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

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

- 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

- 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
Hybrid pathway/regression approach

Gene Expression Profile

Exposure, Time, Silica Particle Size

Gene Cluster

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<tr>
<th>Cluster</th>
<th># of genes</th>
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</table>
Hybrid pathway/regression approach

Gene Expression Profile

Exposure, Time, Silica Particle Size

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

\[
\tau \frac{dy}{dt} (\text{expression}) = \text{Influence} \times [\text{regulator level}] \]

Regulatory Model Of Cluster 15 Transcription

500nm Dose Model

Cluster 15
Estimating Dose For a No Observable Effect Level and Extrapolate to relevant Human Exposure

- 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

Equivalent Human Exposure (#/Macrophage) = 3.9 mg/m³
Equivalent Human Exposure (#/Pulmonary surface area) = 171 mg/m³
Predicted Regulatory Targets Validated in KO Mouse Models of Viral Pathogenesis

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

**Graphs:**
- **H5N1 Avian Influenza infection**
  - Dosage: 1 PFU
  - Graph showing body weight (% of initial) over time after infection (days)
- **SARS-CoV infection**
  - Graph showing percent starting weight over time (days)

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

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

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

Data and Computational Tools are Needed to Extrapolate Response and Exposure

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

Developing PBPK models to capture PAH metabolism, interactions between PAHs, and capture species/life stages
PAHs/Mixtures have Unique Gene Signatures in Skin Post-Initiation

**Tumor Outcome:**
- DBC >>> B[a]P = Mix2 = Mix3 >> Mix1

**Classification:**
- High
- Moderate
- Low

**Fold change (Log2)**

-1.5 0 1.5
Integration of Pathways Classifies the Treatments According to Phenotype

<table>
<thead>
<tr>
<th>Biological Process</th>
<th>BAP</th>
<th>DBC</th>
<th>Mix1</th>
<th>Mix2</th>
<th>Mix3</th>
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<td>Interferon gamma signaling</td>
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</tbody>
</table>

*P-value*

- 1
- 0.05
- 0.001
Integration of Pathways Classifies the Treatments According to Phenotype

**VIBE 2.0: Visual Integration for Bayesian Evaluation**

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

**Biological Process**

- **Response to DNA damage stimulus**
- **Regulation of apoptosis**
- **Cellular response to chemical stimulus**
- **Interferon gamma signaling**

**Posterior Probabilities**
- Low: \( P(G | E) = 0.237 \)
- Moderate: \( P(G | E) = 0.253 \)
- High: \( P(G | E) = 0.266 \)

Proudly Operated by Battelle Since 1965
Developmental Screening in Zebrafish used for HTP analysis of Environmental Mixtures

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

$\sum_{PAH(33)}$  

PAH profile

Sampling Location (River mile)

Concentration-response curves over several dilutions
**RNAseq Analysis of Zebrafish Exposed to PAHs and Mixtures for Phenotype-anchored Analysis**

### 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[a]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

Fish Development

Genomics

Identify Pathways associated with Phenotype and Chemistry

Chemotaxis

Defense Response

Aptosis

Chemistry Data

Subnetwork modules

Pathway Enrichment

Predictive Models

In silico knockdowns

Identify regulators

Target Cell Dose

Feedback Regulation

Adaptation

Lost Regulation

Normal Phenotype

Adverse
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
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