Integrated Testing Strategies for Developmental Neurotoxicity (DNT)

Mamta Behl, Ph.D., DABT
Toxicology Branch
National Institute of Environmental Health Sciences

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Outline

• Background: What is DNT & why do we care?
• Evolution of DNT assessment at the NTP
• Recent Progress
• Where to from here?
What is DNT & why do we care?

- Adverse change in **structure** or **function** of nervous system following chemical exposure during prenatal/ gestational period
- Approx. 400,000-600,000 children born in the United States every year have neurodevelopmental disorders
- DNT causes brain damage often untreatable & frequently permanent

![Graph showing too many chemicals and too little data with a note on why this is so low.](https://example.com/graph.png)

*Courtesy: Tim Shafer, USEPA*
Why do we have such little data on DNT?

- Studying the nervous system is complex!
- Strategies to evaluate DNT are under developed
- *In vivo* DNT Guideline studies: primary method of evaluation
  - Require a trigger- apriori concern (structural relevance, anticipated use), or findings from a acute/subchronic study
  - Time & resource intensive
- Compounds with unknown DNT potential remain untested
  - Existing triggers not adequate → require better triggers
  - Guideline studies alone could not keep pace with untested compounds
At the NTP…

• Up to 2009: No method to evaluate compounds with potential for DNT on a routine basis
  – Case-by-case; mainly animal studies; mostly acute
  – 1 DNT Guideline study completed until 2009

• Increase in “class nominations”
  – e.g. flame retardants, BPA analogs, PFAS, PAHs, herbals
    – Approx 20-50 compounds/class

• Needed efficient approach to identify & characterize compounds with DNT potential
  – Global issue
Development of a Two Prong Approach

• Screen for compounds with potential for DNT
  – Rapid strategies; time & resource honored
    • Prioritize compounds for further testing
    • Complement & refine current DNT Guideline studies based on triggers

• Improve *in vivo* DNT testing
  – Increase automation/ objective measurements in Guideline studies
  – Integrate with routine developmental assessment v/s stand-alone
    • First study completed in 2017 → data QA → Report

Stay tuned for updates on *in vivo* DNT testing
Develop a Comprehensive DNT Screening Strategy

Identify Experts in the field

Identify assays covering MOAs for DT, DNT, acute neurotoxicity

Kristen Ryan  Brad Collins

Pool assays & expertise

Well-characterized Chemical Library

Create Battery for Comprehensive Screening

Unified data analysis strategy

Workshop: September 2017

Raymond Tice  Jui-Hua Hsieh  Fred Parham
Related Assays: General Tox, DT, NT, Neurobehavior

General Toxicity
- Receptor-based HTS
- Tox21

Developmental toxicity
- Zebrafish terata
- Planaria
- c.Elegans
- Stem cell - based early differentiation

Acute Neurotoxicity
- Neuronal Firing
- Protein aggregation
- Astrocyte senescence
- Cell death in NSC, iPSC,
  Neurons, astrocytes
- Receptor-based HTS
  Tox Cast

Neurobehavior
- Zebrafish motor activity
- Planaria
- c.elegans

Coverage: Rat + Mice + Human + zebrafish + planaria + c.elegans
Created an initial battery to integrate assays

What combination of assays is required to cover most aspects of DNT?
Some questions we were interested in:

- Which of these assays are critical for a battery?
  - Evaluation of individual assay & in combination
  - Missing areas
- How can the data be integrated across assays?
- How might this information be used in regulatory decision-making?
  - Limited access for free and open discussion and to protect researchers unpublished data
NTPs Data Integration Approach
NTPs Proposed Approach for Data Analysis

- Several different ways to analyze data
- For battery, need a unified approach
- After considering different approaches, selected a BMC
  - Used in risk assessment

BMC = Benchmark Concentration
NTP Analysis Approach

Chemical

<table>
<thead>
<tr>
<th>No BMC calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active No</td>
</tr>
<tr>
<td>YES</td>
</tr>
<tr>
<td>Calculate BMC</td>
</tr>
<tr>
<td>Active but not selective NO</td>
</tr>
<tr>
<td>YES</td>
</tr>
<tr>
<td>Calculate Selectivity</td>
</tr>
</tbody>
</table>

BMC = Benchmark concentration

GENERAL TOXICITY
- Neuro-specific endpoint
- Cytotoxicity
- Concentration
- Both BMCs similar

SELECTIVE TOXICITY
- BMC (neuro-specific)
- BMC (cytotoxicity)
Integration of Data Visualization
NTP Created a Website for Data Visualization

Database -> NTP Server (temporarily password protected) -> 1 million readouts!

Expected to be made public following primary publications

Ju-Hua Hsieh  Andy Shapiro
Questions that we can begin to ask...

- How do compounds/classes look within an assay?
  - Does one compound within a class look different from the others?

- How do compounds/classes look across assays?
  - Are there assays/combinations that are informing us about potential targets within the nervous system?

- How do assays in the battery compare with each other?
  - Concordance/uniqueness

- How do positive and negative controls perform?

- How do test replicates perform?
Chemical/Class Results within an Assay

Benchmark concentration (BMC) summary by lab

Select an assay(s):
- Biomod 01: zebrafish behavior/development
- Biomod 01: neuron viability
- Biomod 01: neural progenitor aggregation
- Biomod 01: neuron outgrowth
- Biomod 01: neuron migration
- Biomod 01: neuron RNAi

Select readout(s):
- ModDevices: neurite outgrowth
- Total neurite
- Total branch
- Total process

Select BMC model(s):
- H3
- CurveP

Select visuals:
- Activity
- Selectivity

Axis scale:
- Log10
- Linear
- Sort

BMC values: sorted by activity

Benchmark concentration value (µM)

[Graph showing various chemicals and their benchmark concentration values]
Comparing across assays
Some interesting patterns that emerged:

- Total 66 out of 80 compounds active in at least one assay
- Different models & endpoints capture different actives
- Zebrafish development covered largest space
- If a compound is active in more than one assay, may inform about patterns
- Negative controls as expected

Take Home: Different domains contributed something unique
NEED FOR A BATTERY APPROACH
Highlights of Workshop Discussion
Six Critical Areas Discussed: Highlights

- Experimental Design Considerations
  - Dose ranges, time-points, gender, life-stage, experimental conditions

- Biological Coverage: what are we missing?
  - 3-D models, life stages, BBB, glia, other critical processes
  - Integration with other systems toxicity

- Exposure & Metabolism
  - Physico chem properties, barriers & partitioning, internal conc.

- Data analysis & Modeling
  - Challenges with statistical models; relevance to biology

- Regulatory Perspective: Complement, Replace, Develop
Next Steps & Future Directions

- Workshop: initial effort on integration using battery approach
  - Publications & releasing website to public
- Continued global discussion on utility of approach
- Continued discussions on data analysis strategies
- External scientific panel for evaluation of battery
- Continue to refine the battery
  - Solicit assays in missing areas identified
- Implement as routine screening at the NTP
  - Feed into *in vivo* testing
Key Contributors for Screening Efforts

- Ainhoa Alzualde: Biobide
- Julie Andersen: Buck Institute
- Peter Ash: Boston University
- Francis Bailey: Health Canada
- Stan Barone: U.S. EPA
- Linda Birnbaum: NIEHS/NTP
- John Bucher: NIEHS/NTP
- Bradley Collins: NIEHS/NTP
- Eva-Maria Collins: UC San Diego
- Kevin Crofton: U.S. EPA
- Katharina Dach: UC Davis
- Eduardo Gonzalez: UC Davis
- Danielle Hagstrom: UC San Diego
- Susanne Hougaard Bennekou: Danish EPA/EFSA
- Pamela Lein: UC Davis
- Elizabeth Mendez: U.S. EPA
- Rafael Miñana Prieto: ZeClinics
- Arantza Muriana: Biobide
- Johanna Nyffeler: University of Konstanz
- Stephanie Padilla: U.S. EPA
- Ying Pei: Xcell Science Inc.
- Mahendra Rao: Xcell Science Inc.
- Elisa Reaves U.S. EPA
- Magdalini Sachana: OECD
- Timothy Shafer: U.S. EPA
- Nisha Sipes: NIEHS/NTP
- Oksana Sireno: Molecular Devices
- Robert Tanguay: Oregon State University
- Javier Terriente: ZeClinics
- Lisa Truong: Oregon State University
- Molly Vallant: NIEHS/NTP
- Tanja Waldmann: University of Konstanz
- Benjamin Wolozin: Boston University
- Georgia Woods: Buck Institute

National & International Representation from Academia, Government, Industry, and Regulatory community