National Institute of Environmental Health Sciences
Division of Translational Toxicology

OPFR Prioritization Using the Developmental Neurotoxicity In Vitro Test Battery: An Update to OECD IATA Case Study

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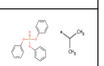
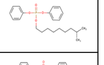
Introduction

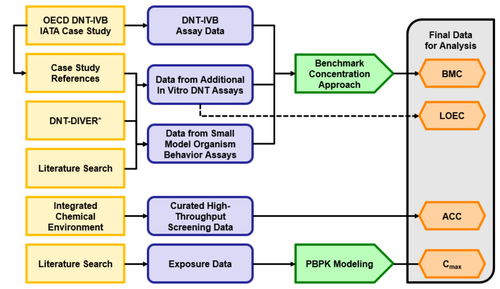
- Organophosphorus flame retardants (OPFRs) are abundant and persistent in the environment due to their extensive use.
- In vivo and in vitro studies suggest that OPFRs may pose health risks. In particular, their structural resemblance to organophosphate pesticides raises concerns about potential acute neurotoxicity and developmental neurotoxicity (DNT).
- The in vivo DNT test guidelines currently used for testing 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 were developed to address these limitations.
- Using OPFRs as an example, the OECD created and published an integrated approach for testing and assessment (IATA) case study for DNT, illustrating how the application of the DNT-IVB can effectively 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 the focus compounds used in the case study.
- In this project, we evaluated potency estimates using data from DNT-IVB assays and assays for DNT endpoints and mechanisms not currently considered in the IVB. We leveraged human exposure data and toxicokinetic modeling to contextualize the potency estimates and chemical comparisons, so it can be applied to prioritize a class of chemicals for further 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

Workflow for collection of in vitro DNT assay data

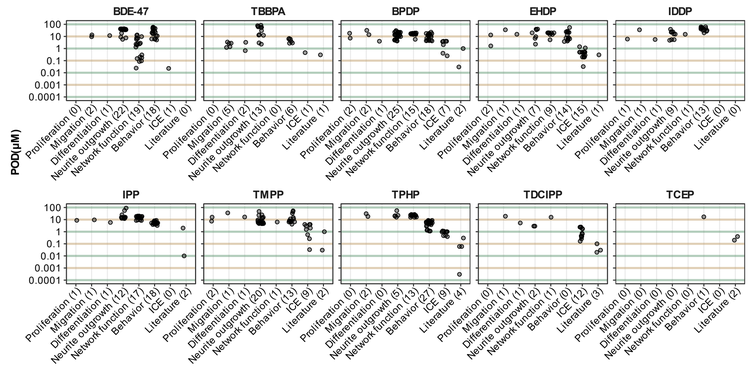


*Developmental NeuroToxicity Data Integration and Visualization Enabling Resource (DNT-DIVER)

- Benchmark concentrations (BMC) were derived for the ten focus chemicals using data from the DNT-IVB.
  - The DNT-IVB included 22 assay data representing key processes of neurodevelopment: proliferation, migration, differentiation, neurite outgrowth, network function, and behavior.
- Lowest observed effect concentrations (LOEC) were derived from the literature based on the concentrations reported in the articles.
- Activity concentration at cutoff (ACC) from in vitro assays not limited to DNT endpoints were obtained from curated high-throughput screening (cHTS) data in NICEATM's Integrated Chemical Environment (ICE).
- Exposure data were collected from the literature and analyzed with physiologically based pharmacokinetic (PBPK) modeling to estimate the maximum plasma concentration (C_{max}).

Distribution of Assay Endpoints

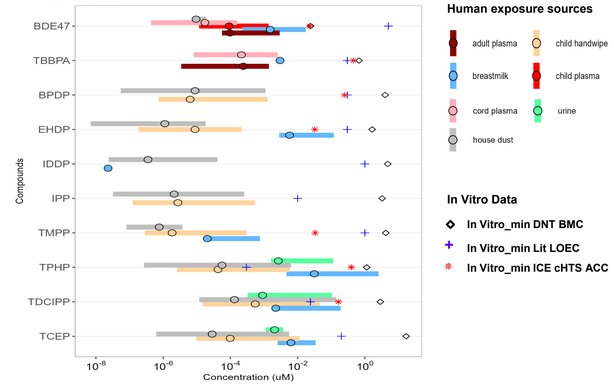
Scatterplots display the distribution of BMCs from assay endpoints with positive hit calls in the DNT-IVB, along with neuronal and non-neuronal ICE and literature endpoints that fell below the most sensitive endpoint for each chemical.



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

Evaluation of Human Exposure to OPFRs — Comparison to In Vitro Data

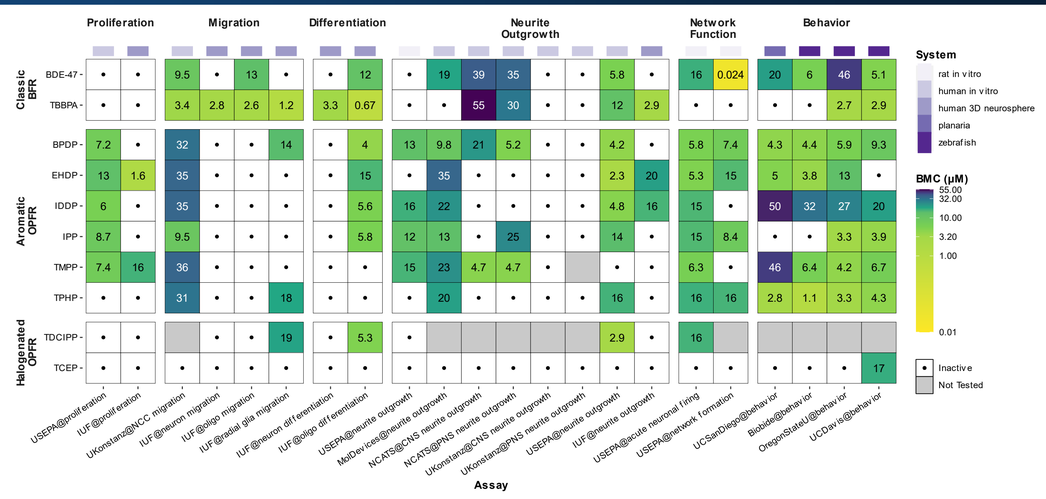
Colored bars display C_{max} values for each chemical estimated from different human exposure sources. Symbols represent the lowest in vitro activity concentration for each in vitro dataset.



- Of exposure sources, exposures through breast milk were the highest, followed by handwipe and house dust.
- For all the FRs except TPHP and TDCIPP, C_{max} values estimated from house dust and handwipe exposure were at least 10-fold lower than the lowest in vitro activity concentrations.
- C_{max} plasma values estimated from biomonitoring data were comparable to the in vitro concentrations for several OPFRs (EHDP, TPHP and TDCIPP). This suggests that human exposure may pose risk for neurodevelopment and endocrine-related effects.

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.
- The aromatic OPFR BPDP affected most endpoints, with activity in 15 assays.
- The BFR BDE-47 and TBBPA was most potent with the lowest BMC, followed by the novel OPFRs TPHP and EHDP.
- 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 noted by current in vitro assays.



Assays are organized based on their associated key neurodevelopmental process (labelled at the top) and cell system (purple).

The colors and numbers in the heatmap represent BMC (μM) of the most sensitive endpoint measured within the assay. White represents inactive; grey, not tested.

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.
- Activity concentrations in the DNT-IVB and other in vitro assays overlap with predicted human exposure for some OPFRs, indicating potential concern for human health.
- Data from ICE and the literature identified other sensitive targets that may lower the point of departure for this class of compounds.
- The DNT-IVB and comparison to human exposure suggest that TPHP should be prioritized for further testing.
- Through integrating additional endpoints, such as endocrine disruption and the inclusion of astrocytes and microglial cell populations, this IATA case study suggests that combining the OECD DNT-IVB with NAMs could improve confidence in DNT assessments.

References and Acknowledgments

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

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