National Institute of Environmental Health Sciences
Division of Translational Toxicology

Integrated Approach for Testing and Assessment for Developmental Neurotoxicity (DNT) to Prioritize Aromatic Organophosphorus Flame Retardants

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Introduction

- Organophosphorus flame retardants (OPFRs) are abundant and persistent in the environment due to their extensive use.
- In vivo and in vitro studies suggest that OPFRs may pose health risks. In particular, their structural resemblance to organophosphate pesticides raises concerns about potential acute neurotoxicity and developmental neurotoxicity (DNT).
- The traditional in vivo DNT guideline studies have challenges including high costs, lengthy timelines, and uncertain human relevance.
- A DNT in vitro battery (DNT-IVB) measuring key processes of neurodevelopment and small model organism behavioral assays was developed to address these limitations. This integrated approach to testing and assessment (IATA) uses an extended battery of DNT in vitro assays and small model organism behavioral assays that inform on cellular processes involved in neurodevelopment and function.
- Using OPFRs as an example, OECD published an IATA case study for DNT, illustrating how application of the DNT-IVB can effectively be used to prioritize testing for compounds within a class (OECD 2022).
- Ten compounds (see table below) including aromatic and halogenated OPFRs and classic brominated flame retardants (BFRs), were used in the case study.
- DNT potential from OPFRs was related to human exposure using physiologically based pharmacokinetic (PBPK) modeling to provide risk assessment recommendations.

Figure 1. Graphical representation of the IATA case study relating human exposure and potential DNT outcomes.

Objectives

- Build upon this IATA for DNT for OPFRs using additional data sources to prioritize chemicals for further testing.
- Evaluate potency estimates using data from DNT-IVB assays and assays for DNT endpoints and mechanisms not currently considered in the IVB.
- Leverage human exposure data and toxicokinetic modeling to contextualize the potency estimates and chemical comparisons.

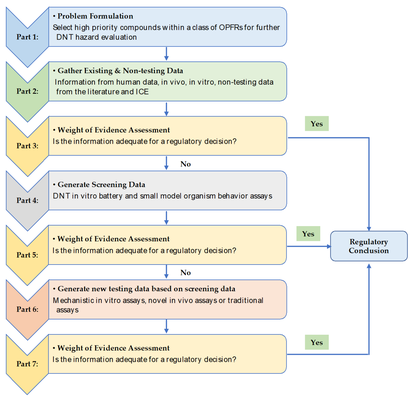

Figure 2. IATA flowchart for prioritization of chemicals for further DNT testing.

Chemicals Assessed in the Case Study

Chemical Type	Chemical Name	Chemical ID	Structure
Classic BFR	2,2'4,4'-tetrabromodiphenyl ether	BDE-47	
	3,3',5,5'-tetrabromobisphenol A	TBBPA	
Aromatic OPFR	triphenyl phosphate	TPHP	
	isopropylated phenyl phosphate	IPP	
	2-ethylhexyl diphenyl phosphate	EHDP	
	tricresyl phosphate	TMPP	
	isodecyl diphenyl phosphate	IDDP	
	tert-butylphenyl diphenyl phosphate	BPDP	
Halogenated OPFR	tris(1,3-dichloro-2-propyl) phosphate	TDCIPP	
	tris(2-chloroethyl) phosphate	TCEP	

Methods

- Benchmark concentrations (BMC) were derived for the 10 case study chemicals using data from the DNT-IVB and small model organism behavioral studies.
- The DNT-IVB included 22 assays that capture key processes of neurodevelopment: proliferation, migration, differentiation, neurite outgrowth, network function, and behavior in small model organisms.
- Lowest observed effect concentrations (LOEC) were derived from the scientific literature.
- Activity concentrations at cutoff (ACC) from in vitro assays (not limited to DNT endpoints) were obtained from the curated high-throughput screening (cHTS) data in NICEATM's Integrated Chemical Environment (ICE).
- Exposure data were collected from the literature and analyzed with PBPK modeling to estimate the maximum human plasma concentration (C_{max}).

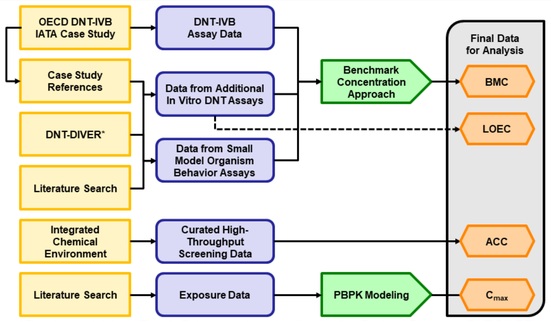

Figure 3. Workflow for collection of in vitro DNT assay data.

Distribution of Assay Endpoints

- Individual BMCs for each of the key process assays (x-axis) of the DNT-IVB showed similar sensitivity across assays, with most endpoints within 10-fold of one another. Lower DNT-IVB BMCs, particularly for network formation, were seen for BDE-47.*
- Intra-assay variation in endpoint sensitivity was greater, such as for key processes of behavior, neurite outgrowth, and network function.
- The point of departure for some compounds was lowered by integrating cHTS data from ICE and in vitro data from the literature. The majority of the most sensitive endpoints found in ICE and the literature were annotated to glial differentiation, gene expression, immune processes, and interaction with the endocrine system.

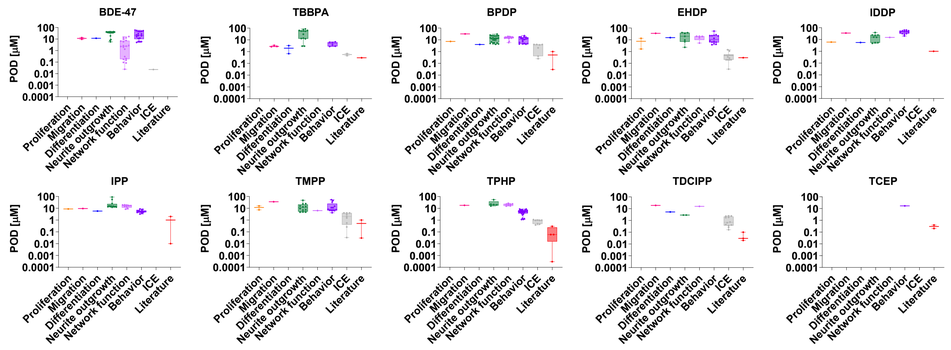

Figure 4. Boxplots display the distribution of BMCs from assay endpoints with positive hit calls in the DNT-IVB, along with neuronal and non-neuronal ICE and literature endpoints that fell below the most sensitive endpoint for each chemical. *Some endpoints missing due to lack of available data.

Summary Heatmap of DNT-IVB Assays for the 10 FRs Tested

- BFRs and aromatic OPFRs were generally more active than halogenated OPFRs. Of 22 total assays, all BFRs and aromatic OPFRs were active in 11-17 assays, while halogenated OPFRs were active in 1-4 assays, though testing of the halogenated OPFRs was limited.
- The aromatic OPFR BPDP affected the most endpoints, with activity in 15 assays.
- The BFR BDE-47 was the most potent with the lowest BMC, followed by the classical BFR, TBBPA, and the novel OPFRs, TPHP and EHDP, which all had similar potency.
- Behavioral, network function, and neurite outgrowth assays were the most sensitive, with zebrafish behavioral assays being more sensitive than planaria assays. This suggests that incorporation of small model organisms into the IVB is important and could provide additional information not detected by the current OECD battery of DNT in vitro assays.
- Overall, the novel OPFRs show comparable activity to BFRs.
- Sources of uncertainty:
  - Lack of concordance within assays outgrowth, migration)
  - Lack of testing across assays (TDCIPP)

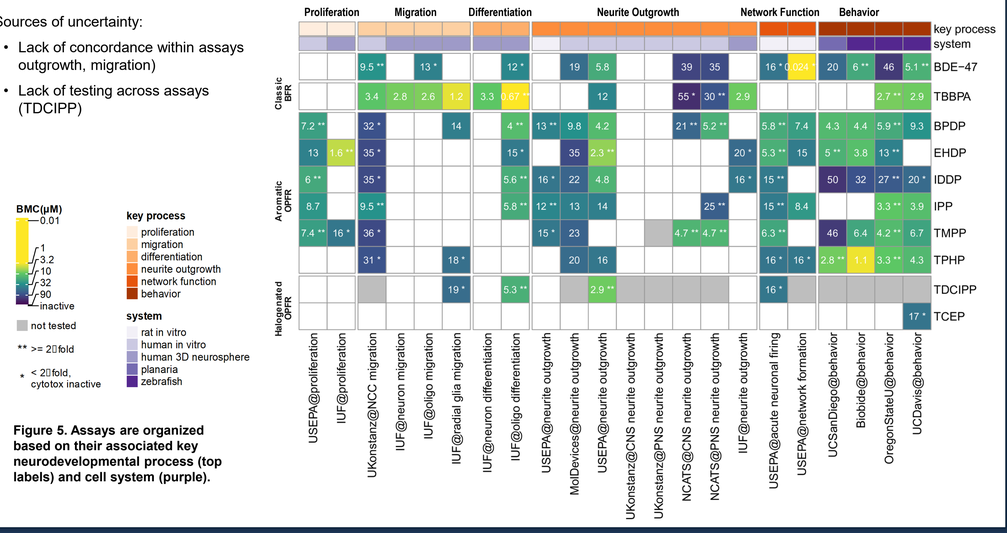

Figure 5. Assays are organized based on their associated key neurodevelopmental process (top labels) and cell system (purple).

Evaluation of Human Exposure to OPFRs — Comparison to Bioactive Concentrations In Vitro and In Vivo

- Of various exposure sources, exposures through breast milk were the highest, followed by handwipes and house dust.
- Plasma C_{max} values estimated from biomonitoring data were comparable to in vitro activity concentrations for several OPFRs (EHDP, TPHP, and TDCIPP—circled in red). This suggests that human exposure may pose a risk for neurodevelopment and endocrine-related effects.
- In vitro activity fell within an order of magnitude of in vivo POD (MRL, BMDL), when available, and was usually more sensitive.

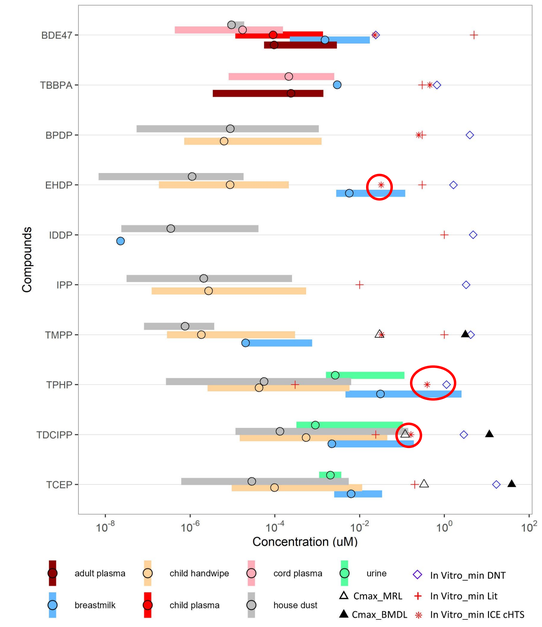

Figure 6. Comparison of in vitro DNT activity data to human exposures. Colored bars display PBPK modeling-derived plasma C_{max} values for each chemical estimated from literature-derived human exposure sources. Symbols represent the lowest in vitro activity concentration for each in vitro dataset. MRL: human minimal risk level, BMDL: lowest benchmark dose in vivo.

Conclusions and Future Directions

- Overall, the aromatic OPFRs had similar activity to the BFRs in the DNT-IVB (network formation assay being an exception), while the halogenated OPFRs showed less activity in the DNT-IVB, though this was based on a limited set of data.
- As a class, the aromatic OPFRs appeared to impact a variety of DNT endpoints.
- Activity concentration in the DNT-IVB and other in vitro assays overlapped with human exposure for some OPFRs, indicating potential concern for human health.
- Data from ICE and the literature identified other sensitive targets that may lower points of departure for this class of compounds.
- The DNT-IVB and comparison to human exposure supports prioritization of TPHP for further testing.
- By integrating additional endpoints, such as endocrine disruption and glial cell populations, this IATA case study suggests that combining the OECD DNT-IVB with additional NAMs could improve confidence in DNT assessments.

References and Acknowledgments

Data retrieved via the Search tool on September 29, 2023, from the National Toxicology Program's Integrated Chemical Environment version 4.0, released March 2023 (https://ice.ntp.niehs.nih.gov/).

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