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National Toxicology Program

Integrated Testing Strategies for Developmental Neurotoxicity (DNT)

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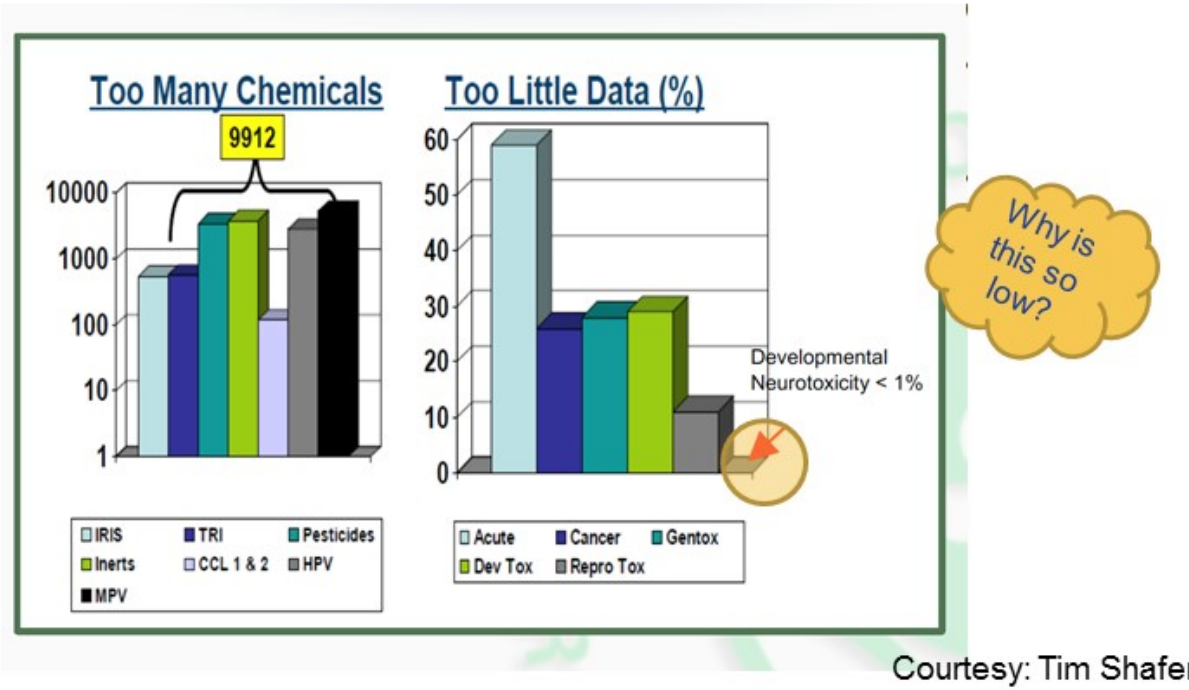
Outline

- 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



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

Create Battery for Comprehensive Screening

Well- characterized Chemical Library

Unified data analysis strategy

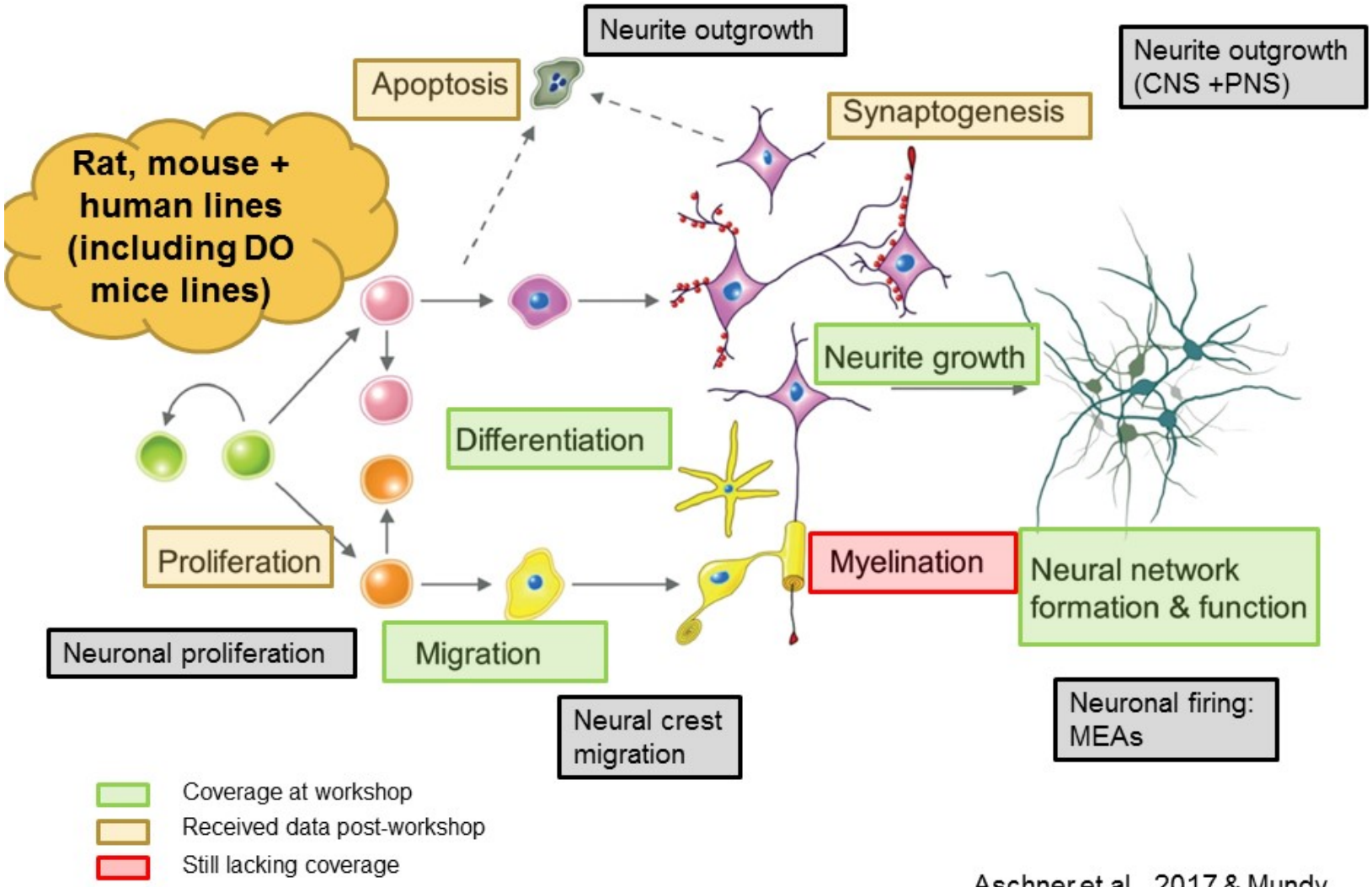
Workshop: September 2017

Raymond Tice

Jui-Hua Hsieh

Fred Parham

Assay identified to evaluate DNT

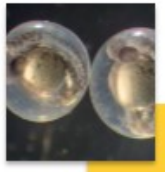


Aschner et al., 2017 & Mundy

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 senescene
- 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

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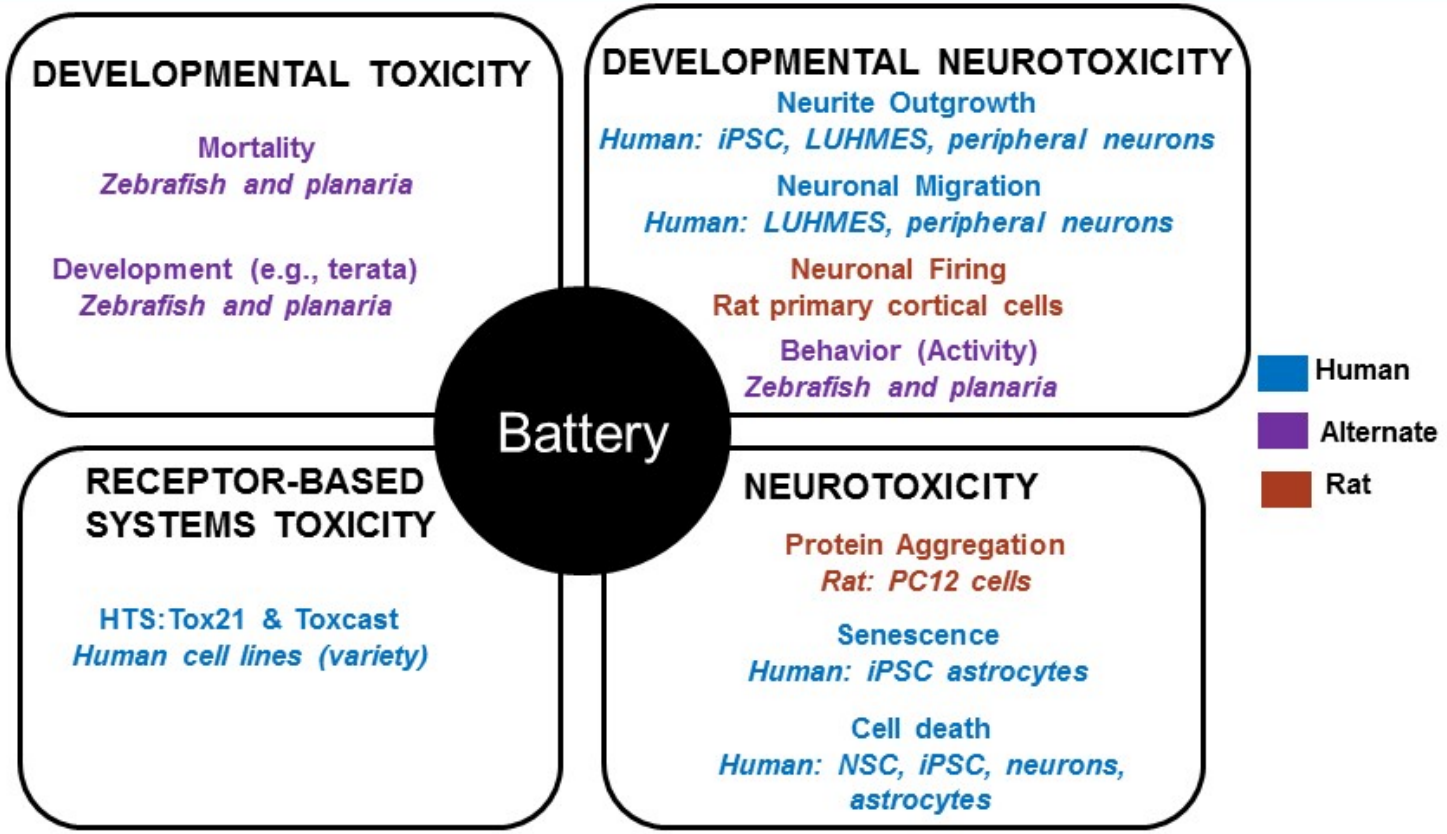
Created an initial battery to integrate assays



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

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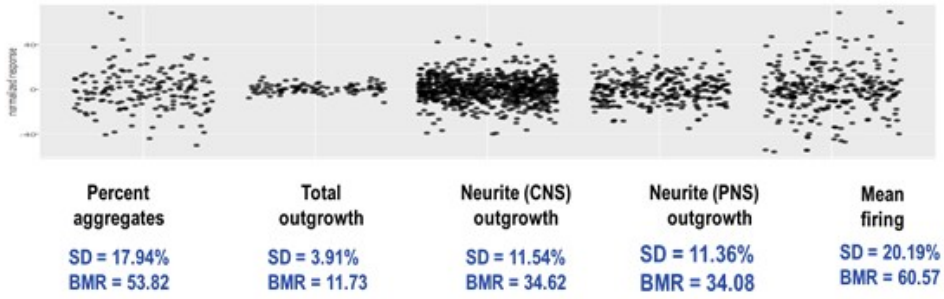
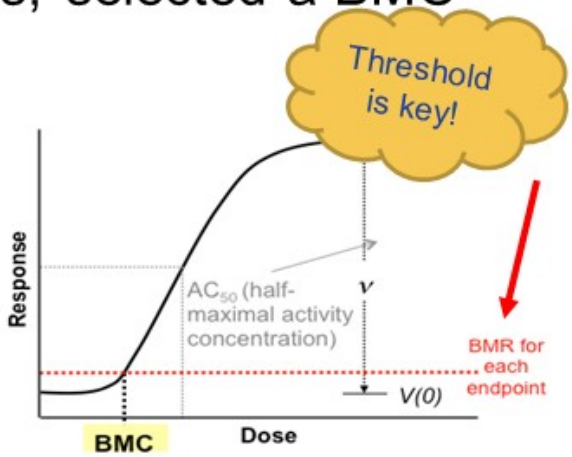
NTPs Data Integration Approach

NTPs Proposed Approach for Data Analysis

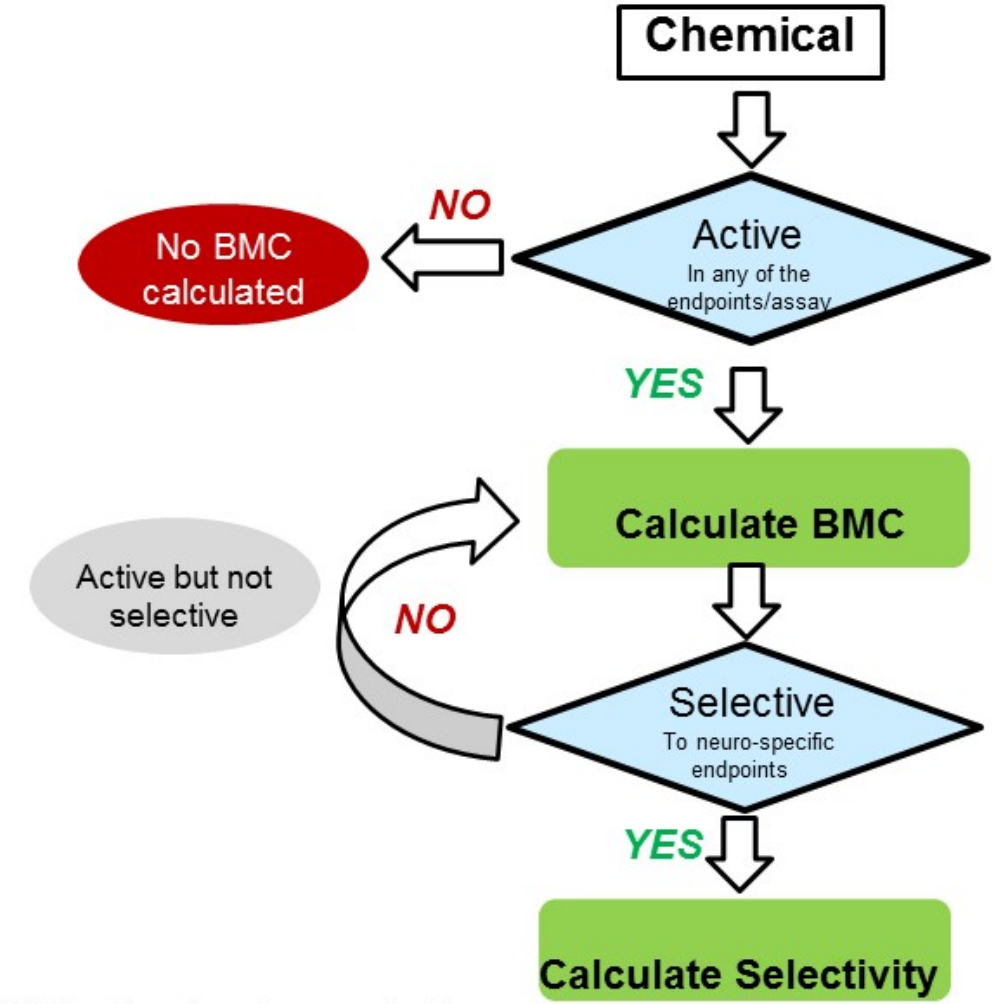
- Several different ways to analyze data
- For battery, need a unified approach
- After considering different approaches, selected a BMC
  - Used in risk assessment

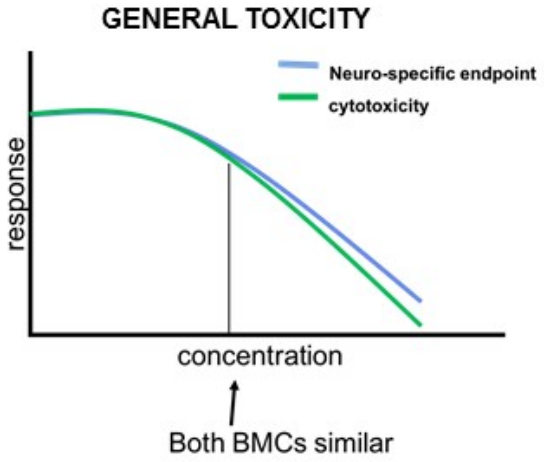


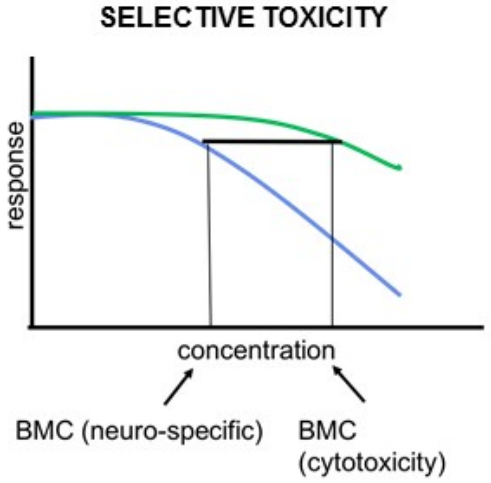


BMC = Benchmark Concentration

NTP Analysis Approach







BMC = Benchmark concentration

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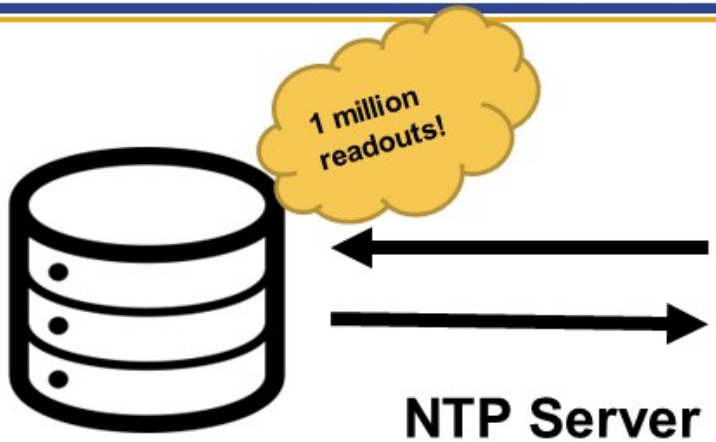
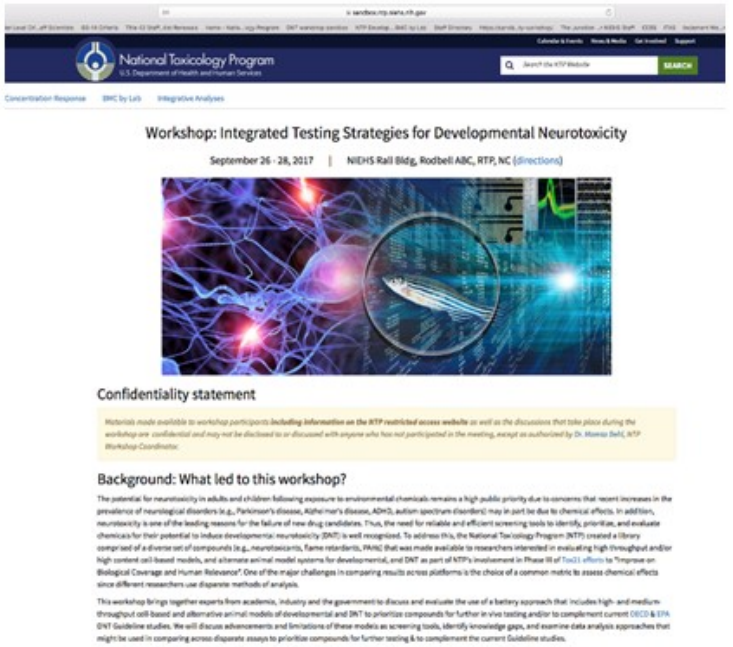
Integration of Data Visualization

NTP Created a Website for Data Visualization





Expected to be made public following primary publications

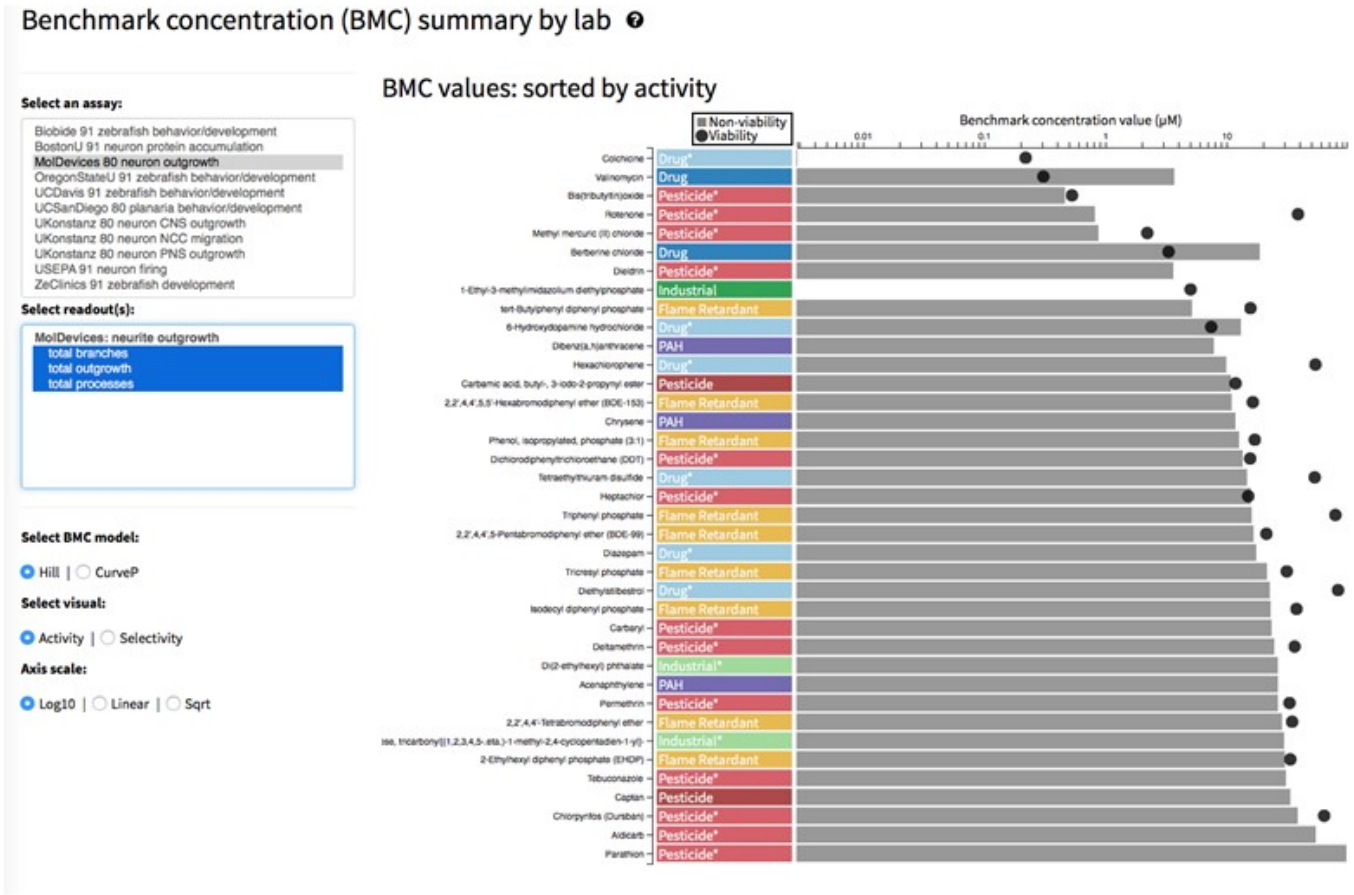
Jui-Hua Hsieh

Andy Shapiro

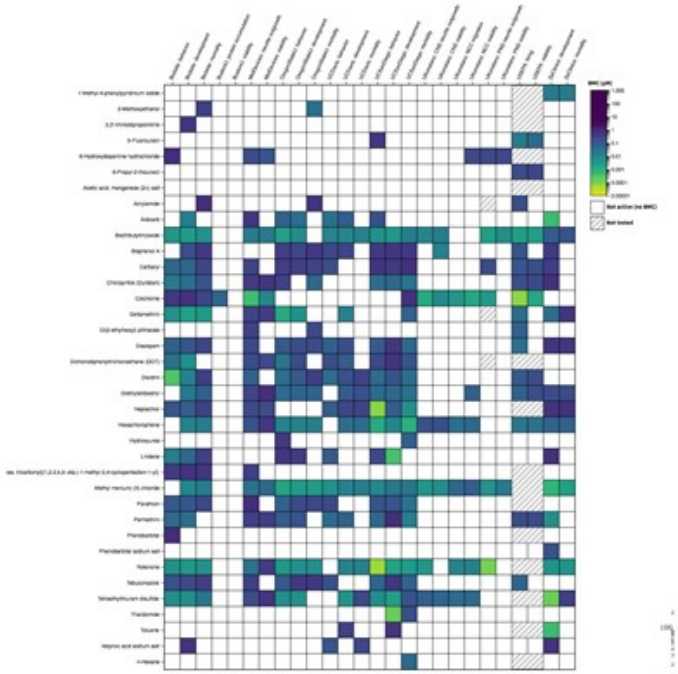
Questions that we can begin to ask…

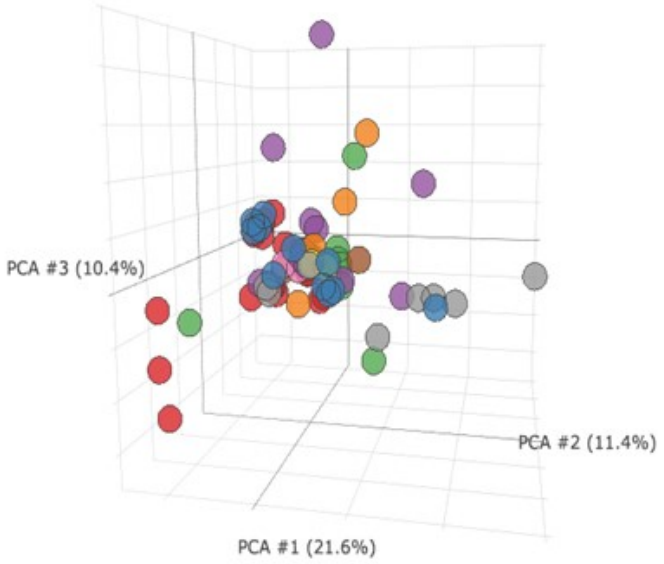
- How do compounds/ classes look within an assay?
  - Does one compound within a class look different from the others?
- How do compounds/ classes look across assays?
  - Are there assays/ combinations that are informing us about potential targets within the nervous system
- How do assays in the battery compare with each other?
  - Concordance/ uniqueness
- How do positive and negative controls perform?
- How do test replicates perform?

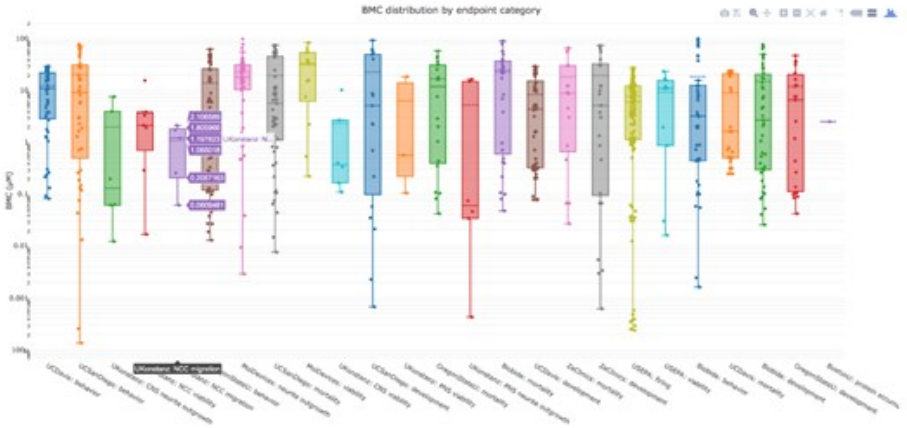
Chemical/Class Results within an Assay



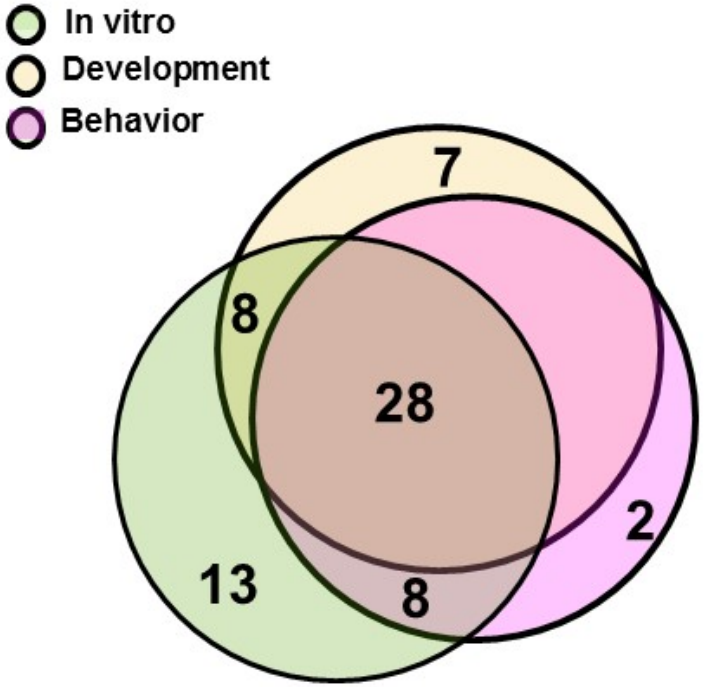
Comparing across assays







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

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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

Mamta Behl
Organizer & Coordinator

Kristen Ryan
Co- Coordinator & Organizing Committee

Jui-Hua Hsieh
Data Analyst & Application Development

Andy Shapiro
Application Lead, Database Lead, & System Administrator

Fred Parham
Data Analyst

Elizabeth A. Maull
Co-organizer

Raymond R. Tice
Co-organizer

Richard S. Paules
Co-Organizer

Paul M. Foster
Organizing Committee

Nigel Walker
Organizing Committee

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
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- Arantza Muriana: Biobide
- Johanna Nyffeler: University of Konstanz
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- Benjamin Wolozin: Boston University
- Georgia Woods: Buck Institute

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