Developmental Neurotoxicity Health Effects Innovation Program

Mamta Behl, PhD, DABT
Division of the NTP
National Institute of Environmental Health Sciences

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• Increase in prevalence of neurodevelopmental disorders in the US and globally
  – WHO: 1 in 6 kids diagnosed at birth

• Strategies to evaluate DNT underdeveloped

• *In vivo* DNT Guideline studies primary method of evaluation
  – Require an *apriori* trigger to be run
  – Time & resource intensive
  – Relevance of animal studies for human translation have been questioned

• Compounds with unknown DNT potential remain untested

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*Centers for Disease Control and Prevention (CDC) prevalence estimates are for 4 years prior to the report date (e.g., 2018 figures are from 2014) Source: autismspeaks.org, “CDC increases estimate of autism’s prevalence by 15 percent, to 1 in 59 children”*
There is a Need for a New Framework for Assessing DNT

“Our system for evaluating scientific evidence and making decisions about environmental chemicals is broken. We cannot continue to gamble with our children’s health”

Consensus statement on the need for innovation, transition and implementation of developmental neurotoxicity (DNT) testing for regulatory purposes
Exposure assessment & clinical translation

• Integrate exposure with study findings using IVIVE* and computational tools
• Enhance communication with stakeholders to understand issues and provide translatable data

DNT Screening

• Prioritize compounds for further testing
• Rapid, high-content, cell-based assays, and complementary animal models
• Complement and refine in vivo

In vivo Testing

• Automated behavior
• Neuroimaging
• Novel Biomarkers

Why Us?

• Cross divisional teams with multidisciplinary expertise
• Building on past experience

* IVIVE = In vitro in vivo extrapolation
Implement a DNT screening battery that covers key neurodevelopmental events

2-D assays
3D- Neurospheres
Zebrafish

DNTP’s Proposed Battery: Initial Assay Selection
What led to selection of the current assays?

- Key neurodevelopmental processes; perturbed → DNT?
- Meet high readiness criteria
  - Biological plausibility
  - Test system description
  - Good robustness
- Implementation for practical applications in industry/regulatory purposes
- High throughput; ready for prime-time

Bal-Price et al., 2018
Objective 1: Building on DNTP’s Past Experience

Pilot: DNTP Workshop, 2017

NTP Chemical Library

Drugs
Flame Retardants
Industrial
PAH
Pesticides
Negatives

Negatives performed as expected

Aschner et al., 2017 & Mundy 2015
Objective 1: Building on NTP’s Past Experience

DNT- Data Integration and Visualization Enabling Resource (DNT-DIVER)

Compare activity of compounds/classes across multiple assays

Example: Flame Retardants

Compare activity of compounds/classes across multiple assays

Individual dose-response curves

Plate and well level information

Control variability in assay

https://sandbox.ntp.niehs.nih.gov/neurotox/
Objective 1: Applications of Battery

Prioritizing for Further Testing

e.g. Flame Retardants

- 2,2′,4,4′,5,5′-Hexabromodiphenyl ether (BDE-153)
- 2,2′,4,4′,5-Pentabromodiphenyl ether (BDE-99)
- 2,2′,4,4′-Tetrabromodiphenyl ether
- 2-Ethylhexyl diphenyl phosphate (EHDP)
- 3,3′,5,5′-Tetrabromobisphenol A (TBBPA)
- Isodecyl diphenyl phosphate (IDDP)
- Isopropylated phenyl phosphates (IPP)
- Trimethyl phenyl phosphate (TMP)
- Triphenyl phosphate (TPHP)
- Tris (2-chloroethyl)phosphate (TCEP)
- Tert-Butylphenyl diphenyl phosphate (BPDP)

Novel replacements show comparable activity to phased-out compounds

Data publicly available on DNT-DIVER
https://sandbox.ntp.niehs.nih.gov/neurotox/
For use in decision-making

1. Novel substitutes have comparable in vitro activity to older flame retardants.
2. In vitro activity within order of magnitude of in vivo point of departure (POD) (when known).
3. Activity lies within range of human exposure (limited exposure data for novel compounds).

When animal studies may not provide the answer…

- Zika infects hiPSC-hNPC
- Zika infection dysregulates cell cycle and transcription in hNPCs
- Zika infection attenuates hNPC growth and induces apoptosis

Guideline studies did not identify effects in humans
MOA discovered using 3-D neurospheres

Olagnier et al. 2016 DNA AND CELLBIOLOGY

Slide Courtesy: Ellen Fritsche
Objective 1: Global Contribution to DNT

How will our battery fill a global niche?

Increase coverage by concerted screening efforts in multiple assays

Address NTP’s DNT-related nominations more efficiently

Assay-specific Compound Lists; Focused on in vivo DNT

Assay 1
Assay 2
Assay 3…

Assay Evaluation
Call for Screening 100 compounds

Objective 1: Recent Progress

~ 300 nominations received from ~ 35 individuals representing different sectors

*DNT Consulting Consortium
R3 Fellows LLC, IDN Consulting, OECD, EFSA, USEPA, Academia, Health Canada
Develop and Implement 7 *in vitro* assays to screen 100 compounds

**Objective 1: Recent Progress**

**Selection of Initial Battery Assays**
- Completed

**Prioritization of Chemicals for Screening**
- Ongoing

**Procurement of Compounds**
- Completed

**Screen compounds (IAA)**
- Not yet started

**Screen Compounds (Contracts)**
- To Do

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IAA: Inter Agency Agreements
Objective 2

Assess novel DNT assays and technologies: In vitro

♂ / ♀ DO Mice  DO ESCs  DO NPCs

Incorporating Genetic Diversity

Microfluidics

Nikolakopoulou et al., 2020
DO Figure Courtesy: Dahea You
Assess novel DNT assays and technologies: In vivo

- Issue: Clinical observations are the primary diagnostic tool in patients, and yet current rodent evaluations are subjective & often miss critical time-periods (e.g., nighttime)

- Proposed solution: Explore automated 24x7 monitoring- overlay artificial intelligence

Linking mechanistic bioactivity to clinical end-points
Issue: Representing the entire brain with limited number of histological sections may not be sufficient to capture subtle changes that may occur in neurodevelopmental disorders.

Proposed Solution: evaluate potential for incorporation of imaging tools to capture structural & functional changes.

Diffusion tensor connectomics to identify alterations in networks.
Establish communication pipelines with stakeholders and public
...Expanding to include clinicians, industry, advocacy groups..
<table>
<thead>
<tr>
<th>Milestone</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
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<tbody>
<tr>
<td>Develop and implement seven <em>in vitro</em> assays</td>
<td>Selection of assays, chemicals, &amp; contractors</td>
<td>Test &amp; Report</td>
<td></td>
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<tr>
<td>Develop and conduct novel automated home-cage monitoring</td>
<td>Purchase cages</td>
<td>Test &amp; Report</td>
<td></td>
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<tr>
<td>Communications (listserv, website, DNT-DIVER)</td>
<td>Establish</td>
<td>Refine</td>
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<td>Establish program for post-doctoral fellow via the Faculty for Advancing Neurosciences</td>
<td>Currently advertised</td>
<td>Implement plan</td>
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<td>Identify functional links between <em>in vitro</em> and <em>in vivo</em> tests</td>
<td>Build</td>
<td>Test &amp; Report</td>
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<td>Contextualize findings from <em>in vitro/in vivo</em></td>
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<td>Test &amp; Report</td>
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<td>Establish strategy for imaging studies</td>
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<td>Design and test</td>
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<td>Integrate DNTP capabilities across translational toxicology pipeline</td>
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<td>Establish process</td>
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Ultimate goal is to more effectively predict DNT for unknown environmental chemicals to prevent neurodevelopmental disorders.
There is currently no comprehensive method to evaluate compounds with unknown DNT potential

Compounds remain largely untested and susceptible populations continue to be exposed

Our effort is an initial step in the long journey of preventing neurodevelopmental disorders due to environmental factors
Thank You!
Question 1

What are you most excited about?
Question 2

Please share your insights about the Program regarding:

a. how the objectives address the problem/opportunity
b. the boldness of the approach to achieve the objectives
c. the alignment of the metrics to the desired impact
Considering DNTP’s capabilities and expertise, what mechanisms do you suggest that we consider to be able to effectively execute against the objectives? With whom might we partner to ensure success?
The disease-focused approach of the Health Effects Innovation Programs is novel in toxicology and hazard assessment. What unique challenges are we likely to encounter in taking that approach for developmental neurotoxicity? What near and mid-term deliverables might reinforce our decision to take that approach?
Question 5

A key theme of the NIEHS Strategic Plan is ‘Data to Knowledge to Action’. At what level of detail do we need to characterize neurodevelopmental hazards to enable public health-protective decisions by individuals, regulatory scientists and policy makers? For example, at the level of bioactivity in the developing central nervous system, induction of adverse changes in morphology or function or at the mechanistic level?