



Interagency Coordinating Committee on the Validation of Alternative Methods

Presentation Abstracts and Background Materials

SCIENTIFIC ADVISORY COMMITTEE ON ALTERNATIVE TOXICOLOGICAL METHODS

Session III: Developmental Neurotoxicity (DNT)

Wednesday, September 18, 2024

International Efforts Implementing NAMs for Assessing DNT in Chemical Risk Assessment

Presenter: Dr. Iris Mangas, European Food and Safety Authority (EFSA)

In June 2023, the OECD published the Test Guidelines Initial Recommendations for evaluating the DNT In Vitro Battery (DNT IVB) data [ENV/CBC/MONO (2023)13]. In addition, a series of Integrated Approaches to Testing and Assessment (IATA) case studies using the DNT IVB data in current regulatory frameworks for single chemical hazard characterization is available. This is the result of a major endeavour from various regulatory and academic groups over the last years to standardise a battery of 17 in vitro assays measuring Key Neurodevelopmental Processes (KNP) in human relevant test systems with the ultimate goal to assess any regulated chemical for DNT and minimize the need for in vivo DNT studies. In the available case studies, the DNT IVB demonstrated to provide relevant, high-quality data on information regarding DNT potential of chemicals that are perturbing early cellular processes and that are difficult to measure in vivo.

For EFSA, the Adverse Outcome Pathways (AOP) informed IATA framework, using NAMs and exposure models are the proposed tools to change the current Risk Assessment approach and to consciously include NAMs in the process. AOPs can serve as the basis for designing NAM testing strategies and that the data generated by NAMs mapped in AOPs could be used to identify a Reference Point (RP, also known as Point of Departure, PoD) within the AOP-informed IATA framework. In the EU context of the assessment of agrochemicals, DNT is a front running in testing this new paradigm paving the way for other domains. EFSA AOP informed IATA workflow for DNT allowed regulatory acceptance of data from the DNT IVB in combination for hazard characterization.

Currently, limited number of the KNP in the DNT IVB are mapped into OECD endorsed AOPs and the development of AOP informed IATA when a non-endorsed AOP exists is a resource tool for assessing a huge number of chemicals. Ongoing EFSA and international efforts are further testing of hundreds of chemicals in the DNT IVB, transferability of the assays and more proof-of-concept case studies. This will allow to advance in the understanding of how chemical exposures impact brain health and disease during development and importantly to implement this knowledge for protecting human health. The final aim is to have an internationally agreed interpretative guidance and an agreed standard approach facilitating the mutual use in the regulatory frameworks for quantitative risk assessment while retaining necessary flexibility depending on the context of use and advancement of science. This future work will continue to imply international and collaborative effort paving the way also for a broader use of the new paradigm in EFSA.

Background

- OECD. 2023. Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In-Vitro Testing Battery (pages 1-42). Series on Testing and Assessment Number 377. Paris: Organisation for Economic Co-operation and Development. https://www.oecd.org/en/publications/initial-recommendations-on-evaluation-of-data-from-the-developmental-neurotoxicity-dnt-in-vitro-testing-battery_91964ef3-en.html.
- EFSA PPR Panel, Hernandez-Jerez A, Adriaanse P, Aldrich A, Berny P, Coja T, Duquesne S, Focks A, Marinovich M, Millet M, Pelkonen O, Pieper S, Tiktak A, Topping C, Widenfalk A, Wilks M, Wolterink G, Crofton K, Hougaard Bennekou S, Paparella M, Tzoulaki I. 2021. Development of integrated approaches to testing and



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assessment (IATA) case studies on developmental neurotoxicity (DNT) risk assessment. EFSA J 19(6):6599. <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2021.6599>.

- EFSA. 2023. Tender Specifications: Environmental Neurotoxins – Advancing Understanding on the Impact of Exposure on Brain Disease. <https://etendering.ted.europa.eu/cft/cft-document.html?docId=149444>.
- EFSA. 2023. Environmental Neurotoxins [Presentation]. <https://www.efsa.europa.eu/sites/default/files/2023-05/presentation-tender.pdf>.

Integrated Approaches to Testing and Assessment (IATA) Case Studies for DNT

Presenter: Dr. Helena Hogberg, Division of Translational Toxicology (DTT), National Institute of Environmental Health Sciences (NIEHS)

Recently, the Organisation for Economic Co-operation and Development (OECD) released a crucial guidance document titled “Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In-Vitro Testing Battery (IVB)” providing essential insights into the implementation and interpretation of the DNT IVB. Moreover, integrated approaches to testing and assessment (IATA) case studies have been developed to exemplify diverse regulatory requirements including weight of evidence for hazard assessment, screening and prioritization. The OECD is currently working on an IATA Framework Templates for DNT to standardize and promote the development of new IATA case studies. This presentation will give an overview of AOP-informed and non-AOP-informed IATA case studies and how the IATA framework template can be applied as an intermediate step towards a defined approach for DNT.

Background

- Kreutz A, Oyetade OB, Chang X, Hsieh JH, Behl M, Allen DG, Kleinstreuer NC, Hogberg HT. 2024. Integrated approach for testing and assessment for developmental neurotoxicity (DNT) to prioritize aromatic organophosphorus flame retardants. *Toxics* 12(6):437. <https://doi.org/10.3390/toxics12060437>.

The DNT In Vitro Battery; Establishing Confidence in and Using Data from the Battery

Presenter: Dr. Timothy J. Shafer, Rapid Assay Development Branch, Biomolecular and Computational Toxicology Division Center for Computational Toxicology and Exposure, U.S. Environmental Protection Agency Office of Research and Development

The OECD approved the initial recommendations for the DNT in vitro battery in April of 2023, and this has increased interest in using the battery for regulatory activities. This presentation will briefly present the assays in the battery, discuss how these assays meet criterion for confidence outlined in a 2024 ICCVAM report, summarize case-studies where data from DNT-IVB have been used for regulatory activities and briefly discuss approaches to analysis and interpretation of data from the battery.

Background

- OECD. 2023. Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In-Vitro Testing Battery (pages 1-42). Series on Testing and Assessment Number 377. Paris: Organisation for Economic Co-operation and Development. https://www.oecd.org/en/publications/initial-recommendations-on-evaluation-of-data-from-the-developmental-neurotoxicity-dnt-in-vitro-testing-battery_91964ef3-en.html.
- Sachana M, Shafer TJ, Terron A. 2021. Toward a better testing paradigm for developmental neurotoxicity: OECD efforts and regulatory considerations. *Biology* 10(2):86. <https://doi.org/10.3390/biology10020086>.
- Van der Zalm A, Barroso J, Browne P, Casey W, Gordon J, Henry TR, Kleinstreuer NC, Lowit AB, Perron M, Clippinger AJ. 2022. A framework for establishing scientific confidence in new approach methodologies. *Arch Toxicol* 96(11):2865–2879. <https://doi.org/10.1007/s00204-022-03365-4>.



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Integrating Screening Level Developmental Neurotoxicity (DNT) Information of Chemical in a New Approach Methods (NAMs) Battery to Identify Compounds for Future Study

Presenter: Dr. Christopher McPherson, NIEHS DTT

Evidence indicates that chemical exposure contributes to neurodevelopmental disorders. However, most chemicals have not been tested for developmental neurotoxicity (DNT) due to the limitations of current test guidelines, which rely on traditional *in vivo* studies. These studies are not commonly used because they are expensive, require large numbers of animals, and the results are not always considered reliable for regulatory purposes. To address this, the Division of Translational Toxicology established the DNT Health Effects Innovation (HEI) program in 2019. The program aims to evaluate the risks of chemical exposure to the developing nervous system. One of its objectives is to implement a DNT screening battery that covers key neurodevelopmental events, providing timely data for decision-making and prioritizing compounds with potential DNT for further study. This screening battery includes 2D and 3D human and rodent *in vitro* assays that measure proliferation, cell migration, neurite growth, neural network formation and function, as well as a zebrafish embryo neurobehavior assay. Based on nominations from various stakeholders, the DNT HEI program selected and distributed over 200 chemicals for testing. The program is now developing a unified data analysis pipeline, including multi-objective optimization and toxicological prioritization index (ToxPi) analysis, to integrate data from individual assays, evaluate DNT hazards, and prioritize chemicals for further in-depth evaluation within an integrated DNT testing framework. This strategy demonstrates the applicability of the current DNT battery for prioritizing chemicals for further studies and provides a method to contextualize data derived from the DNT screening battery.

Background

- Behl M, Ryan K, Hsieh JH, Parham F, Shapiro AJ, Collins BJ, Sipes NS, Birnbaum LS, Bucher J, Foster PMD, Walker NJ, Paules RS, Tice RR. 2019. Screening for developmental neurotoxicity at the National Toxicology Program: The future is here. *Toxicol Sci* 167(1):6-14. <https://doi.org/10.1093/toxsci/kfz036>.
- Hsieh JH, Ryan K, Sedykh A, Lin JA, Shapiro AJ, Parham F, Behl M. 2018. Application of benchmark concentration (BMC) analysis on zebrafish data: a new perspective for quantifying toxicity in alternative animal models. *Toxicol Sci* 167(1):92-104. <https://doi.org/10.1093/toxsci/kfy258>.

Considerations and Challenges Associated with the Transfer and Implementation of Select DNT *in vitro* Battery Assays in a New Laboratory

Presenter: Dr. Megan Culbreth, U.S. Food and Drug Administration

The prevalence of neurodevelopmental disorders (e.g., autism spectrum disorder, attention-deficit hyperactivity disorder) has increased in recent decades. Although the precise cause of this increase has not been identified, enhanced evaluation and diagnosis likely are not the only determinants. It has been speculated that environmental factors (e.g., exposure to compounds in the maternal diet) may contribute. As such, the need to comprehensively evaluate the potential developmental neurotoxicity (DNT) of a compound is paramount. At present, there is a paucity of data on DNT potential for most commercially available compounds. Furthermore, regulatory agencies do not require DNT testing for new or re-registration of compounds. Need for a DNT *in vivo* test is only triggered when other animal studies indicate possible neurodevelopmental effects. It is important to note, however, that the current regulatory accepted paradigm for *in vivo* DNT testing (i.e., OECD 426) has ethical limitations, is time intensive, and not cost-effective. These *in vivo* studies also provide limited mechanistic information.

In response to the need for more efficient evaluation of DNT potential, an international effort was initiated to develop more rapid *in vitro* new approach methods (NAMs) that capture fundamental neurodevelopmental processes. The culmination



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of this effort was a publication by the Organisation for Economic Co-operation and Development (OECD) on initial recommendations on the application of data from these in vitro NAMs, now deemed the DNT in vitro battery (IVB). However, for these initial recommendations to be accepted as an OECD guidance document, several steps were outlined by international stakeholders which included the transfer to and implementation of these DNT NAMs in new laboratories. As part of this step, the U.S. Food and Drug Administration (FDA) Center for Food Safety and Applied Nutrition (CFSAN) has initiated transfer of select DNT IVB assays inclusive of those developed at the U.S. Environmental Protection Agency (EPA) and the Leibniz Research Institute for Environmental Medicine. Importantly, this transfer is not one-to-one, i.e., direct technology transfer. As such, there are several considerations and challenges that have impacted this process, of which this presentation will focus on two: cell models and proficiency compounds.

First, of the DNT IVB assays that CFSAN selected to transfer, none of the cell models utilized are commercially available. Moreover, the laboratories do not have access to primary animal-derived cells necessary for select assays. As such, each approach must be adapted for a new cell model, which can potentially have a significant impact on the performance of an assay. Second, there is not a consensus on proficiency compounds to evaluate assay performance, i.e., whether the new laboratory can reliably detect compounds expected or not expected to produce an effect on any endpoint. FDA has consulted with assay developers from the EPA about proficiency compounds, however, to further the transfer effort worldwide, a consensus list for each DNT IVB assay must be agreed upon. Aside from these considerations and challenges, there are other factors not presently addressed which include data analysis and across-laboratory comparison that will be briefly presented.

Background

- OECD. 2023. Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In-Vitro Testing Battery (pages 1-42). Series on Testing and Assessment Number 377. Paris: Organisation for Economic Co-operation and Development. https://www.oecd.org/en/publications/initial-recommendations-on-evaluation-of-data-from-the-developmental-neurotoxicity-dnt-in-vitro-testing-battery_91964ef3-en.html.
- OECD. 2023. Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In-Vitro Testing Battery (Appendices A and B9). Series on Testing and Assessment Number 377. Paris: Organisation for Economic Co-operation and Development. https://www.oecd.org/en/publications/initial-recommendations-on-evaluation-of-data-from-the-developmental-neurotoxicity-dnt-in-vitro-testing-battery_91964ef3-en.html.