Integrated Approaches for Testing and Assessment for Developmental Neurotoxicity

Organophosphorus flame retardants: A Case Study

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ICCVAM Public Forum
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ICCVAM and IATAs

- IATAs integral component of ICCVAM; various applications
  - Skin Sensitization, Endocrine disruption
- Expansion of Biological Space for IATAs
- OECD DNT Expert Group developing a guidance document using IATAs for DNT that can be used for regulatory decision-making
  - Recent Meeting held in April 2020
- NTP developing a DNT IATA case study for the OECD guidance document
  - Efforts could feed into ICCVAM

General IATA Template

- Introduction
- Purpose
- Chemical tested
- End-points
- Hypothesis
- Approaches used
- Findings & Interpretation
- Relevance to human exposure
- Application of IATA
- Uncertainties
- Conclusion

Today: Flame Retardants as a case example of a DNT IATA for hazard characterization and prioritization

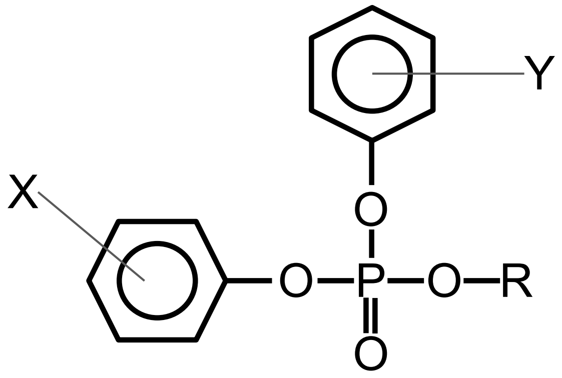
Introduction: Exposure to Flame Retardants

Introduction: Phased out vs Novel Replacements

- Projected increase in exposure & use of organophosphate flame retardants (OPFRs) following:
  - Voluntary phase-out of polybrominated diphenyl ethers (BDEs)
  - CPSC petition to ban organohalogens in 2017; NAS report generated in response in 2019
- Concerns for DNT in infants and toddlers- car seats; mouthing
- Lack of toxicity data on hazard characterization & risk assessment
  - Regrettable substitutes?

Introduction: Why IATA for OPFRs?

- Projected increase in exposure
- 20-50 compounds in class including commercial and isomeric mixtures
- Cannot test our way through all combinations using traditional animal guideline studies
- Need strategy to prioritize compounds for further in-depth hazard characterization

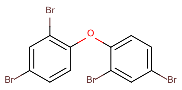
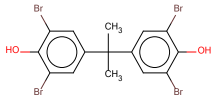
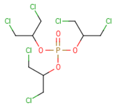
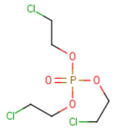
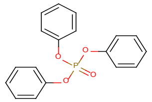
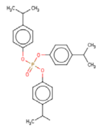
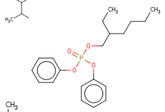
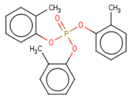
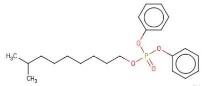
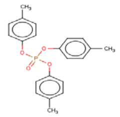
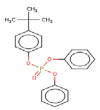


R = aryl or alkyl

Purpose of the IATA

- Screen compounds for prioritization for further testing
- Hazard ID/ characterization
- Timely dissemination of information

Chemicals in IATA

CAS	Chemical Name	Chemical.ID	Structure
	Representative Brominated FRs (BFRs)		
5436-43-1	2,2'4,4'-Tetrabromodiphenyl ether	BDE-47	
79-94-7	3,3',5,5'-Tetrabromobisphenol A	TBBPA	
	Organophosphorous FRs (OPFRs)- aliphatic, halogenated		
13674-87-8	Tris(1,3-dichloro-2-propyl)phosphate	TDCIPP	
115-96-8	Tris(2-chloroethyl) phosphate	TCEP	
	Organophosphorous FRs (OPFRs)- Aromatic		
115-86-6	Triphenyl phosphate	TPHP	
68937-41-7	Phenol, isopropylated, phosphate (3:1)	IPP*	
1241-94-7	2-Ethylhexyl diphenyl phosphate	EHDP*	
1330-78-5	Tricresyl phosphate	TMPP*	
29761-21-5	Isodecyl diphenyl phosphate	IDDP	
56803-37-3	tert-Butylphenyl diphenyl phosphate	BPDP*	
78-30-8	Tri-o-cresyl phosphate	TOCP	

representative isomer in mixture is shown as structure

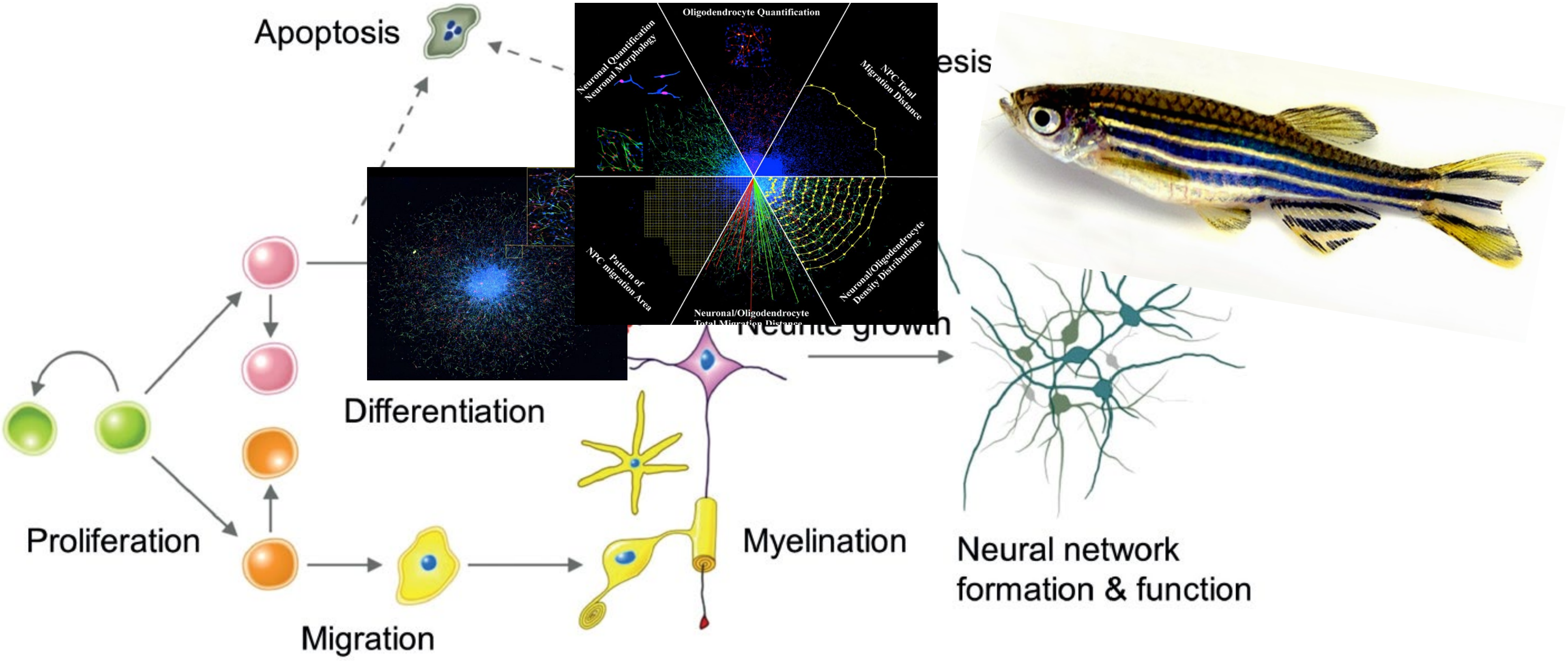
BDEs *(Phased-out)*

TBBPA *(Extensively used)*

Aliphatic organohalogens *(petition to ban)*

Aromatic phosphates *(novel replacements)*

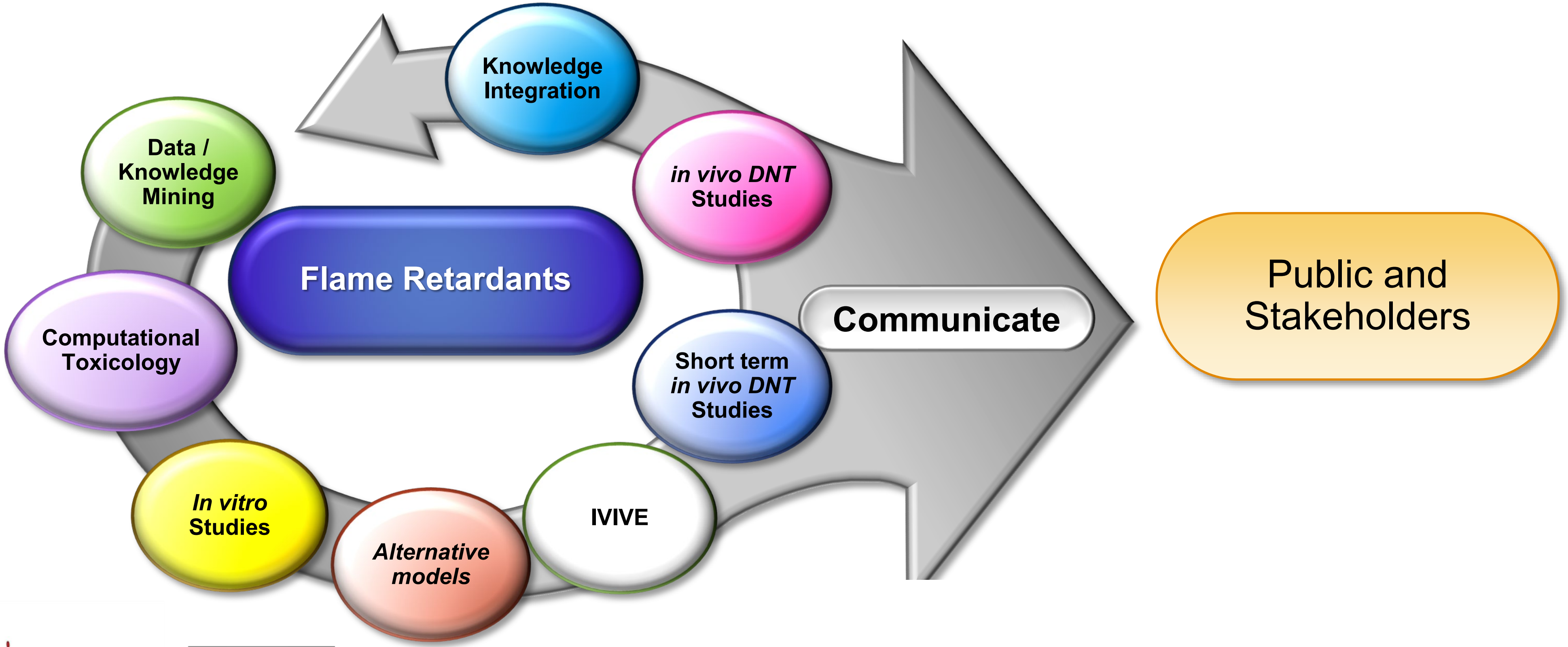
NTPs DNT Screening Battery

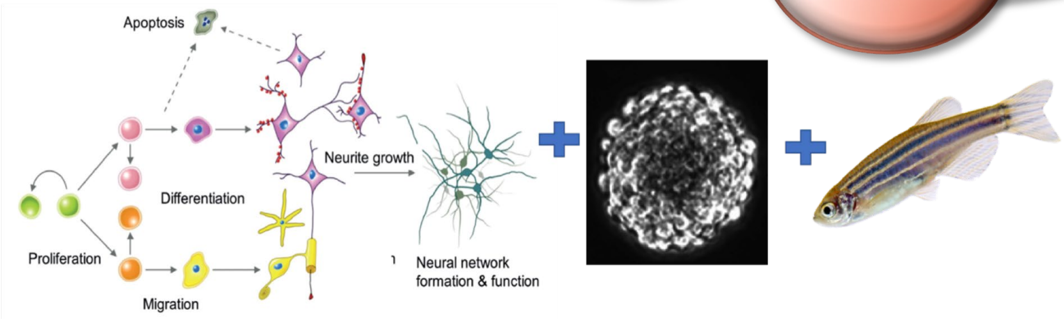


2-D in vitro assays + 3-D neurospheres + Zebrafish

Part of the Bigger Picture

Applying our capabilities in deliberate, integrated and complementary ways.



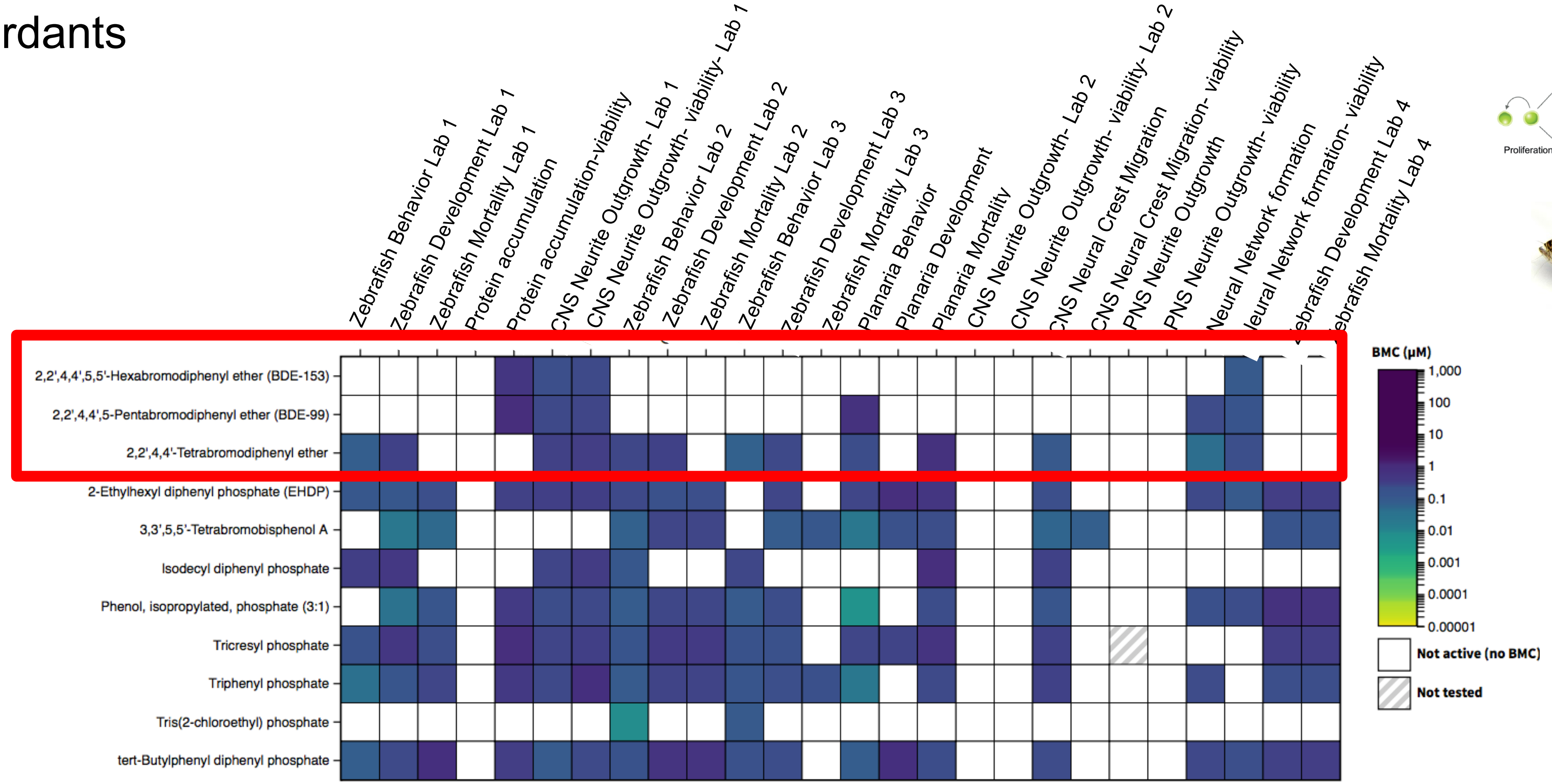


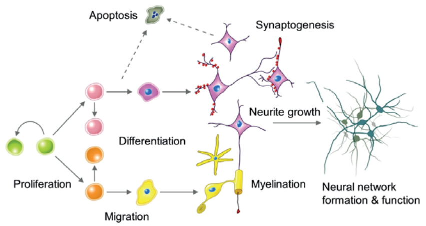
Hypothesis

The replacement aromatic OPFRs are less active(toxic) than the phased-out BDEs and hence are currently being used as substitutes

Comparison of OPFRs with phased-out compounds (2D + Behavior)

Flame Retardants

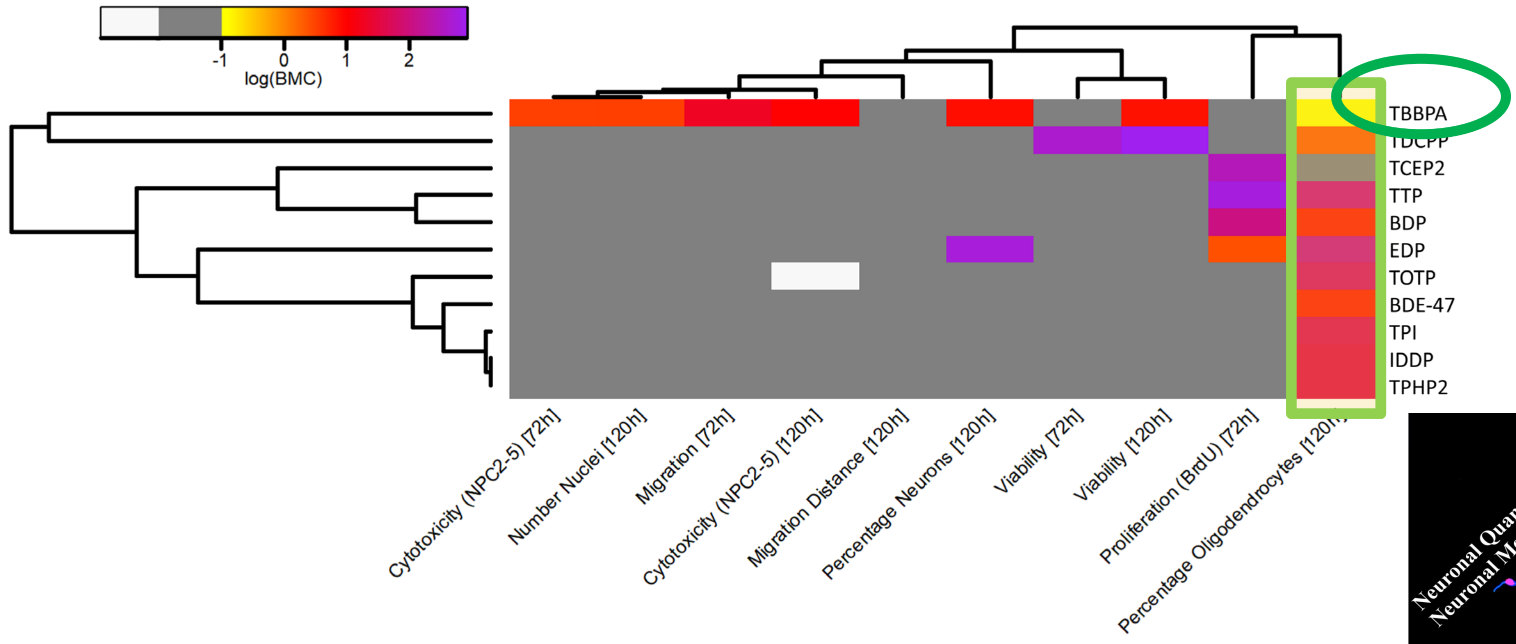




Novel replacements show comparable activity to phased-out compounds

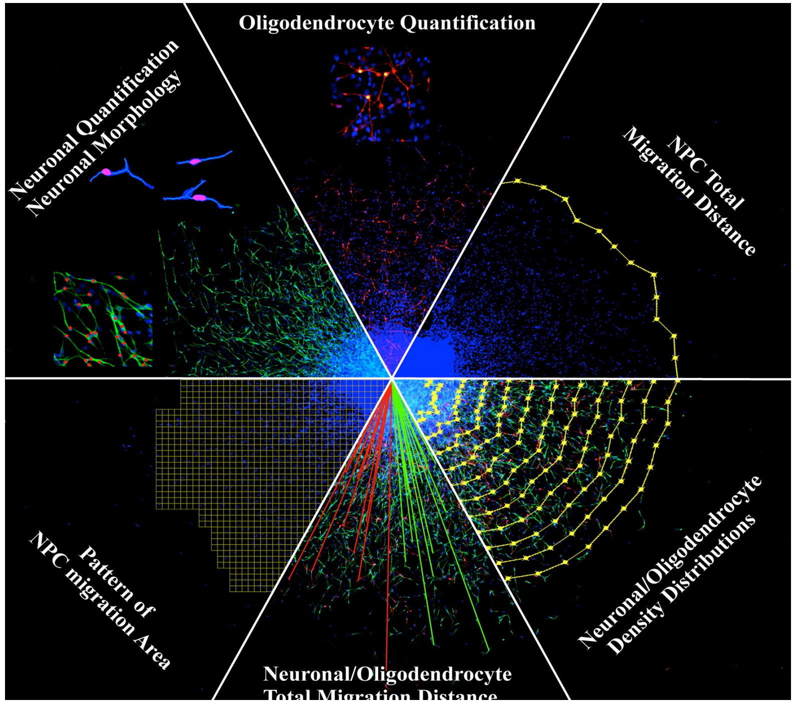
Data publicly available on DNT-DIVER
https://sandbox.ntp.niehs.nih.gov/neurotox/

Comparison of OPFRs with phased-out compounds (3D Neurospheres)



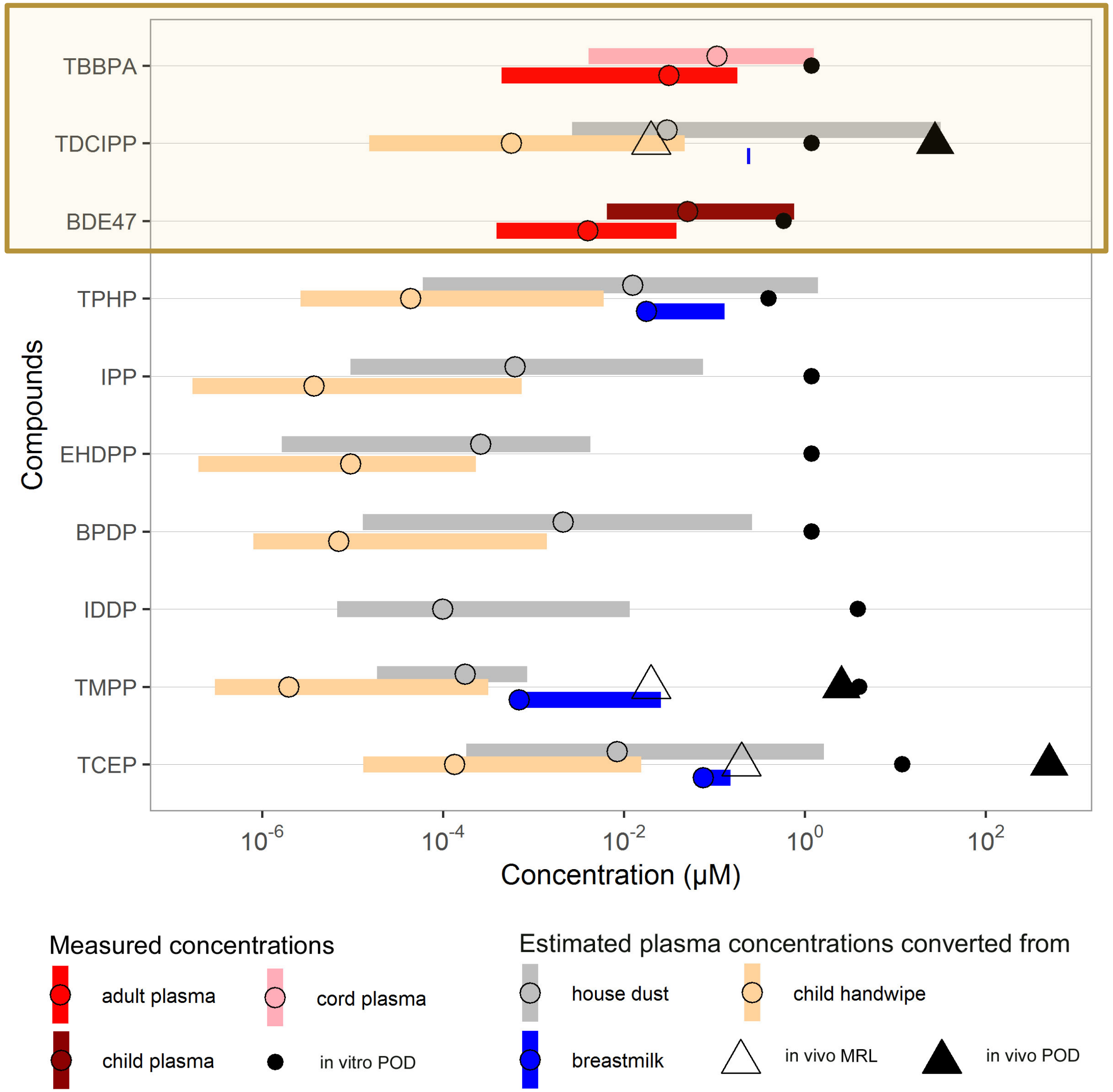
white = no data
grey = no effect

Oligodendrocytes most sensitive end-point



3-D models used to capture additional DNT- related biological space

Relevance to Human Exposures



Extensively used (TBBPA), phased-out (BDE47)

Nov

1. Novel substitutes have comparable in vitro activity to older FRs
2. In vitro activity within order of magnitude of in vivo POD (when known)
3. Activity lies within range of human exposure (limited exposure data for novel compounds)

Blum et al., (2019) Environ. Sci. Technol. Lett. 6, 638−649

Ongoing Efforts at the NTP

- NTP conducting guideline DNT studies on 2 representative compounds to compare findings in the battery with *in vivo* studies
- Evaluating novel short-term behavioral screens that may replace DNT Guideline studies
  - Minimal experimenter interference, automated, social housing
  - Applying principles of artificial intelligence

Uncertainty

- IATA being used to prioritize compounds for further *in vivo* testing
  - Collectively discussing what else is required for prediction
- Assumptions of in vitro & alternative animal models
  - Kinetics, metabolism, internal dose, absence of BBB, genetic diversity, gender
- Data analysis pipeline can influence results
- Assumptions in IVIVE modeling
  - Assumptions in clearance; BBB

What is the alternative approach for timely regulation and protection of susceptible populations?

Application of IATA

- Generate data for hazard characterization in a timely manner

ELSEVIER

Neurotoxicology and Teratology

Volume 52, Part B, November–December 2015, Pages 181-193

Use of alternative assays to identify and prioritize organophosphorus flame retardants for potential developmental and neurotoxicity ☆

Mamta Behl[a], Jui-Hua Hsieh[d], Timothy J. Shafer[b], William R. Mundy[b], Julie R. Rice[a], Windy A. Boyd[a], Jonathan H. Freedman[c], E. Sidney Hunter III[b], Kimberly A. Jarema[b], Stephanie Padilla[b], Raymond R. Tice[a]

Environmental Science & Technology LETTERS

Review

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pubs.acs.org/journal/estlcu

Organophosphate Ester Flame Retardants: Are They a Regrettable Substitution for Polybrominated Diphenyl Ethers?

Arlene Blum,[†,‡] Mamta Behl,[§] Linda S. Birnbaum,[∥] Miriam L. Diamond,[⊥] Allison Phillips,[●] Veena Singla,[#] Nisha S. Sipes,[§] Heather M. Stapleton,[@] and Marta Venier[*,∇]

- Use for prioritizing compounds for further testing

Conclusion

- IATA demonstrates how a battery may be used for prioritization, timely data dissemination, and (depending on the user) decision-making
- Appears that the *in vitro* activity for some of the OPFRs (i.e., TDCIPP and TPHP) is comparable to that of the phased-out BDEs (e.g., BDE-47) and lies within the range of human exposure (TPHP)
- The *in vitro* activity appears to be at levels comparable to the *in vivo* BMCs (PODs) for some compounds (e.g., TDCIPP)
  - Data exist only for select compounds
- Important to consider IATAs to provide rapid and timely relevant information for human health protection especially for sensitive populations
  - Complement time and cost intensive animal studies

The Team

- Jui-Hua Hsieh, NTP
- Fritsche Lab, IUF
- Magdalini Sachana, OECD
- Tim Shafer, USEPA
- Nisha Sipes, NTP

Thanks to other NTP staff and Global Collaborators!

Thank you for your attention!