Developmental Neurotoxicity Health Effects Innovation Program

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Problem Statement

There is global concern that neurodevelopmental disorders (e.g., autism, attention-deficit hyperactivity disorder (ADHD), and other learning disabilities) are rising in populations worldwide, and that environmental exposures may be contributing factors. Current methods to evaluate environmental compounds with unknown developmental neurotoxicity (DNT) potential remain largely ineffective due to the complexity of neurodevelopment with its multiple key processes, one or more of which might be perturbed by an environmental agent. An integrated testing strategy for DNT that incorporates novel and innovative methods could better inform public health decisions on DNT hazards and how they might contribute to the etiology of neurodevelopmental disorders.

Objectives

The Developmental Neurotoxicity Health Effects Innovation (DNT HEI) Program is structured around the following three objectives:

1. Implement a DNT screening battery that covers key neurodevelopmental events. The goals of this battery are to provide timely data to the public for decision-making, to prioritize compounds with potential for DNT for further short-term in vivo studies, and ultimately to reduce and refine the use of animal models.
    a. Identify key neurodevelopmental events and initial assays that have high readiness criteria to be incorporated as part of the Division of the National Toxicology Program's (DNTP's) Screening Battery. Selected assays include proliferation and apoptosis of human neural progenitor cells (hNPC), migration of human neural crest cells (NCC), neurite outgrowth in human induced pluripotent stem cells (iPSCs), synaptogenesis and network formation/maturation in multi-electrode array (MEA) using rat primary cortical neurons, zebrafish behavior during early development (total distanced and larval movement pattern), and 3D neurosphere assay using hNPCs (proliferation, apoptosis, migration and differentiation) to allow for interaction of multiple brain cell types.[1]
    b. Screen a set of approximately 100 compounds using these assays to provide weight-of-evidence evaluation of DNT potential and to prioritize compounds for further study (e.g., using short-term in vivo behavioral, in vivo neuroimaging, and mechanistic studies).
    c. Compare in vitro assay point-of-departure potencies to known or estimated in vivo studies (i.e., rodent models and/or human exposure data) by utilizing in vitro-to-in vivo extrapolation (IVIVE) methods.

[1] Bal-Price *et al.* (2018) Recommendation on test readiness criteria for new approach methods in toxicology: Exemplified for developmental neurotoxicity. ALTEX 35: 306-352 (supplemental table). https://doi.org/10.14573/altex.1712081

2. Assess novel DNT assays and technologies for potential adoption into the current battery to address coverage gaps. The goals of this effort are to analyze novel in vitro and in vivo models to be used toward redefining current DNT testing.
    a. Evaluate in vitro assays with potential for future implementation into the screening battery and/or to provide further in-depth mechanistic information. Such assays may include but are not limited to glial maturation, myelination, blood brain barrier and blood-cerebrospinal fluid (CSF) barrier models, glial co-cultures, brain-on-a-chip, microfluidics, complex 3-D organoid models, and models incorporating genetic diversity.
    b. Evaluate short-term in vivo rodent behavioral tracking with artificial intelligence (AI) methods (e.g., 24/7 behavioral monitoring) to improve the ability to track subtle behavioral perturbations, reduce experimenter variability, and potentially link the behavioral perturbations to alterations in neural networks using in vivo imaging.
3. Establish communication pipelines with stakeholders to allow for concerted global progress of DNT, enable the knowledge generated by DNTP to be used in further evaluations (e.g., for decision-making), and to inform the public about the latest advancements through a range of diverse media.
    a. Identify and engage stakeholders through DNT HEI hosted meetings, emails communicated using the DNT HEI listserv, and other ad hoc workshops to inform about DNTP assay results, the DNT HEI Program strategy, and global efforts.
    b. Integrate data into DNTP's free and publicly available Developmental NeuroToxicity Data Integration and Visualization Enabling Resource (DNT-DIVER), a data integration and visualization tool, to serve as a global data integration, visualization, and hosting resource.
    c. Develop outreach plans to inform the public about issues and latest advancements with respect to DNT.

Rationale

Public Health Context

There is growing concern backed by substantial scientific evidence linking toxic environmental chemicals to neurodevelopmental disorders such as autism spectrum disorder, ADHD, intellectual disability, and learning disorders. Currently, it can take over a decade for regulations to be put into effect from the time a compound is identified as potentially developmentally neurotoxic due to the rigor required for decision-making. In the interim, susceptible populations continue to be exposed to environmental chemicals that may result in neurodevelopmental disorders.

The DNT HEI program is a high priority initiative that aims to effectively develop a comprehensive method to evaluate DNT of environmental compounds with unknown DNT potential, which number in the thousands. There are concerns about the current framework of DNT assessment, which largely comprises rodent guideline studies. These studies are time and resource intensive, and are performed only when there is an a priori trigger—for example, clinical observations or histopathological changes in the brain noted from acute or subchronic studies, and structural and/or use patterns of concern to known DNTs (such as extensive exposure to pesticides with an organophosphate backbone in children). As a result, environmental compounds with unknown potential to cause DNT remain largely untested. Even in cases with in vivo DNT data, uncertainties remain in the current DNT test guidelines due to limitations with respect to sensitivity, reproducibility, and relevance when extrapolating data from rodents to humans for complex diseases like autism or ADHD.

Alignment with Mission, Goals, Strategic Pipeline

The DNT HEI program aims to alleviate some of these concerns by using novel, relevant tools and technologies that incorporate a tiered strategy in line with the DNTP pipeline. The program will use new approach methodologies (NAMs), short-term in vivo studies, and exposure information to provide reliable data to stakeholders for timely protection of children's health.

The DNT HEI Program is aligned with the National Institute of Environmental Health Sciences (NIEHS) 2018–2023 strategic plan and supports the goal of predictive toxicology and the cross-divisional focus area of neuroscience. The development of innovative, human-relevant, novel tools to predict potential hazards for regulators and the public in a timely manner aligns with the DNTP mission.

Stakeholder Interest and Engagement

DNTP initiated a proof-of-concept on the use of a DNT-screening battery with a set of 91 compounds that was presented at a 2017 workshop[2] at which the strategy gained a high level of global visibility. Subsequently, the Organization of Economic Cooperation and Development (OECD) initiated a global DNT expert group, composed of researchers from OECD as well as from the European Food Safety Association (EFSA), U.S. Environmental Protection Agency (EPA), Health Canada, Danish EPA, DNTP, and several other collaborators, to develop guidance on the implementation of NAMs into DNT screening. These assays are currently largely evaluated individually and not in combination, thereby creating a challenge with respect to identifying compounds with differing modes of DNT. In recognizing this challenge, DNTP and other collaborators, including EPA and EFSA, are working toward integrating these assays into a screening battery. DNTP's proposed screening battery will complement global efforts by expanding the limited set of compounds that have been tested across different assays within the battery to better understand the relative contribution of individual assays.

Furthermore, we plan to serve as a forum to foster communication and collaboration among these and other external stakeholders, including clinicians and child health advocacy groups, as well as internal NIEHS stakeholders, such as DNTP, Division of Intramural Research, and Division of Extramural Research and Training. Some strategies for engagement include but are not limited to:

- Hosting workshops and seminars
- Sharing information via listservs and social media
- Engaging the National Institutes of Health Institutes/Centers and NIEHS partners
- Working with the NIEHS Office of Communications and Public Liaison and DNTP Office of Liaison, Policy, and Review to develop factsheets, video, or other informational resources for outreach

Steps Taken to Engage Stakeholders

DNTP continues to engage subject matter experts, building on the momentum of the DNTP-hosted workshop described above, via email communication and meetings to present progress and obtain input for moving the field forward. For example, we have requested feedback on our DNT HEI strategy and on nominations of chemicals to test in the assay battery. The DNT HEI Program has created a listserv and

[2] Workshop Report on Integrated Testing Strategies for Developmental Neurotoxicity. National Toxicology Program. September 26-28, 2017. https://sandbox.ntp.niehs.nih.gov/static/resources/neurotox/DNT%20Workshop%20Report.pdf

mailbox to inform all partners about advancements of the program (including the creation of the screening battery) and solicit feedback.

Ongoing and Continuing Interactions

Stakeholder	Issue	Role of Stakeholder
EPA	DNTP requires assay expertise	Partner and end-user: Mutual interest in predictive DNT; Subject matter experts on 2D assays: neurite outgrowth, network formation proliferation, and apoptosis assays
Academia	DNTP requires assay expertise	Technical advisors: Subject matter experts in neural crest migration, 3-D neurosphere mixed cultures, and zebrafish behavior
Health Canada	Need for regulatory perspective on utility of the battery	Technical advisor and end-user: regulatory subject matter expert
OECD	Need for input on complementary models to evaluate DNT	Partner: Mutual interest in complementary models; OECD reached out to Dr. Behl to solicit expert advice and input on creating a guidance document using complementary models to evaluate compounds with DNT potential using Integrated Approaches to Testing and Assessment (IATAs); DNTP is contributing a case study on flame retardants that evaluates a class of compounds with unknown DNT potential
EFSA	Need for guidance on alternative DNT screening methods using NAMs	Partner and end-user: Mutual interest in DNT and DNT in vitro screening
CPSC, DNTP, DNT Consulting Consortium*, NRDC, TENDR, Academia	Request for chemical nominations	Partners, end users, and nominators of screening battery chemicals
Others: Click here to see complete list of contributors involved in DNTP's 2017 workshop.[3]		

Input Received

In response to our call for nominations, we received approximately 300 compounds by 35 contributors representing DNTP Program Management Teams/Branches, EFSA, EPA, Consumer Products Safety Commission (CPSC), Natural Resources Defense Council, Inc. (NRDC), Project Targeting Environmental Neuro-Developmental Risks (TENDR), academia (United States and Europe) and the DNT Consulting Consortium* (members include R3 Fellows LLC, IDN Consulting, OECD, EFSA, EPA, academia, and Health Canada).

[3] Ibid. Appendix C.

There is a concerted global need and enthusiasm among stakeholders that aligns with DNTP DNT HEI's idea of using a pragmatic, integrated approach to screen large sets of compounds to identify environmental compounds with unknown DNT potential, followed by more targeted in vivo testing to better characterize hazards and understand underlying mechanisms. There was also a high level of interest in DNT-DIVER, which will serve as a central repository to host global DNT data.

Milestones and Metrics

Key initial decisions for the DNT screening assay battery include:

1. Selecting a set of 100 chemicals to screen in the assay battery from nominations received by stakeholders
2. Developing acquisition packages for external contracts, and interagency agreements (IAAs) with EPA to screen the compounds in (1) above

Initial products will include:

1. Chemical set with controls ready to ship (stock solutions of chemicals that have undergone chemical identification and purity analysis and been aliquoted and packaged)
2. Finalized contracts and IAAs to run the battery

We will also inform stakeholders of the chemical set to be tested to avoid redundancy of effort.

Milestones for the overall program within the next 3 years will be represented by completion of these successful projects:

- Develop and implement seven in vitro assays (proliferation, migration, apoptosis, synaptogenesis, network formation, 3-D neurospheres, zebrafish behavior) for DNT screening (Years 1–2: build, Year 3: test)
- Develop and conduct novel automated home-cage monitoring and incorporate social housing in close-to-natural conditions to improve in vivo DNT evaluation (Years 1–3)
- Identify functional links between in vitro and in vivo tests (Year 1–2: build, Year 3: test)
- Establish strategy for imaging studies to identify phenotypes linked to in vitro studies, and compare with traditionally evaluated neurohistopathology (Year 1–3)
- Establish program for postdoctoral fellow via the Faculty for Advancing Neurosciences (an NIEHS cross-divisional focus area group) (Year 1: establish funding, Year 2–3: implementation)
- Integrate DNTP capabilities across the Translational Toxicology Pipeline (Year 1–3)
- Contextualize findings from in vitro/in vivo (Years 2–3)

Data from the screening battery will be hosted in DNT-DIVER, for analysis and visualization. Chemicals causing damage to neurodevelopmental processes represented in the screening battery will be identified and prioritized for further testing (in vivo and/or mechanistic testing). Evaluating species sensitivity and comparing to in vivo data and human exposure using IVIVE will be done when data are available. Assessment of the effectiveness of the strategy, the results, and lessons learned will be incorporated into a white paper for publication.

Subsequent products resulting from novel in vivo strategies described above will be determined before initiation of those future phases of the program.

Value Proposition and Summary

The DNT HEI program aims to improve the current state of DNT testing by providing timely and relevant information to the public by delivering:

1. Screening-level information employing NAMs for compounds with unknown DNT potential and interactive web-based data reporting using DNTP's free, interactive, and publicly available data analysis and visualization tool, DNT-DIVER—information that may be used as an interim means to evaluate hazards and to prioritize chemicals for further in-depth evaluation
2. Short-term in vivo studies that further address complex neurodevelopmental issues resulting from environmental exposures by incorporating more human-relevant behavioral and brain network assessments
3. Reports on contextualizing in vitro and in vivo findings with human exposure using IVIVE and in silico approaches to provide more relevant and translatable information that can be used to protect children's health

Due to the complexity of the developing nervous system, a battery of assays is required to capture perturbations caused by compounds via different modes of action. Currently, assays are still largely evaluated individually or in limited combinations, thereby creating gaps in understanding the effects of chemical exposure. We aim to screen a battery of assays that cover major events occurring during neurodevelopment. DNTP is uniquely qualified to perform this type of screening because we have multi-disciplinary in-house expertise, established relationships with global partners, previous experience with screening a set of compounds, contracting capabilities, and DNT-DIVER, that we have developed to host the data.

An important value to DNTP is that this program will complement global DNT efforts and aid in moving the DNT field forward. This work will be conducted using research and development contracts, and with government partners using IAAs. Ultimately, this coordinated DNT strategy is designed to provide a biological and mechanistic understanding of environmentally related neurodevelopmental disorders.