Developmental Neurotoxicity (DNT)
Health Effect Innovation (HEI)

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NTP Board of Scientific Counselors Meeting
June 18, 2019
• Program Management Team
• DNT-HEI Aims
• DNT Public Health Problem
• Environmental Links to Neurodevelopmental Disorders
• DNT History and Testing Challenges
• DNT Modeling Opportunities and New Framework
• DNT Screening, *In Vivo* and Translational Strategies
Developmental Neurotoxicity-HEI Aims

- Have global public health impact by identifying environmental chemicals that have the greatest potential to affect susceptible populations (developing embryo/fetus, infants, children)

- Provide a forum for collaborations among NIEHS scientists, NIH, EPA, and FDA’s National Center for Toxicological Research; engagement with external stakeholders, including clinicians and children health advocacy groups

- Training the next generation of scientists on all aspects of neurodevelopmental disorders related to environmental exposures and on the very latest technology
Public Health Problem

Increasing prevalence of learning and behavioral disabilities and neurodevelopmental disorders in children


Attention Deficit and Hyperactivity Disorder (ADHD)

1 in 10 children in the US are estimated to have ADHD (CDC 2012)

Sources: abcnews.go.com, “ADHD rates in kids have increased over the past 20 years, new study says”; Journal of the American Medical Association (JAMA)

Autism Spectrum Disorder

Estimated Autism Prevalence 2018

* 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”

• Economic costs associated with neurodevelopmental disorders is staggering, estimated to be approximately $461 billion by 2025
Leading scientists came together to issue a call to action to reduce widespread exposures to chemicals that interfere with fetal and children’s brain development.

Summarized environmental links to neurodevelopmental disorders:

- Organophosphate pesticides
- Polybrominated diphenyl ether flame retardants
- Combustion-related air pollutants
- Phthalates
- Lead
- Mercury
- PCBs

Source: Environmental Health Perspectives, July 2016, Vol. 124, No. 7
• A goal of the DNT-HEI program is to develop a new framework for identifying environmental chemicals that can contribute to neurodevelopmental disorders and thereby reduce the potential for learning disabilities in our future generation.
- Stand alone DNT study
- Created guidance document for DNT studies
- DNT studies incorporated into perinatal inhalation and reproductive studies
- Conducting and reporting studies
DNT Study Design - Continuous Exposures

- GD 6
- GD 21
- Wean
- PND 28: Detailed clinical observations: 2x/d every 2-3 days until weaning and weekly thereafter

Limitations:
- Number of DNT studies conducted: 4

Challenges:
- DNT studies resource intensive - time, money and number of animals
- Limited potential for understanding mechanisms of neurodevelopmental disorders

- PND 30-34: Motor activity
- Motor Function
- Motor and sensory function

- PND 60-70: Motor activity
- Motor Function
- Motor and sensory function
- Morris water maze

- ~PND 120: Brain weights
- Neurohistopathology (CNS/PNS)
DNT Modeling Opportunities

Understanding mechanisms linked to major neurodevelopmental process

- NTP is developing a battery of *in vitro* assays anchored to key neurodevelopmental processes for evaluating DNT effects

**Timeline of Major Events in Human Brain Development**

Source: Tan, G.Z., et. al., Neuropsychopharmacology Reviews, 5:147-168, 2010
There is a Need for a New Framework for Assessing DNT

- Rapid and balanced assessment strategy for evaluating behavior, structural and chemical disruption, and understanding mechanisms of DNT

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

Project TENDR Consensus: Targeting Environmental Neuro-Developmental Risks.
Source: http://dx.doi.org/10.1289/EHP358
• Integrated Translational Toxicology Pipeline is key to addressing DNT public health problems

• Applying our capabilities in deliberate, integrated and complementary ways
Overview

- Program Management Team
- DNT-HEI Aims
- DNT Public Health Problem
- Environmental Links to Neurodevelopmental Disorders
- DNT History and Testing Challenges
- DNT Modeling Opportunities and New Framework
- DNT Screening, *In Vivo* and Translational Strategies
Global Rise in Neurodevelopmental Disorders

Official report from Japanese Ministry of Education, 2017

http://www.mext.go.jp/a_menu/shotou/tokubetu/__icsFiles/afieldfile/2018/05/14/1402845_03.pdf

Attention deficit hyperactivity disorder
Learning disability
Autism
Emotional disorder
Hearing, Amblyopia, etc
Speech disorder

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Slide Courtesy: Kentaro Tanemura
NTP’s Integrative Testing Strategy for DNT

**Exposure assessment & clinical translation**
- Integrate exposure with NTP study findings using IVIVE and computational tools
- More direct communication with stakeholders to understand issues and provide translatable data

**In vivo Testing**
- Automated behavior
- Imaging techniques
- New Biomarkers

**DNT Screening**
- Prioritize compounds for further testing
- Rapid high-content cell based assays + alternative animal models
- Complement & refine *in vivo*

**Exposure assessment & clinical translation**
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**DNT Screening**
- Prioritize compounds for further testing
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DNT Screening
DNT Screening: What have we done so far?

(Not so) Historic Perspective

Stand-alone DNT study

Created guidance document for DNT studies

- DNT studies incorporated into perinatal inhalation and reproductive studies
- Conducting and reporting studies


Created library to screen assays targeting neurodevelopmental processes

First NTP paper published on concept and application of battery for DNT screens

Workshop: Integrated Testing Strategies for DNT

Updated BSC on workshop goals and outcomes

Updated BSC on interactive web-based tool (DNT-DIVER)
• The NTP created a free, publicly available interactive web-based tool known as DNT- DIVER
  – Developmental NeuroToxicity Data Integration and Visualization Enabling Resource

• First data integration and visualization resource that allows for a comparison of chemical effects across assays that cover critical nodes of neurodevelopment

• Created due to the recognition that neurodevelopment is complex; one assay will not be sufficient to address the complexity of the nervous system
  – Need for a battery

https://sandbox.ntp.niehs.nih.gov/neurotox/
DNT Screening: What questions can we now address?

- How do compounds/classes with unknown DNT potential look across a battery of assays?
  - Overlapping vs unique assays

- How do compounds/classes look within an assay?
  - QSAR vs Biology

- How does data with the knowns compare with that found from in vivo studies?
  - Can this method actually be used to prioritize compounds?
Current *In Vitro* Coverage in DNT Screening

Human-derived high content, cell-based functional assays

- **Compounds Library Categories**
  - Drugs: 18%
  - Flame Retardant: 22%
  - Industrial: 6%
  - PAH: 20%
  - Pesticides: 17%
  - Negative: 17%

**Current In Vivo Coverage in DNT Screening**

- **Human-derived high content, cell-based functional assays**
  - **Apoptosis**
    - Paraquat, Rotenone, Colchicine
  - **Synaptogenesis**
    - Neurite growth
    - Paraquat, Rotenone, Colchicine
  - **Proliferation**
    - 5-FU
  - **Differentiation**
    - Valproic acid, Lead acetate
  - **Migration**
  - **Myelination**
    - Hexachlorophene, DDT, BPDP, BDE-47, Carbaryl
  - **Neural network formation & function**
    - BDE-99, BDE-47, EHDP

**Aschner et al., 2017 & Mundy**
Current Alternative Model Coverage in DNT Screening

• Behavior in complementary animal models
  – Zebrafish: Motor activity
    • Other assays being evaluated in lower throughput systems (startle, L&M)
  – Planaria: gliding and swimming, phototaxis, thermotaxis, and scrunching
Most of these fall under “high readiness "criteria as recently determined by OECD (OECD 2017)
For further details..

FORUM

Screening for Developmental Neurotoxicity at the National Toxicology Program: The Future Is Here

Mamta Behl,*† Kristen Ryan,* Jui-Hua Hsieh,† Frederick Parham,* Andrew J. Shapiro,* Bradley J. Collins,* Nisha S. Sipes,* Linda S. Birnbaum,* John R. Bucher,* Paul M. D. Foster,* Nigel J. Walker,* Richard S. Paules,* and Raymond R. Tice†
For further details...

ONLINE COLLECTION IN TOXSCI

https://academic.oup.com/toxsci/pages/ntp_collection

DNT-DIVER

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

Research shows that a child's developing nervous system is far more vulnerable to chemical exposures than an adult nervous system. Recent increases in the rise of neurodevelopmental disorders such as attention deficit hyperactivity disorder (ADHD), dyslexia, and autism spectrum disorder have prompted scientific interest in the potential contribution of environmental toxicants to these disorders.

Traditional animal, or in vivo, studies provide important information about developmental neurotoxicity (DNT) but they are time and resource intensive. NTP has also developed more rapid screening tools that use human cell-based, or in vitro, assays, as well as alternate animal models such as zebrafish and planaria to identify toxicants with potential for DNT. Multiple tests, or assays, are often required to represent the complexity of the developing nervous system, but that can make it challenging to compare and summarize results.

NTP designed the Developmental NeuroToxicity Data Integration and Visualization Enabling Resource (DNT-DIVER) to analyze, compare, and visualize multiple DNT assays in an interactive web-application.

Within DNT-DIVER, comparisons are organized on different tabs including experimental design summary, quality control, chemical-specific concentration response curves, ranking of chemical toxicity per lab/assay, and comparison of results across assays. We also provide downloadable resources including raw and final data, along with the NTP Chemical Library (Microsoft Excel) that was tested by collaborators in their respective models.

Suggestions?
We are currently refining assays, analyses, and visualizations, and would appreciate your input! Please direct questions or suggestions to Mamta Behl.

https://sandbox.ntp.niehs.nih.gov/neurotox/

ToxSci NTP Collection

Screening for Developmental Neurotoxicity at the National Toxicology Program: The Future is Here
Mamta Behl, Kristen Ryan, Jui-Hua Hsieh, et al.

Comparative Analysis of Zebrafish and Planarian Model Systems for Developmental Neurotoxicity Screens Using an 87-Compound Library
Danielle Hagstrom, Lisa Truong, Siqi Zhang, Robert Tanguay, and Eva-Maria S. Collins

Multi-Behavioral Endpoint Testing of an 87-Chemical Compound Library in Freshwater Planarians
Siqi Zhang, Danielle Hagstrom, Patrick Hayes, Aaron Graham, and Eva-Maria S. Collins

International Regulatory and Scientific Effort for Improved Developmental Neurotoxicity Testing
Magdalini Sachana, Anna Bal-Price, Kevin M. Crofton, et al.

Functional and Mechanistic Neurotoxicity Profiling Using Human iPSC-Derived Neural 3D Cultures
Oksana Sirenko, Frederick Parham, Steven Dea, et al.

Teratological and Behavioral Screening of the National Toxicology Program 91-Compound Library in Zebrafish (Danio rerio)
Katharina Dach, Bianca Yaghoobi, Martin R. Schmuck, et al.
DNT Screening: Where to from here?

• Plan on building on our data-base and expanding to:
  – Incorporate more chemicals
  – Improve assay coverage (3-D models, mixed culture, barriers)
  – Evaluate status of current assays

• In parallel working on lower throughput, relevant assays
  – Genetically diverse mouse and humans lines
In vivo DNT Testing
**Current in vivo Design**

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- **GD 21**
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**Limitations:**
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- **~PND 120**
  - Brain weights
  - Neurohistopathology (CNS/PNS)
• Capture comprehensive information from same/ lesser animals by continuous monitoring
  • Overlay with artificial intelligence
In vivo DNT testing: Where to from Here?

- Incorporate imaging tools (MRI)
- Clinically relevant biomarkers of brain injury

More comprehensive & objective
Reduce time & number of animals
Improve translation
Exposure Assessment & Clinical Translation
Translation & Communication

- Incorporation of tools to contextualize exposure with toxicity noted in our studies
  - Models to inform *in vitro in vivo* extrapolation (IVIVE)
  - Use of *in silico* approaches for data profiling
- Engage trans- NTP groups on data mining/ systematic evaluation of literature
- Engage with stakeholders relatively early on during problem formation and continue communication throughout process
  - Cohorts on exposure assessments, clinicians, collaborative expert groups (e.g. TENDR)
Applying our capabilities in deliberate, integrated and complementary ways.
What do we ultimately hope to accomplish?

• Improve methods to identify compounds in the environment with unknown neurotoxic potential

• Provide data for timely public protection

• Better understand how *in vitro* studies can inform evaluation in animals & humans
Questions and Comments