Integrated Testing Strategies for Developmental Neurotoxicity (DNT)

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• 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.

**Diagram:**

- **Too Many Chemicals**
  - 9912
  - IRIS, TRI, Pesticides, Inerts, CCL 1 & 2, HPV, MPV
- **Too Little Data (%)**
  - Developmental Neurotoxicity < 1%
  - Acute, Cancer, Sentox, Dev. Tox, Repro. Tox

*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
- \textit{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 $\rightarrow$ 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

1. Identify Experts in the field
2. Identify assays covering MOAs for DT, DNT, acute neurotoxicity
3. Pool assays & expertise
4. Create Battery for Comprehensive Screening
5. Workshop: September 2017

Well-characterized Chemical Library
Unified data analysis strategy
Assay identified to evaluate DNT

Rat, mouse + human lines (including DO mice lines)

Apoptosis -> Neurite outgrowth

Synaptogenesis

Neurite growth

Myelination

Neural network formation & function

Neuronal firing: MEAs

Proliferation

Differentiation

Migration

Neuronal proliferation

Coverage at workshop
Received data post-workshop
Still lacking coverage

Aschner et al., 2017 & Mundy
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

Battery

DEVELOPMENTAL TOXICITY
- Mortality
  - Zebrafish and planaria
- Development (e.g., terata)
  - Zebrafish and planaria

DEVELOPMENTAL NEUROTOXICITY
- Neurite Outgrowth
  - Human: iPSC, LUHMES, peripheral neurons
- Neuronal Migration
  - Human: LUHMES, peripheral neurons
- Neuronal Firing
  - Rat primary cortical cells
- Behavior (Activity)
  - Zebrafish and planaria

RECEPTOR-BASED SYSTEMS TOXICITY
- HTS:Tox21 & Toxcast
- Human cell lines (variety)

NEUROTOXICITY
- Protein Aggregation
  - Rat: PC12 cells
- Senescence
  - Human: iPSC astrocytes
- Cell death
  - Human: NSC, iPSC, neurons, astrocytes

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

- No BMC calculated
- Active: In any of the endpoints/assays
  - YES
  - Calculate BMC
  - NO
  - Active but not selective

Calculate BMC

Selectivity To neuro-specific endpoints

Calculate Selectivity

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

SELECTIVE TOXICITY
- BMC (neuro-specific)
- BMC (cytotoxicity)

BMC = Benchmark concentration
Integration of Data Visualization
NTP Created a Website for Data Visualization

1 million readouts!

Database

NTP Server (temporarily password protected)

Expected to be made public following primary publications

Jui-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:
- Biocide 01: ratfish behavior/development
- Dermal 01: neuron protein accumulation
- Melanoma 02: neuron outgrowth
- 0/3-3/9 axolotl behavior/development
- UCSD 80: planaria behavior/development
- UCSD 90: neuron CNS outgrowth
- UCSD 108: neuron CNS migration
- UCSD 90: neuron CNS outgrowth
- USEPA 01: neuron firing
- Zebra 03: neuron development

Select readouts:
- MoaDevices: neuron outgrowth
- Total branches
- Total outgrowth
- Total processes

Select BMC models:
- HI
- CurveP

Select visuals:
- Activity
- Selectivity

Axis scales:
- Log10
- Linear
- Sort

BMC values: sorted by activity

Benchmark concentration value (µM)
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
The NTP Workshop Team

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Key Contributors for Screening Efforts

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- Peter Ash: Boston University
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National & International Representation from Academia, Government, Industry, and Regulatory community