TERATOLOGY v2.0
– building a path forward

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Joseph Warkany Lecture
Teratology Society - June 29, 2014
Bellevue, WA

Disclosure Slide

DISCLAIMER: The views expressed in this presentation are those of the presenter and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

No conflicts of interest to disclose.
PhD training in Dave Kochhar’s lab hammered on the importance of approaching teratogenesis as a multiscale problem: the integration of genetic-biochemical-mechanical factors over space and time is of fundamental concern.

Having an incredibly supportive wife allowed my focus to remain on that problem for over 35 years - Thank you, Cyn!
Pushing the Boundaries: potential ‘Game-Changers’

One-liners solicited from ~35 Teratology Society members from different sectors, diverse expertise and age ranges; their responses in a nutshell (http://www.Wordle.net):

- “<advancing the> mechanistic understanding of gene-environment interactions.” – gd
- “<identifying> a single major sensitivity gene for a given exposure <for> pre-conceptional genetic testing and counseling ...” - rf
- “<defining what is normal given the> virtually limitless combination of alleles and environments ... in a global culture” – cc
- “<having> the tools and knowledge to understand the causes of most birth-defects rather than the minority that we do today.” – any
- “<funding> new initiatives for understanding developmentally-mediated disorders” collaboratively.” – ezf
We can ‘Push the Boundaries’ by:

• “<applying> synthetic biology <to> the relationships between mechanistic effects and phenotypic consequences.” – sh

• “<developing a pregnant> human-on-a-chip <platform> that incorporates microfluidics and is amenable to HTS.” – nk

• “<when a> computer gives birth to a virtual infant.” – any

• “modeling neurodevelopmental pathways in rodents, primates and humans, with extrapolation to C. elegans and zebrafish.” – emf

• “<having> a unified dose response approach to cancer and non-cancer endpoints ... raising the value of research in our field.” – any

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We can ‘Push the Boundaries’ by:

• “… determining which species was correctly predicting human response for each different exposure ...” – rc

• “… determining the molecular basis, or a refutation thereof, of a single unifying mechanism of teratogenesis”. – bb

• “… <shifting> the emphasis toward a collaborative effort to find plausible predictive mechanistic models.” – ns

• “<replacing> conventional descriptive methods with systems biology-based approaches ...” - ec

• “<using> a systems level in silico model as the basis for a regulatory decision involving a developmental hazard.” – any
Systems Toxicology: **decoding the toxicological blueprint** of active substances that interact with living systems

- Detailed mechanistic, quantitative and dynamic understanding of toxicological processes;
- Permitting **prediction** and accurate **simulation** of complex (emergent) adverse outcomes.

HTS/HCS platforms, advanced analytical tools, and computational models are transforming toxicology to a data-rich science.

<table>
<thead>
<tr>
<th>LTS</th>
<th>MTS</th>
<th>HTS</th>
<th>uHTS</th>
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<tr>
<td>10s – 100s/yr</td>
<td>1000s/day</td>
<td>10,000s – 100,000s/day</td>
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</tr>
<tr>
<td>gene expression</td>
<td>zebrafish</td>
<td>stem cells</td>
<td></td>
</tr>
<tr>
<td>Human Relevance</td>
<td>cost/complexity</td>
<td>throughput/ simplicity</td>
<td></td>
</tr>
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</table>
Chemical Testing under ToxCast and Tox21

- **ToxCast**: EPA research effort profiling >1060 chemicals across >800 in vitro assays (27M data points, ~1.7M conc. response curves).
  
  [http://www.epa.gov/ncct/toxcast/](http://www.epa.gov/ncct/toxcast/)

  - **Phase-I**: 310 data-rich chemicals (primarily pesticides) having over 30 years of traditional animal studies valued at $2B (completed 2011).
  - **Phase-II**: adds 767 chemicals (eg, industrial and consumer products, food additives, failed drugs) extend the broader chemical landscape (2014).
  - **Phase-IIIa**: adds 1001 compounds in a subset of ~100 assays (2014 - ); E1K adds 880 chemicals in ~50 endocrine-related assays.

- **Tox21**: partnership of federal agencies.
  - 8193 chemicals in dozens of HTS assays (ongoing)
  - brings total number of chemicals to ~10,000

iCSS Dashboard: public delivery portal for ToxCast data

[http://actor.epa.gov/dashboard/](http://actor.epa.gov/dashboard/)
EPA’s Children’s Environmental Health (CEH) Research Roadmap (see P40 by Sipes et al. in Tuesday’s poster session)

**Overarching research goal:** To provide the Agency and others with the information needed to incorporate consideration of early lifestage susceptibility and vulnerability into decision making.

**Research questions:**
- By what biological Adverse Outcome Pathways do environmental contaminants contribute to important childhood health outcomes (adverse birth outcomes, obesity, cognitive disorders, asthma)?
- What are the systems-level influences of the chemical, natural and built environments on these health outcomes?
- How can we evaluate the cumulative risk of chemicals including the contribution of non-chemical stressors?

**Priority research areas:**
1. Knowledge infrastructure to provide early lifestage-specific data and information.
2. Systems understanding of the relationship between environmental exposures and health outcomes across development.
3. Methods and model fit for purpose to evaluate early lifestage-specific risks and to support decisions protective of all early lifestages.
4. Translational research to incorporate CEH into tools fit for purpose to inform community actions and decisions.

**Predictive model:** prenatal developmental toxicity (phase-I)

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAR</td>
<td>Retinoic Acid receptor</td>
<td>0.58</td>
</tr>
<tr>
<td>GPCR</td>
<td>G-Protein-Coupled Receptors</td>
<td>0.55</td>
</tr>
<tr>
<td>TCF7L</td>
<td>Transforming Growth Factor β</td>
<td>0.38</td>
</tr>
<tr>
<td>MT</td>
<td>Microtubule organization</td>
<td>0.30</td>
</tr>
<tr>
<td>SENS_CYP</td>
<td>Cytochrome P450 (sensitive)</td>
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</tr>
<tr>
<td>API</td>
<td>Activator protein 1</td>
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</tr>
<tr>
<td>SLC19</td>
<td>Organic anion transporter 1B1</td>
<td>0.11</td>
</tr>
<tr>
<td>CYP</td>
<td>CYPs (other)</td>
<td>0.36</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>MHC complex</td>
<td>-0.38</td>
</tr>
<tr>
<td>PXR</td>
<td>Pregnan X receptor</td>
<td>-0.24</td>
</tr>
<tr>
<td>IL8</td>
<td>Interleukin 8</td>
<td>-0.23</td>
</tr>
<tr>
<td>PGE2</td>
<td>Prostaglandin E2 response</td>
<td>-0.18</td>
</tr>
</tbody>
</table>

**Multivariate Rat Model**
71% balanced accuracy

**Multivariate Rabbit Model**
74% balanced accuracy

**SOURCE:** Sipes et al (2011) Toxicol Sci 124
DevTox Model: features mapped to GO Biological Process

univariate DevTox features
multivariate DevTox features

Processes related to neovascularization (vasculogenesis and/or angiogenesis)

Toxicity Prioritization Index (ToxPi) based on ToxCast for vascular disruption

1060 Chemicals in ToxCast Ranked by pVDC score

Thalidomide structural analogue - disrupts angiogenesis
5HPP-33 (0.683)

Mitocide/insecticide - mitochondrial respiratory chain
Pyridaben (0.667)

Herbicide/weed control - acetohydroxyacid synthesis
Imazamox (0.02)
**AOPs:** putative vascular disrupting compounds (pVDCs) and their predicted impact on embryogenesis.

Minibrains and 3D Organotypic Culture Models

SOURCE: W Murphy, U Wisconsin


Minibrains and 3D Organotypic Culture Models

* Scale bars represent 250 µm

Hallmarks of Transformation

- **Embryogenesis** is a multicellular process – we need to visualize, analyze and model the dynamic nature of cellular interactions.

- so is **Teratogenesis** - even a few cell-cell interactions, disrupted at a critical time in development, can have an impact children’s health.

- major future **Challenge** - integrate the dynamics of these processes at different spatial scales during normal and abnormal development.

- a **Predictive** understanding depends on a global strategy to interact ‘big-data’ with ‘principles of teratology’ at a systems level.
Agent-Based Model (ABMs)

*Biological rules assigned to low-level ‘agents’ that then interact in a shared environment to display high-order (emergent) features.*

*In vitro*  
*In silico*

SOFTWARE: [www.CompuCell3D.org](http://www.CompuCell3D.org)  
James Glazier and colleagues, Indiana U

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FINS to LIMBS (... and back)

*Evolution and Development of the pentadactylous autopod*

HoxD (fin rays) → Gli3R (pentadactyly) → Gli3R (polydactyly) → HoxD ('fin' rays)

*Boot et al. (2008) Nat Met 5: 609*  
*Vogel (2012) Science 338: 1406*  
*Sheth et al. (2012) Science 338: 1476* (with permission from J Sharpe)
Morphogenesis: \textit{limb development}

PRE-PATTERNING (specification) \rightarrow SIGNALING (organization) \rightarrow INDUCING (differentiation)

\textbf{HoxD} \hspace{1cm} \textbf{D10} \hspace{0.5cm} \textbf{D11} \hspace{0.5cm} \textbf{D12} \hspace{0.5cm} \textbf{D13}

Limb outgrowth: \textit{control network}
Agent-Based Model: *CompuCell3D* simulation of key spatial temporal gradients patterning limb-bud outgrowth

*Source: Ahir, Knudsen et al. (NCCT)*

*ISH (mouse literature) vs ABM*
HYPOTHESIS

A computer model that executes the spatial and temporal dynamics of biological networks in the embryo can be used predictively to simulate developmental toxicity.
Translating *in vitro* data into simulation

**Impact of 5FU on the Shh-cell lineage**

**Impact of retinoids on the Grem1-lineage**

**Failability: running the models backward to reveal the earliest signs of failure (e.g., onset of Shh expression)**

Run #3
Onset of Shh expression is slightly ahead in this run, leading to an posterior bias in the FGF10 domain.

Run #4
Onset of Shh expression is slightly behind in this run, leading to an anterior bias in the FGF10 domain.
The Multiscale Problem

- Small disturbances at the cellular-molecular level might cascade into big effects as the system evolves to higher scales;

- (or large disturbances might be buffered prior to any observable outcome).

- Uncertainty on the microscopic scale (e.g., how disruption in one cell impacts the behavior of others) hinders our ability to predict the outcome at a macroscopic scale.


FLIPPING THE EGF/TGFβ3 SWITCH

<table>
<thead>
<tr>
<th>KEY EVENT:</th>
<th>TGFβ3</th>
<th>EGF</th>
<th>normal</th>
<th>TGFβ knockout</th>
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</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCDD (AhR Activation)</td>
<td>TGFβ3</td>
<td>EGF</td>
<td>WeaK dose</td>
<td>Strong dose</td>
</tr>
</tbody>
</table>

SOURCE: Hutson et al. (manuscript in preparation).
Blood vessel development disrupted by Thalidomide

Limb-bud outgrowth disrupted by SFU-induced mesenchymal loss

Genital tubercle outgrowth disrupted by AR-knockout

Palatal fusion disrupted by TCDD-induced AhR hyperactivation

Benefits and challenges of computational model for predicting developmental toxicity

- reconstructing spatial dimension and function (systems response)
- predicting impacts of cellular changes on dynamics (trajectories)
- quantifying the ‘un-measureable’ (lesion propagation)
- parameter sweeps to isolate key elements (sensitivity analysis)
- high-throughput hypothesis testing (mechanistic understanding)
- pinpointing nascent events underlying ‘emergent’ biology
- surrogate for missing data or information (knowledge gaps)
- probing pathway interactions (convergence, cumulative)
- simulating different exposure scenarios (ADME)
- not a living entity (can only code rules as we understand them)
- finding sweet-spot to enable, but not over-specify performance
- how complex do these systems model need to be (reality check)
- extending them for lifestage considerations / life-course model