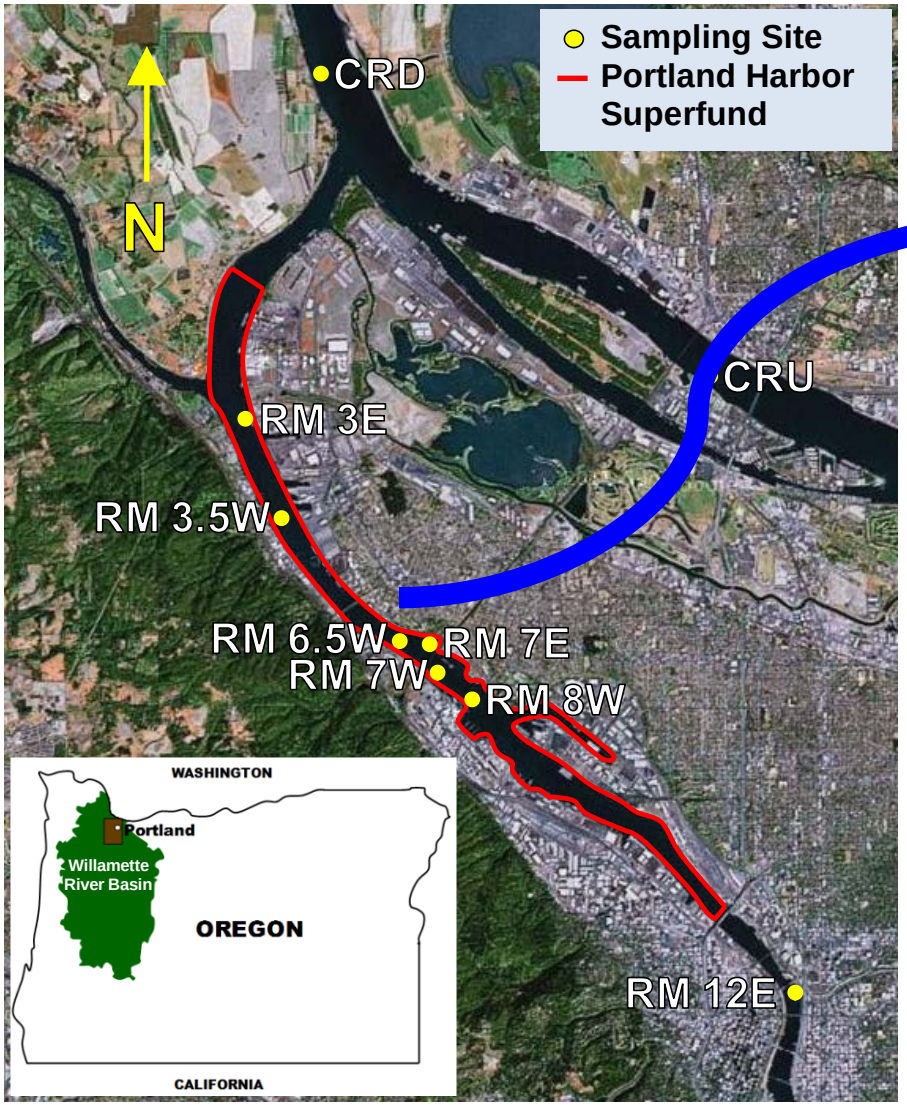
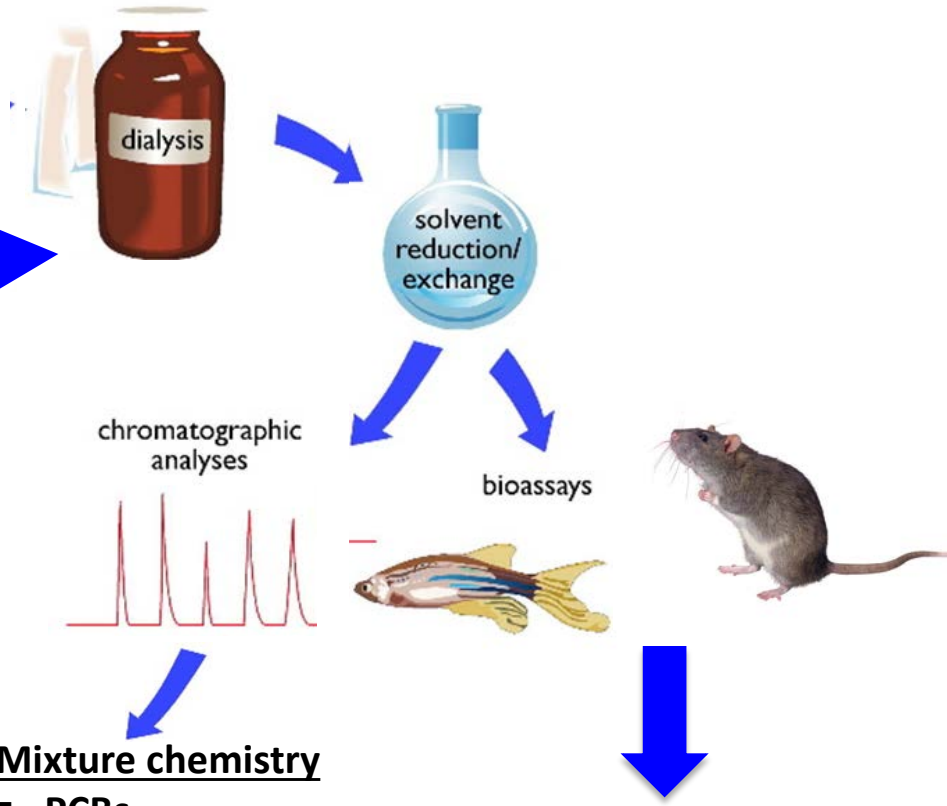




Mixture chemistry

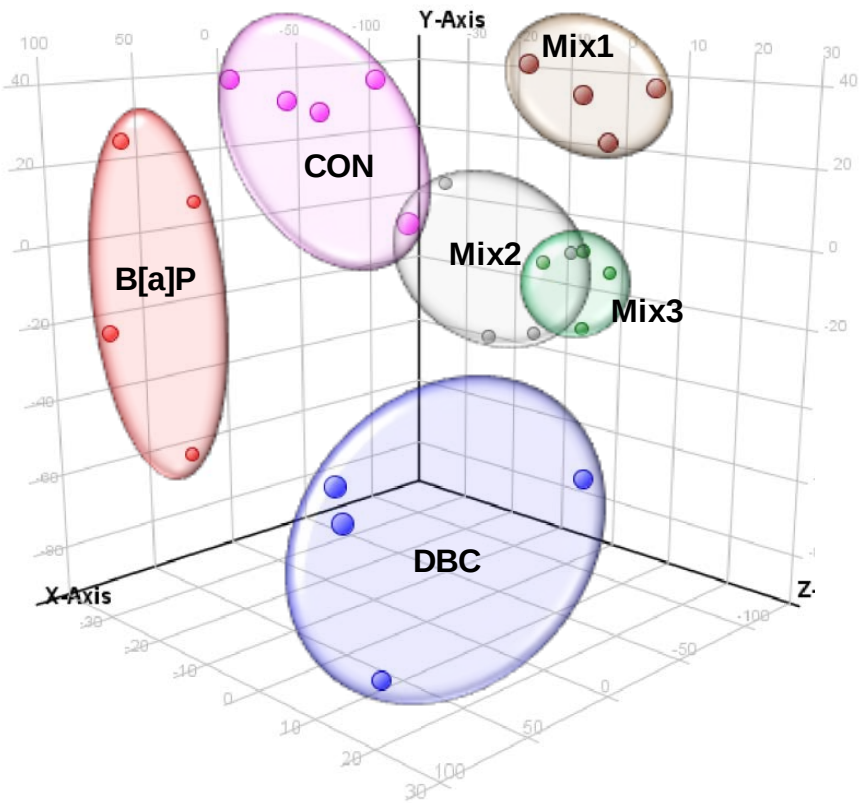
- PCBs
- PAHs
- PAH-metabolites
- Oxy-PAH

Toxicological response

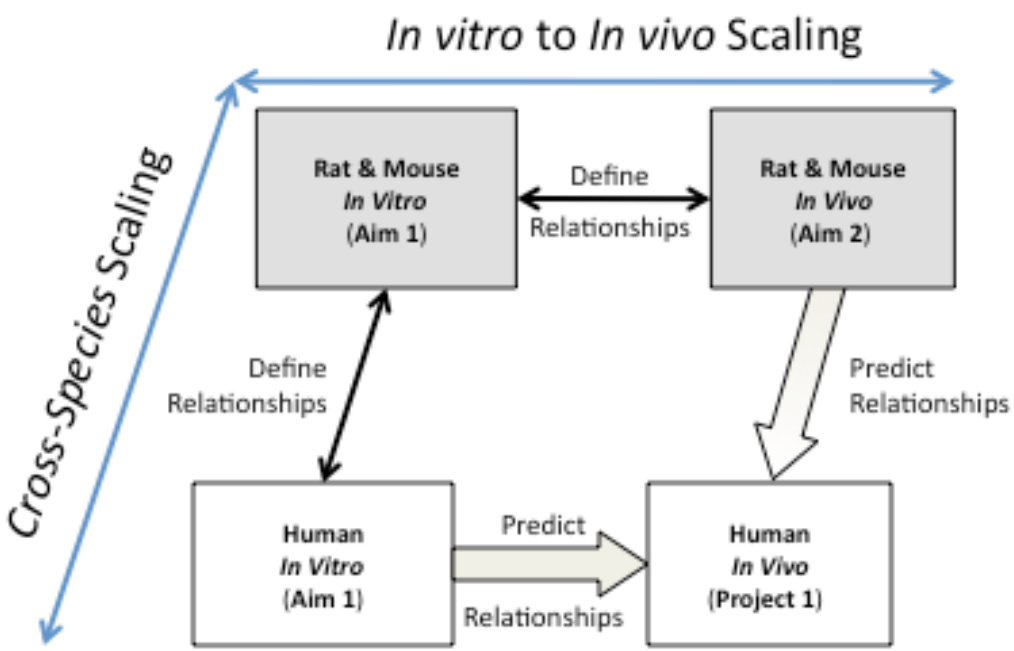
- Mortality
- Carcinogenesis
- Developmental Morphology
- Behavior
- Microarrays/RNAseq

Adapted from Allan et al. (2012) Environ. Toxicol. Chem.

Data and Computational Tools are Needed to Extrapolate Response and Exposure

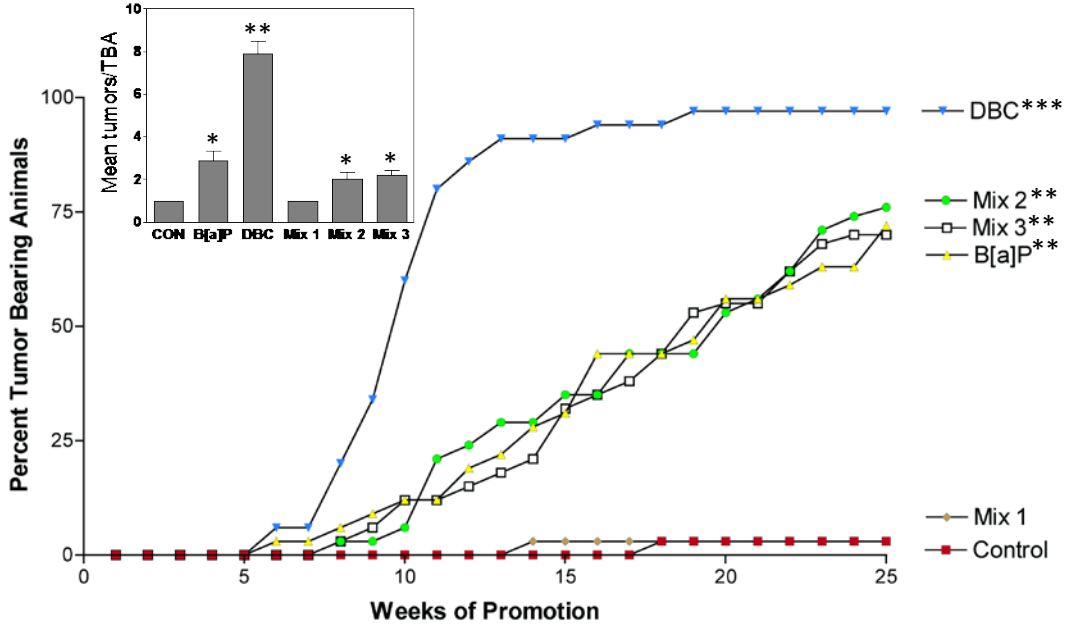


Developing PBPK models to capture PAH metabolism, interactions between PAHs, and capture species/life stages

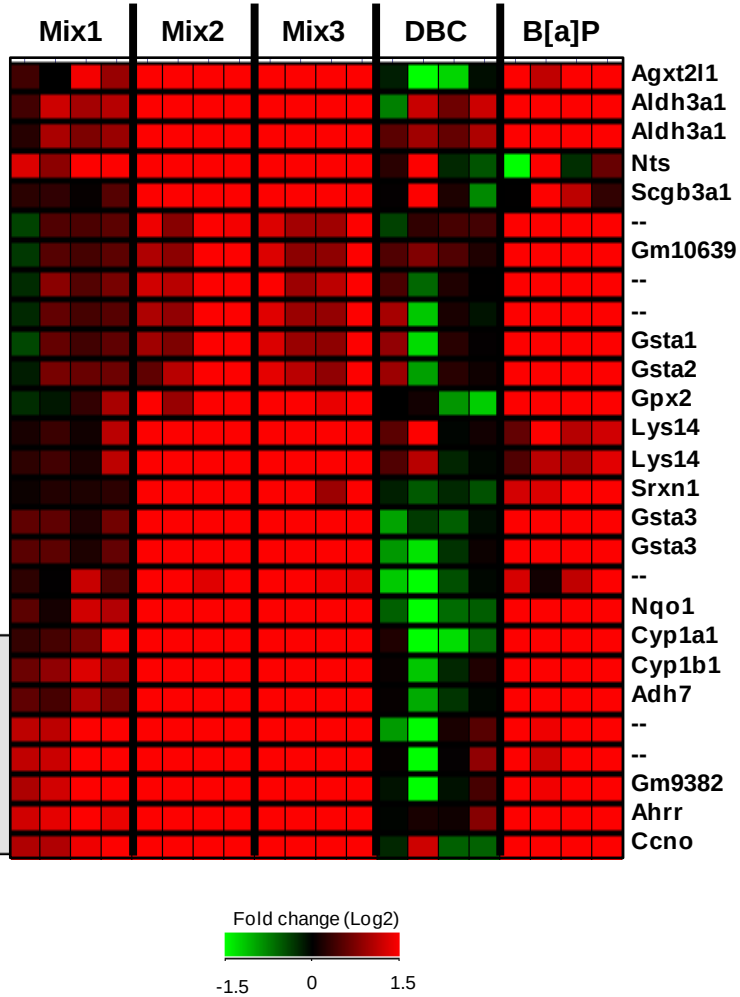


Using Statistical and Bioinformatics tools to identify MOA for PAHs and mixtures in mouse and zebrafish

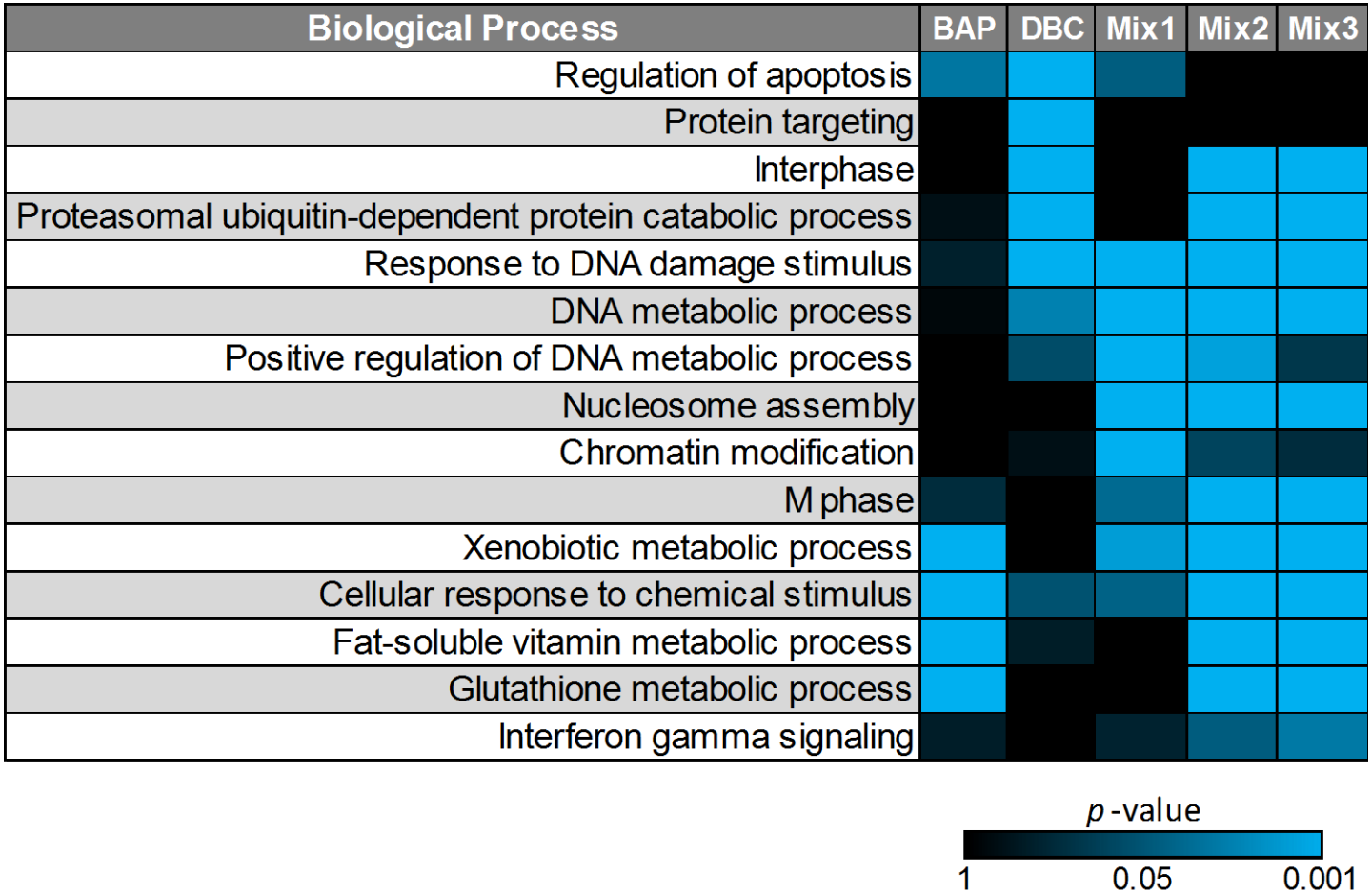
PAHs/Mixtures have Unique Gene Signatures in Skin Post-Initiation



Tumor Outcome:	DBC >>>	B[a]P = Mix2 = Mix3	>> Mix1
Classification:	High	Moderate	Low



Integration of Pathways Classifies the Treatments According to Phenotype



Integration of Pathways Classifies the Treatments According to Phenotype

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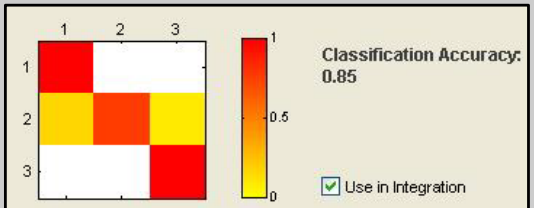
Biological Process	BAP	DBC	Mix1	Mix2	Mix3
Proteasomal ubiqu					
Posit					

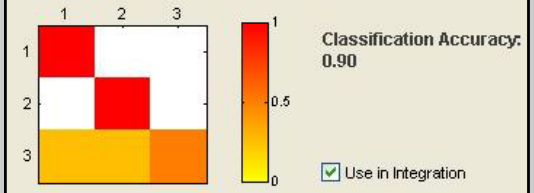
VIBE 2.0: Visual Integration for Bayesian Evaluation

Response to DNA damage stimulus

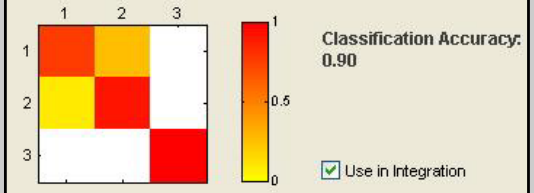


Regulation of apoptosis

Cellular response to chemical stimulus

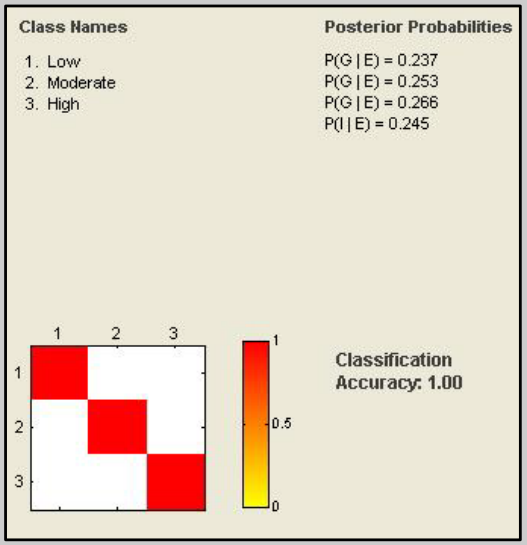


Interferon gamma signaling



Tumor classification:

1. Low (Mix1)
2. Moderate (B[a]P, Mix2, Mix3)
3. High (DBC)



Developmental Screening in Zebrafish used for HTP analysis of Environmental Mixtures

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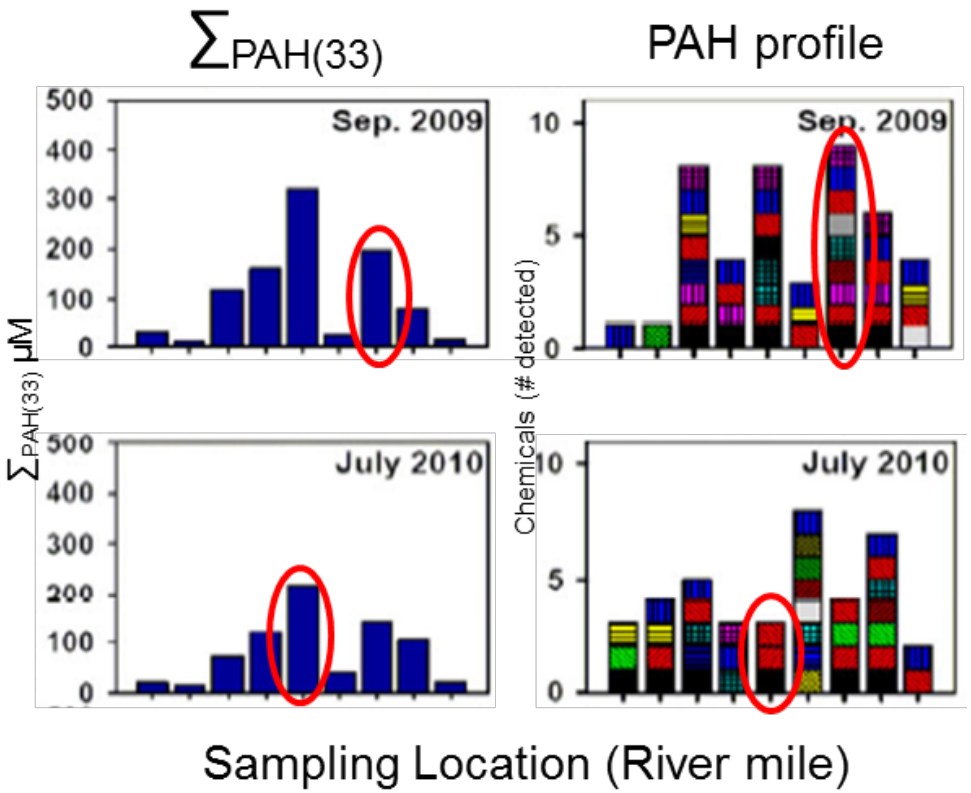
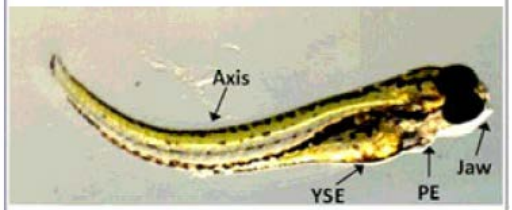
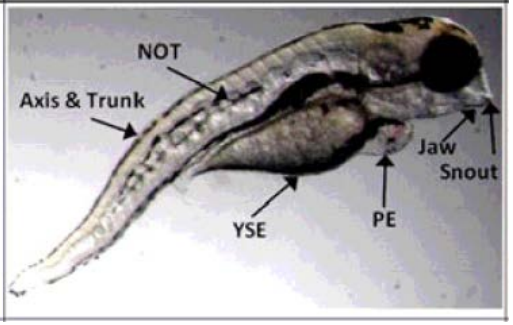
Two extracts with same total PAHs (µM), but different chemical profiles and phenotypes



Sample	Representative developmental abnormalities
Vehicle control (1% DMSO)	
RM 7W Sept. 2009	
RM 6.5W Jul. 2010	

Concentration-response curves over several dilutions

RNAseq Analysis of Zebrafish Exposed to PAHs and Mixtures for Phenotype-anchored Analysis

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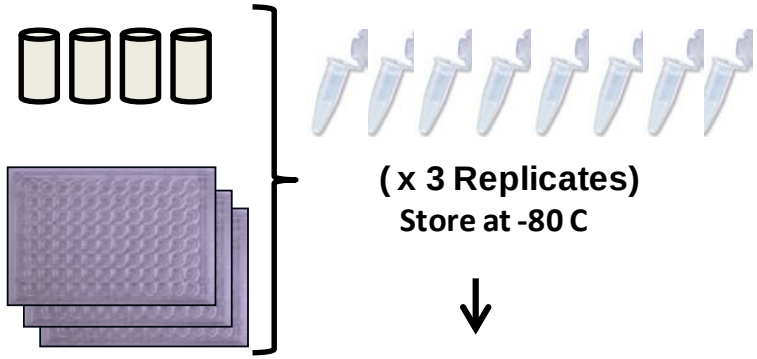
6 hpf Exposure

3 biological replicates, 20 embryos per treatment group

1) 1% DMSO Control
2) 10 uM BEZO
3) 10 uM 7,12B[a]AQ
4) PH RM 7W sample
5) PH RM 6.5W sample
6) 1.2 uM 9,10-PHEQ
7) 1% DMSO Control
8) 10 BaP
9) 10 DB[al]P
10) 1 BaP

48 hpf Homogenize in RNAzol



(x 3 Replicates)
Store at -80 C

Isolate total RNA from all samples
Quantify and QC with nanodrop and bioanalyzer

mRNA purification, sample prep, rnaSeq (50 bp paired end reads) performed by University of Oregon Core

Portland harbor mixtures compared to PAH standards and metabolites

Evolution of Systems Models from Integrated Data

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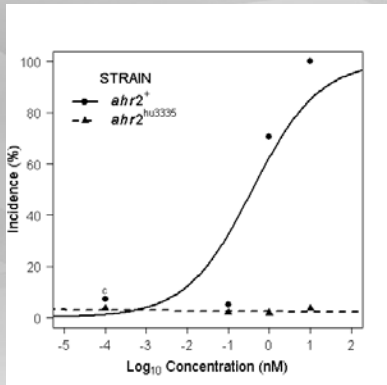
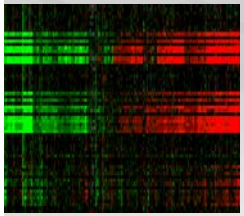
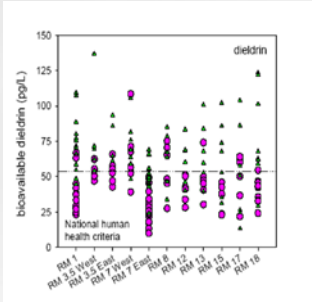


Fish Development

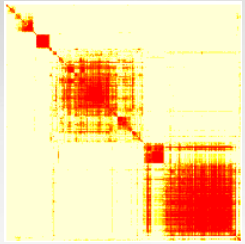


Genomics

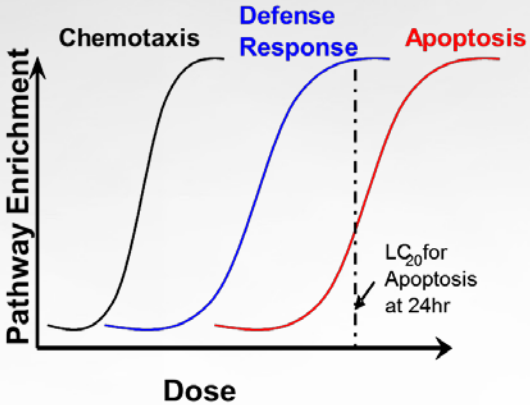


Chemistry Data

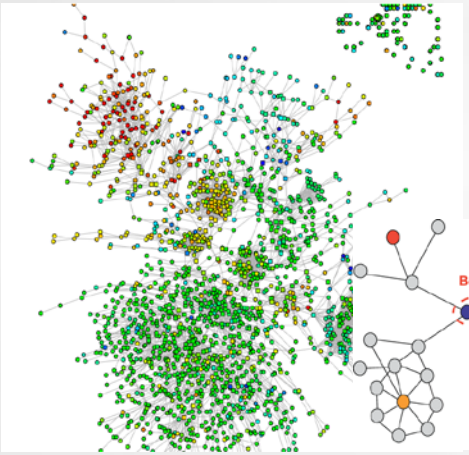
Identify Pathways associated with Phenotype and Chemistry

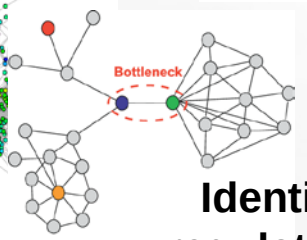


Subnetwork modules

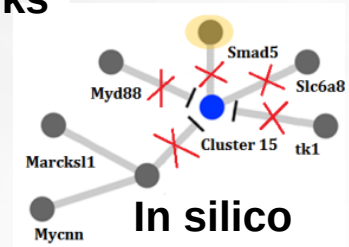


Regulatory Networks



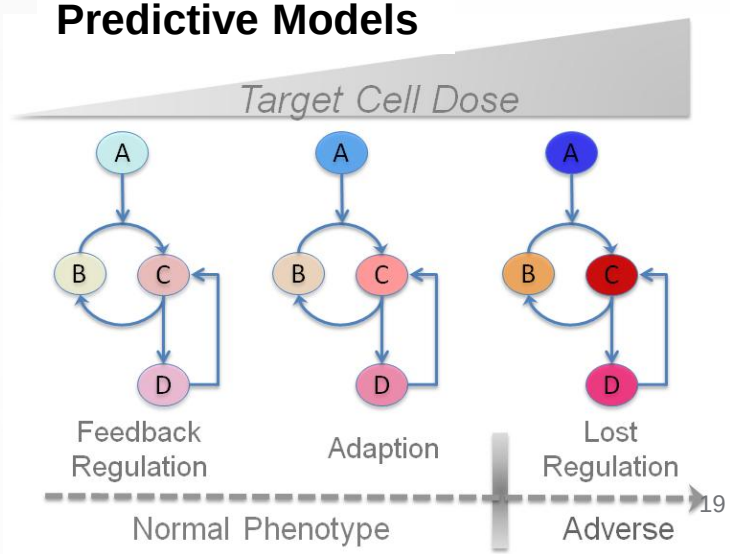


Identify regulators

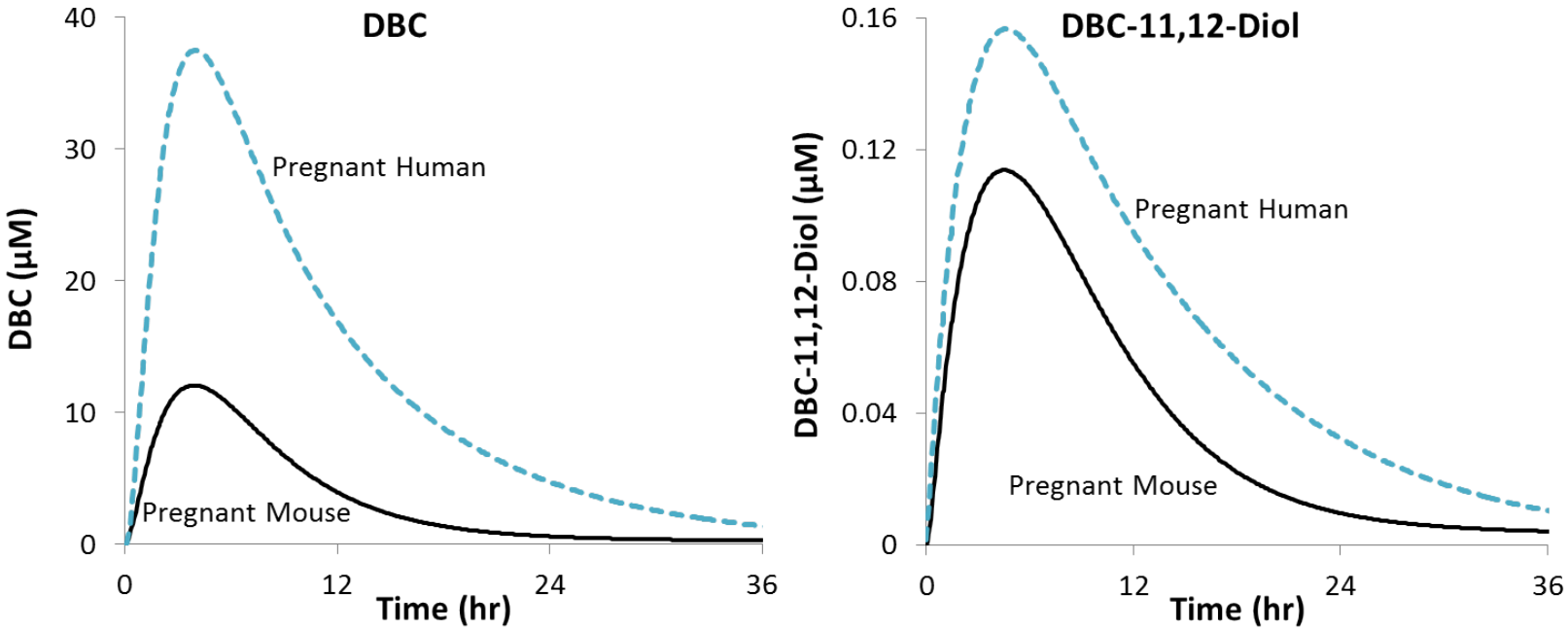


In silico knockdowns

Predictive Models



Use PBPK Studies to Extrapolate Relevance of Zebrafish Studies to Human Exposure



Simulated maternal blood concentrations of DBC and DBC-11,12-Diol in pregnant mice and humans following a single oral dose of 15 mg/kg.

Conclusions & Research Needs

- Systems models can be useful to unravel the time dependence on dose response but calculation of NOEL is tricky
- Validation through experimentation is key to all areas of science, particularly those that involve computational modeling
- Modeling of mixtures will require more data on dose-response, temporal evolution of response and comparison to pure compounds to deconvolute pathway interactions
- Start simple: synthetic mixtures with known concentrations of constituents will be needed for computational methods development
- Investment in computational models for experimental systems without exposure assessment and comparison of dosimetry in humans is not interpretable for realistic extrapolation of risk

Acknowledgements

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Computational Biology
Jason McDermott
Harish Shankaran
Hugh Mitchell
Susan Tilton
Bobbie-Jo Webb-Robertson
Melissa Matzke
Elena Peterson
Michelle Archuleta

Systems Toxicology
Justin Teeguarden
Richard Corley
Susie Crowell
Kevin Minard
Chuck Timchalk
Brian Thrall
Joel Pounds

OSU Superfund Program
Dave Williams
Robert Tanguay
Kim Anderson
Beth Siddons
Britton Goodale
Bill Baird
Staci Simonich
Anna Harding

Funding sources:
NIEHS U19 ES019544 PNNL Center for Nanotoxicology
NIEHS P42 ES016465 OSU/PNNL Superfund Research Program
NIAID Systems Biology of Infectious Diseases Program under contract # HHSN272200800060C

PNNL-SA-91685

OSU Oregon State UNIVERSITY

Superfund Research Program