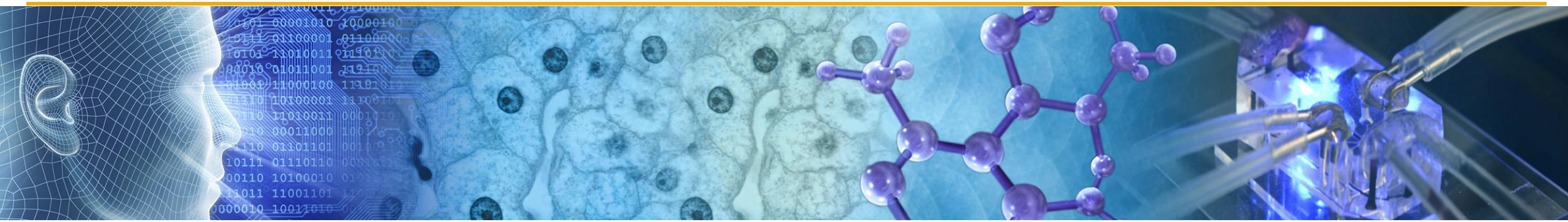
NTP
National Toxicology Program



Activities of the Developmental Neurotoxicity Health Effects Innovation Program

Helena Hogberg on behalf of the team

Division of the NTP, National Institute of Environmental Health Sciences
NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

ICCVAM Public Forum May 26, 2022

UNITED STATES ICCVAM Advancing Alternatives to Animal Testing

DNT Health Effect Innovation (HEI) Program Management Team

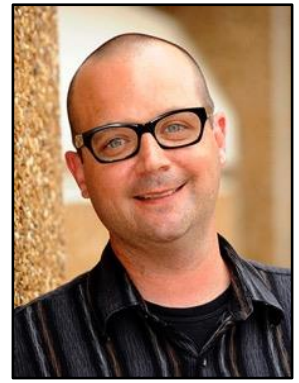
DNTP

Mamta Behl*
Former Team Lead

Jeremy Erickson
PTB

Laura Hall
OPO

Helena Hogberg
PTB



Chris McPherson
MTB

Robert Sills
CMPB, Liaison

Shannah Witchey**
Former STB

Neurobiology Core
DIR

Jesse Cushman
NL/DIR

Briana Bernstein
NL/DIR (adjunct)

Leslie Wilson
NL/DIR (adjunct)

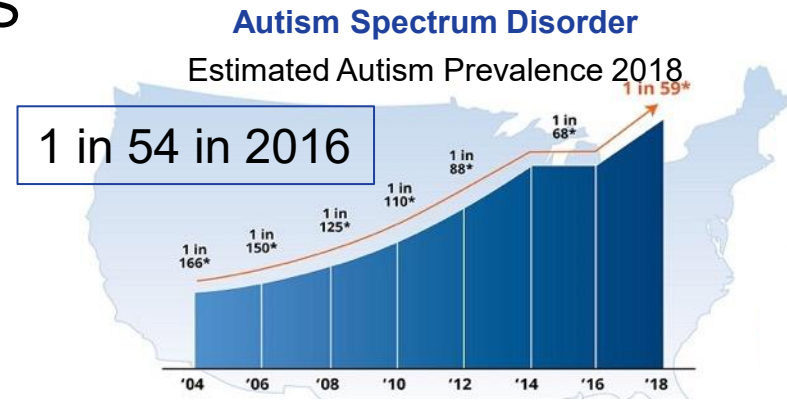
Division of the National Toxicology Program (DNTP)
ODS (Office of Data Science)
OPO (Office of Program Operations)
CMPB (Comparative & Molecular Pathogenesis Branch)
MTB (Mechanistic Toxicology Branch)
STB (Systems Toxicology Branch)
PTB (Predictive Toxicology Branch)

Division of Intramural Research (DIR)
NL (Neurobiology Laboratory)

*Now Neurocrine Biosciences
**Now with Inotiv

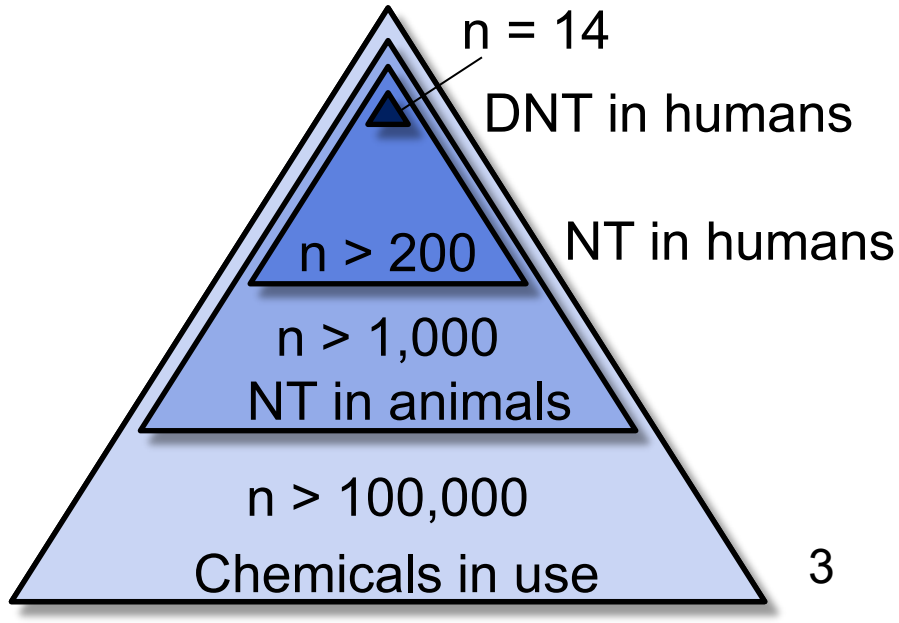
Importance of Assessing Developmental Neurotoxicity (DNT)

- Increase in prevalence of developmental disorders in the US and globally
  - WHO: 1 in 6 kids diagnosed
- *In vivo* DNT Guideline studies primary method of evaluation
  - Require an *a priori* trigger to be run
  - Time & resource intensive
  - Relevance of animal studies for human translation have been questioned
- Compounds with unknown DNT and NT potential remain untested



* Centers for Disease Control and Prevention (CDC) prevalence estimates are for 4 years prior to the report date (e.g. 2018 figures are from 2014)
Source: autismspeaks.org, "CDC increases estimate of autism's prevalence by 15 percent, to 1 in 59 children"

Need for a new Framework!!



Revised from Grandjean and Landrigan 2006, Lancet

Regulatory Focus on Developing New Frameworks

Perspectives | Brief Communication

Project TENDR: Targeting Environmental Neuro-Developmental Risks. The TENDR Consensus Statement

http://dx.doi.org/10.1289/EHP358

Toxicology and Applied Pharmacology 354 (2018) 3–6

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journal homepage: www.elsevier.com/locate/taap

Consensus statement on the need for innovation, transition and implementation of developmental neurotoxicity (DNT) testing for regulatory purposes

Ellen Fritsche[a], Philippe Grandjean[b], Kevin M. Crofton[c], Michael Aschner[d], Alan Goldberg[e,w], Tuula Heinonen[f], Ellen V.S. Hessel[g], Helena T. Hogberg[h], Susanne Hougaard Bennekou[i], Pamela J. Lein[j], Marcel Leist[k], William R. Mundy[l], Martin Paparella[m], Aldert H. Piersma[n], Magdalini Sachana[o], Gabriele Schmuck[p], Roland Solecki[q], Andrea Terron[r], Florianne Monnet-Tschudi[s], Martin F. Wilks[t], Hilda Witters[u], Marie-Gabrielle Zurich[s], Anna Bal-Price[v,*]

OXFORD

SOT | Society of Toxicology

www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 167(1), 2019, 45–57

doi: 10.1093/toxsci/kfy211

Advance Access Publication Date: November 23, 2018

Forum

FORUM

International Regulatory and Scientific Effort for Improved Developmental Neurotoxicity Testing

Magdalini Sachana,*,[1] Anna Bal-Price,† Kevin M. Crofton,‡ Susanne H. Bennekou,§ Timothy J. Shafer,¶ Mamta Behl,‖ and Andrea Terron‖

FIFRA Scientific Advisory Panel Meeting Minutes and Final Report No. 2020-02

Peer Review of the Use of New Approach Methodologies (NAMs) to Derive Extrapolation Factors and Evaluate Developmental Neurotoxicity for Human Health Risk Assessment

September 15-18, 2020

FIFRA Scientific Advisory Panel Meeting

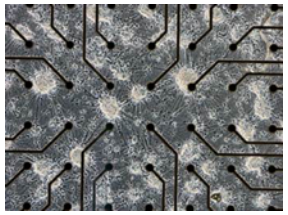
Battery of test: 12 *in vitro* assays + Zebrafish

136 compounds

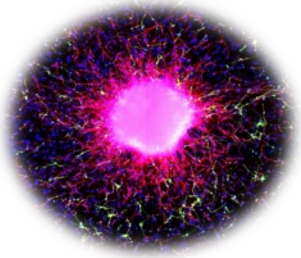
91 + 115 compounds

Bal-Price et al., 2018 ALTEX

2D assays

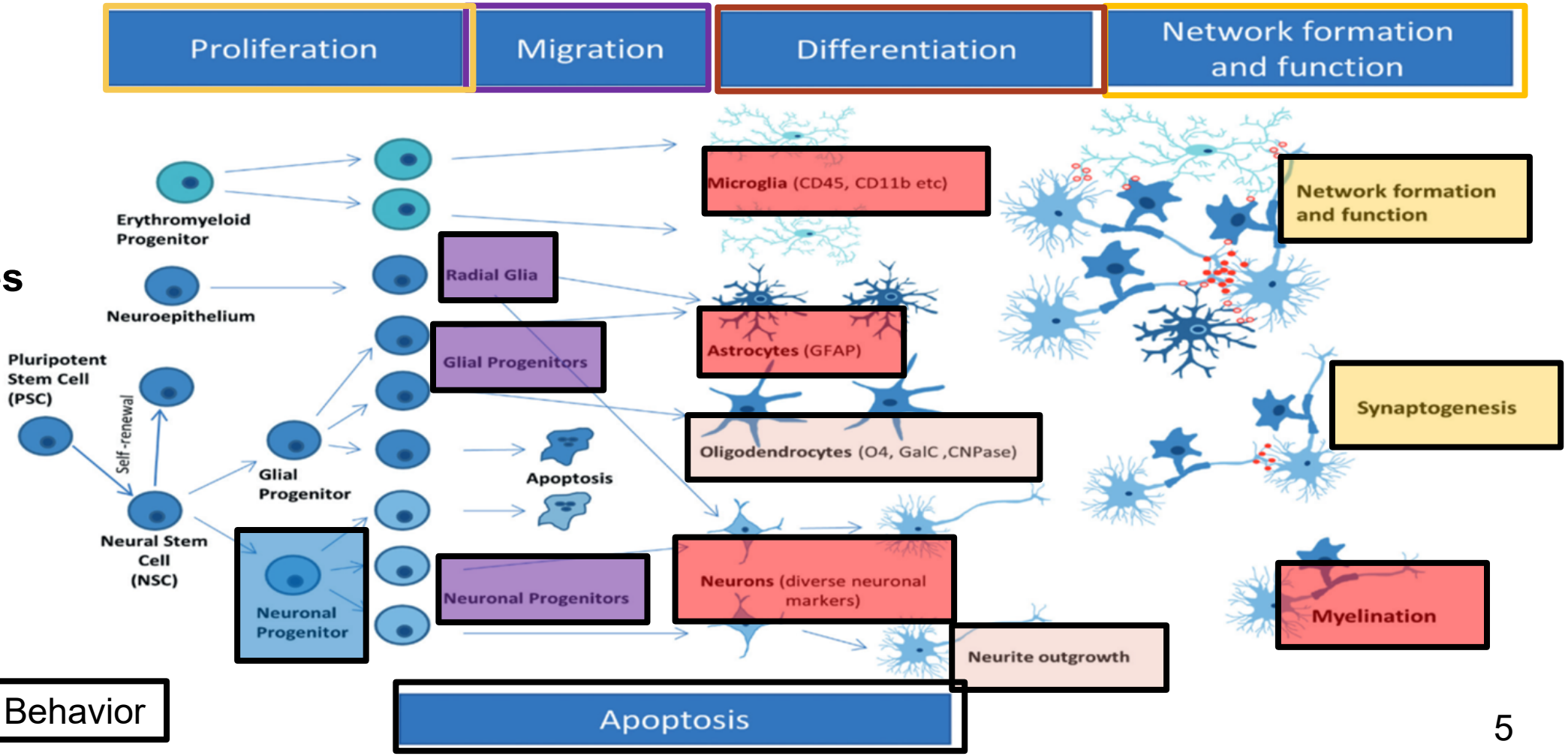


3D Neurospheres



Zebrafish

Behavior

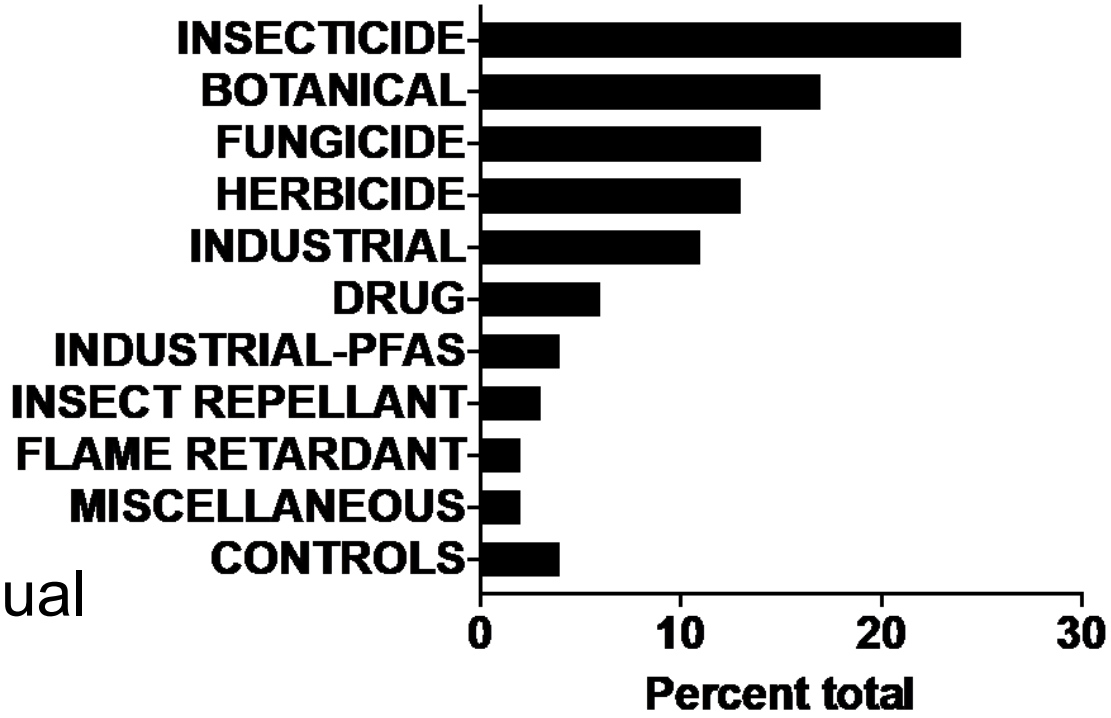


Stakeholder Nominated Chemical Library

Phase 1: 115 chemical set

Selection Criteria

- Evidence of DNT *in vivo*
- Known human exposure
- Guideline study complete, lacking *in vitro*
- Suggested by multiple stakeholders
- Incomplete *in vitro* battery data



Currently tested in the battery in the individual labs, to be finalized in early fall

Phase 2: Chemical nominations received, selection is ongoing

Phase I chemicals
https://www.niehs.nih.gov/research/atniehs/assets/docs/developmental_neurotoxicity_screening_assay_chemical_list_508.pdf

Integrated Approach to Testing and Assessment (IATA) for DNT

Expert Group on DNT

OECD

Guidance Document

Crofton and Mundy 2020

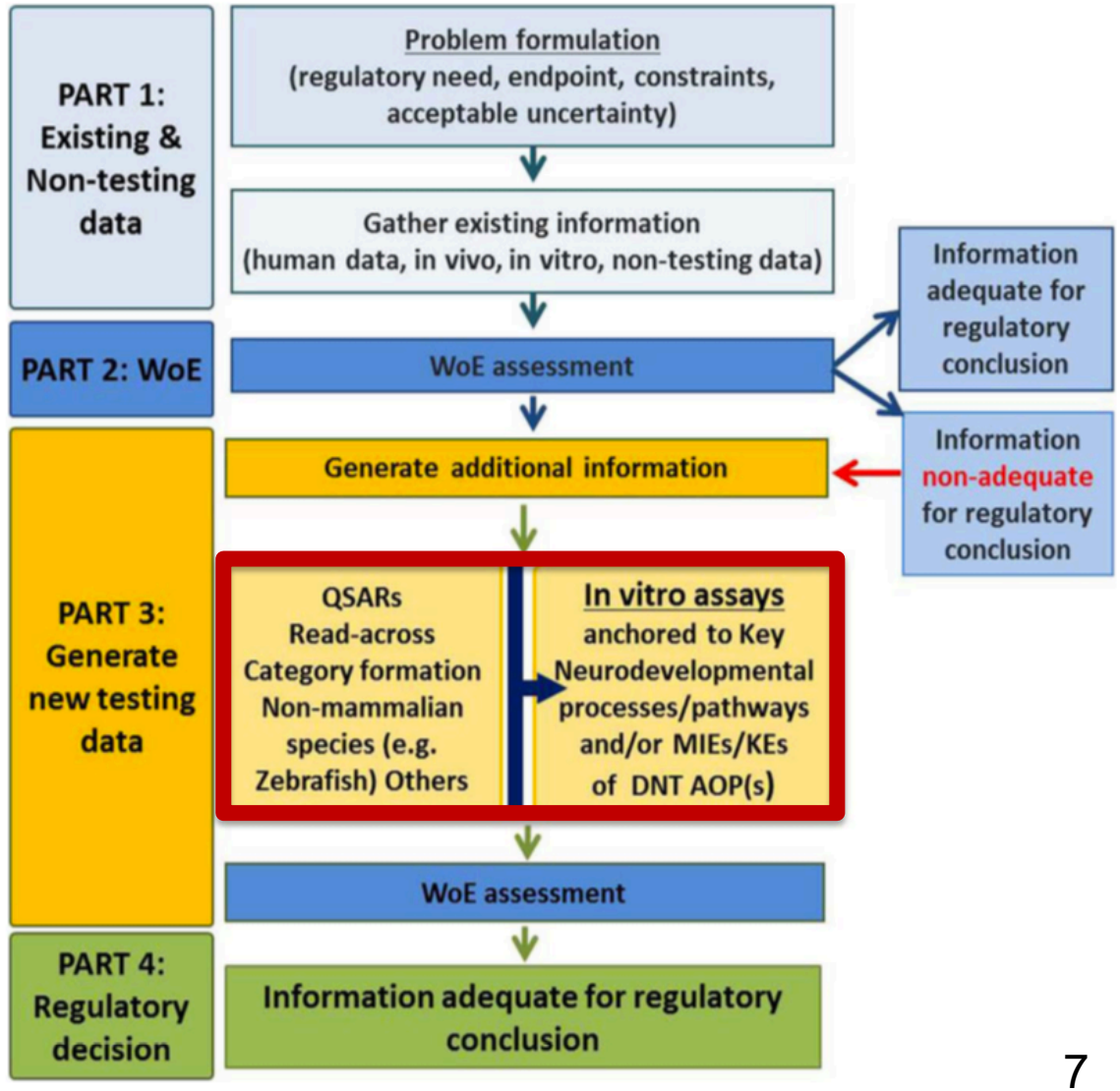
To inform on the testing battery, its usage and interpretation. Case studies exemplifying different regulatory need e.g., prioritization, hazard assessment

FIFRA review NAMs for DNT

2021- EPA Uses NAMs for DNT to support waiving a guideline DNT study

Dobrenieck et al., 2022 Regulatory Toxicology and Pharmacology

Bal-Price et al., 2018 ALTEX



Case study led by DNTP

Title: Organophosphorus flame retardants, a case study on the use of IATA for DNT to prioritize a class of compounds

Authors: Helena Hogberg, Jui-Hua Hsieh, Xiaoqing Chang, Nisha Sipes, Tim Shafer, Mamta Behl

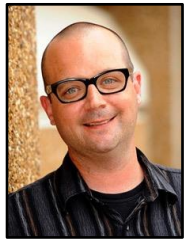
- To be included in the OECD Guidance document on the use and interpretation of DNT battery for the aromatic OPFR
- Intended to provide an example for the use and application of the DNT battery for prioritization of a class of compounds
- Help inform organizations who are evaluating NAMs for use in prioritization and ultimately decision-making

Phased-out (BDE) or Extensively used (and studied): 2

Aromatic phosphates (non-halogenated): 6

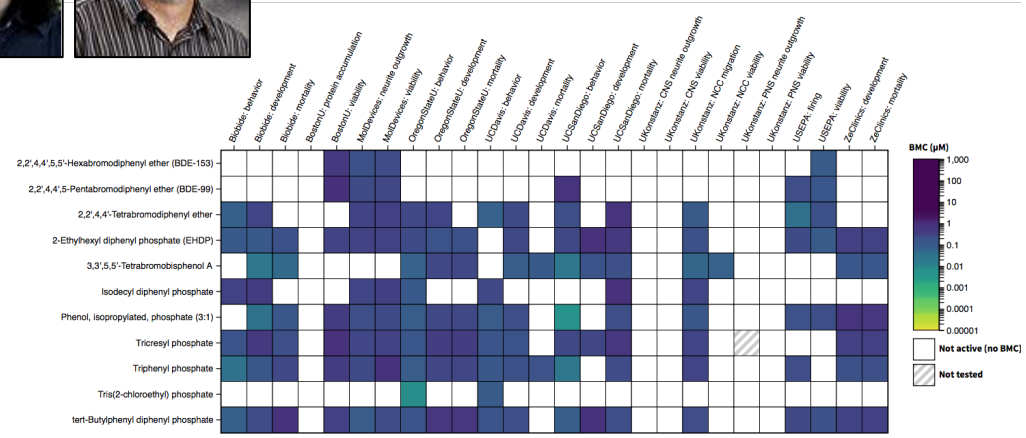
Aliphatic organohalogens: 2

DNT- Data Integration and Visualization Enabling Resource

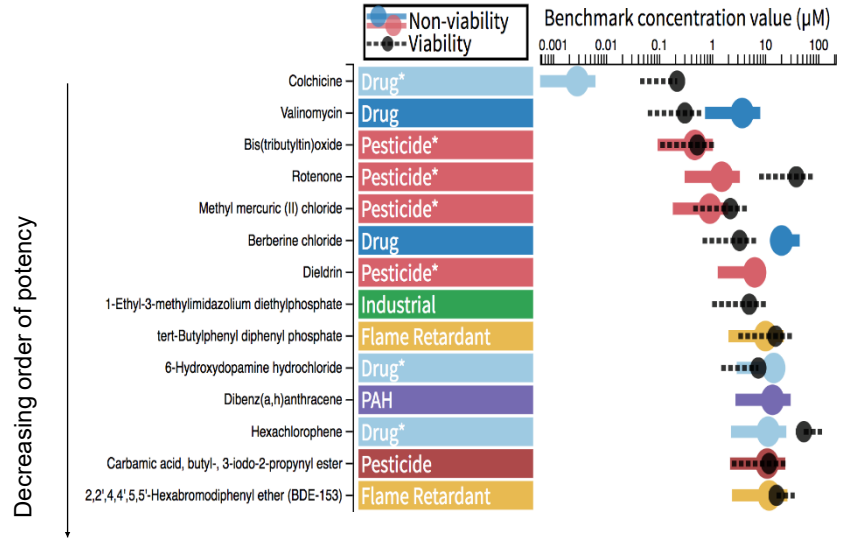




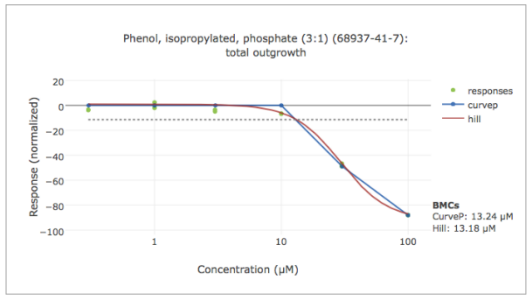

What can you do in DNT-DIVER?



Compare activity of compounds/classes across multiple assays



Compare activity of compounds within an assay



Individual dose-response curves

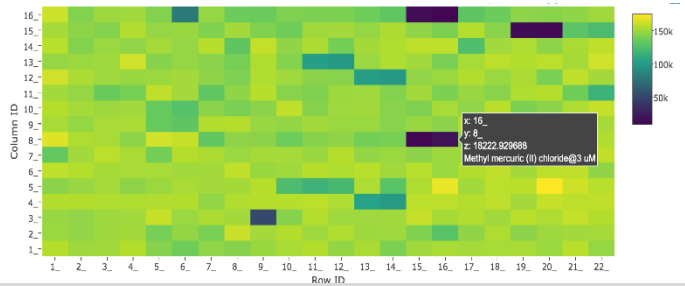
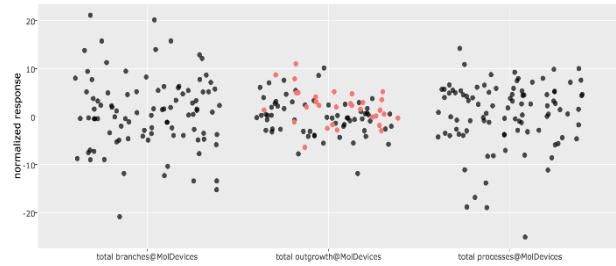


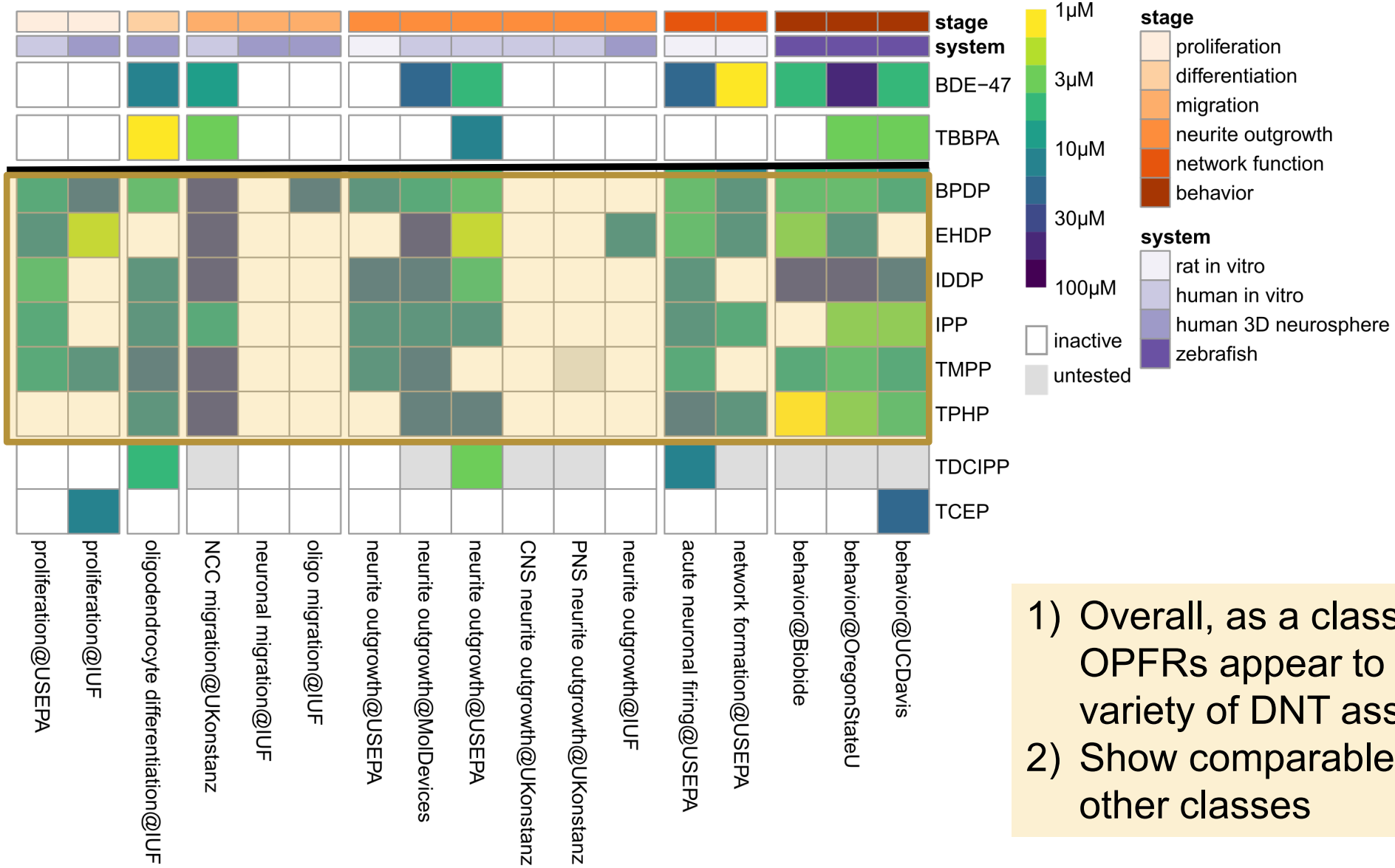
Plate and well level information



Control variability in assay

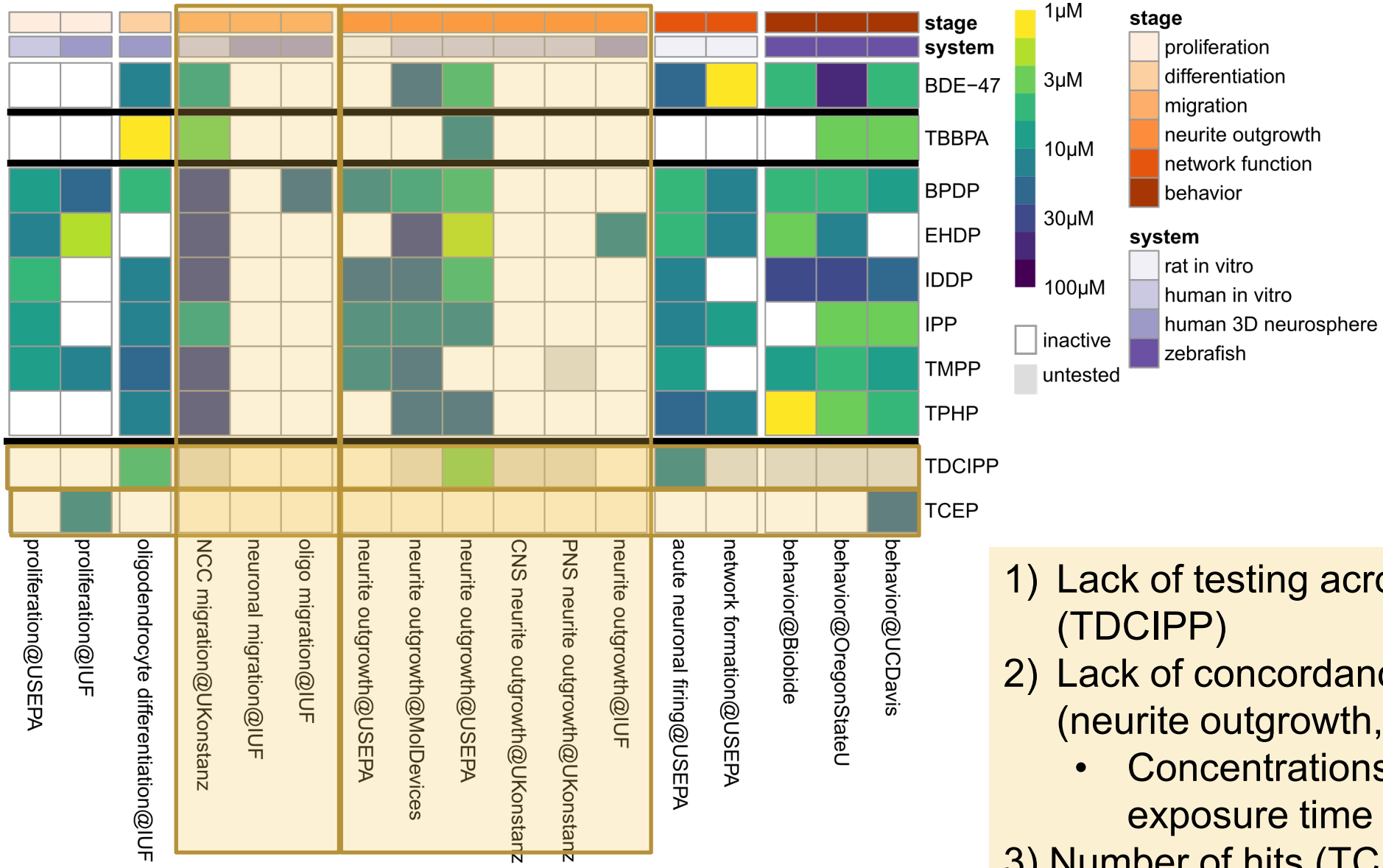
https://sandbox.ntp.niehs.nih.gov/neurotox/

Summary of Findings



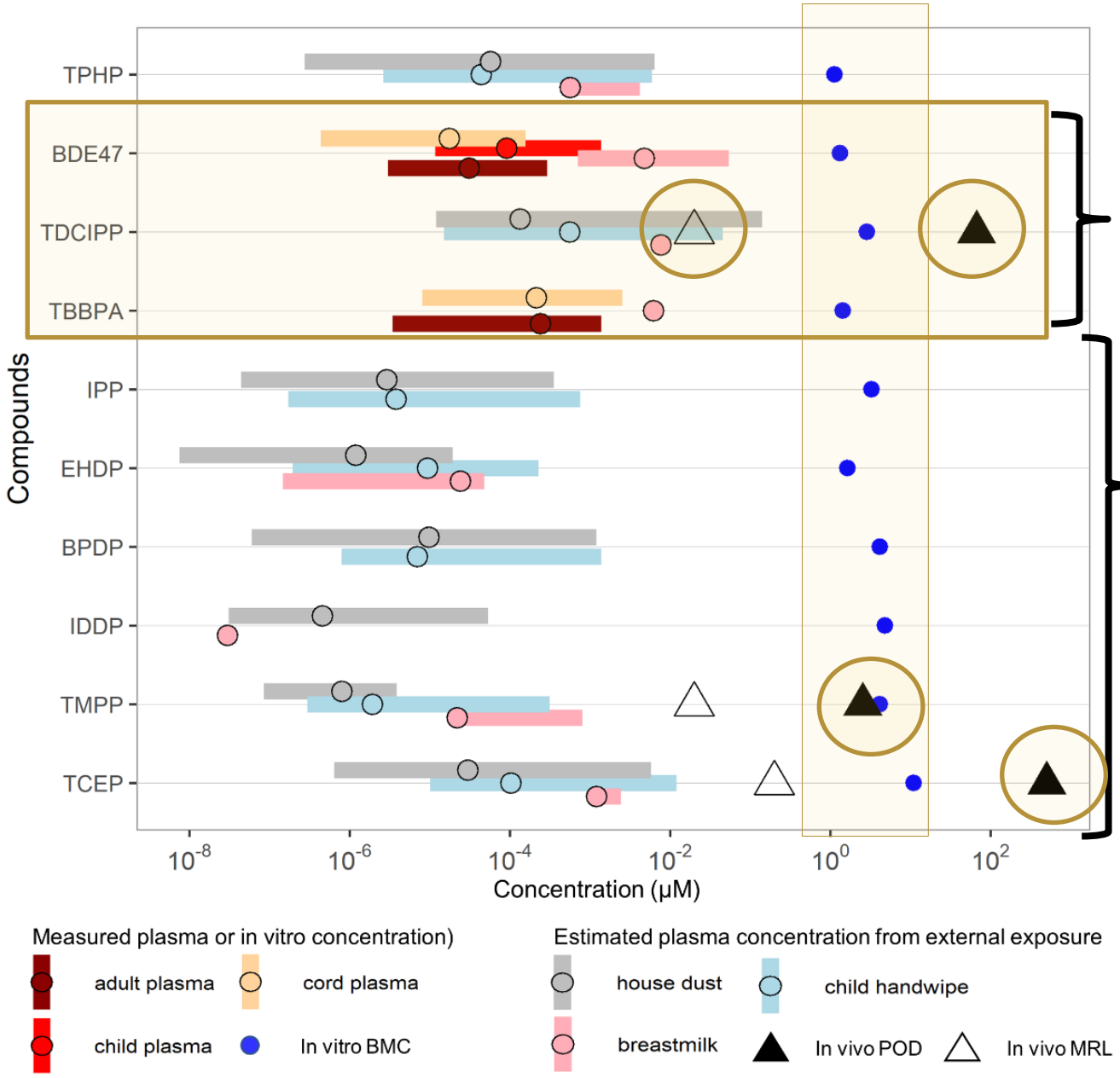
1) Overall, as a class the aromatic OPFRs appear to be active in a variety of DNT assays
2) Show comparable activity to other classes

Sources of Uncertainty



1) Lack of testing across assays (TDCIPP)
2) Lack of concordance within assays (neurite outgrowth, migration)
   - Concentrations, models, exposure time
3) Number of hits (TCEP)

Relevance to Human Exposures



Novel Substitutes

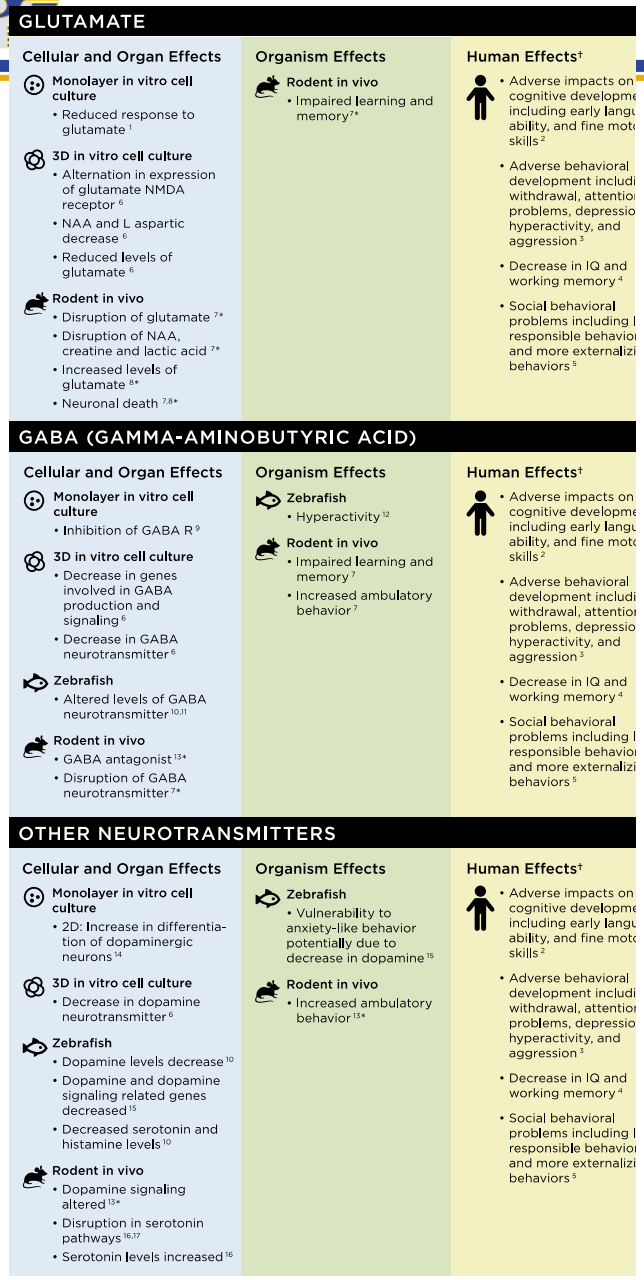
Extensively used/ phased-out

Novel Substitutes

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. In vivo MRL lies within range of human exposure for TDCIPP (limited exposure data for novel compounds)

Updated figure from
Blum et al., 2019, Environ. Sci. Technol.

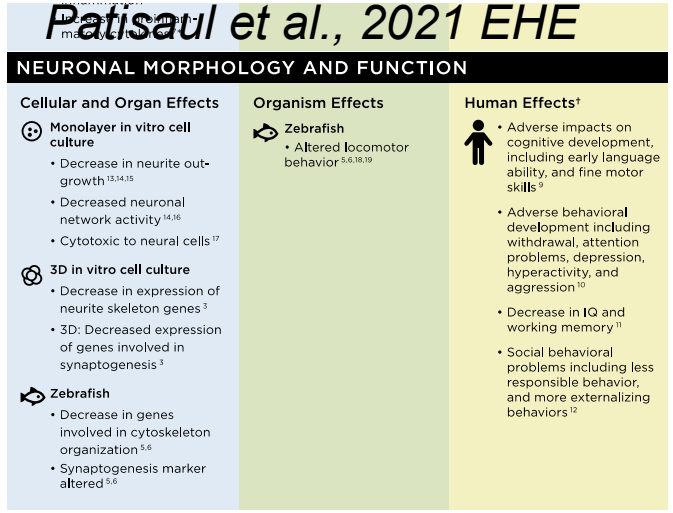
Consideration for Further Development of AOP



Beyond Cholinesterase Inhibition: Developmental Neurotoxicity of Organophosphate Ester Flame Retardants and Plasticizers

Heather B. Patisaul,[1] Mamta Behl,[2,3] Linda S. Birnbaum,[2,3,4] Arlene Blum,[5,6] Miriam L. Diamond,[7] Seth Rojello Fernández,[5] Helena T. Hogberg,[8] Carol F. Kwiatkowski,[5,9] Jamie D. Page,[10] Anna Soehl,[5] and Heather M. Stapleton[4]

Patisaul et al., 2021 EHE



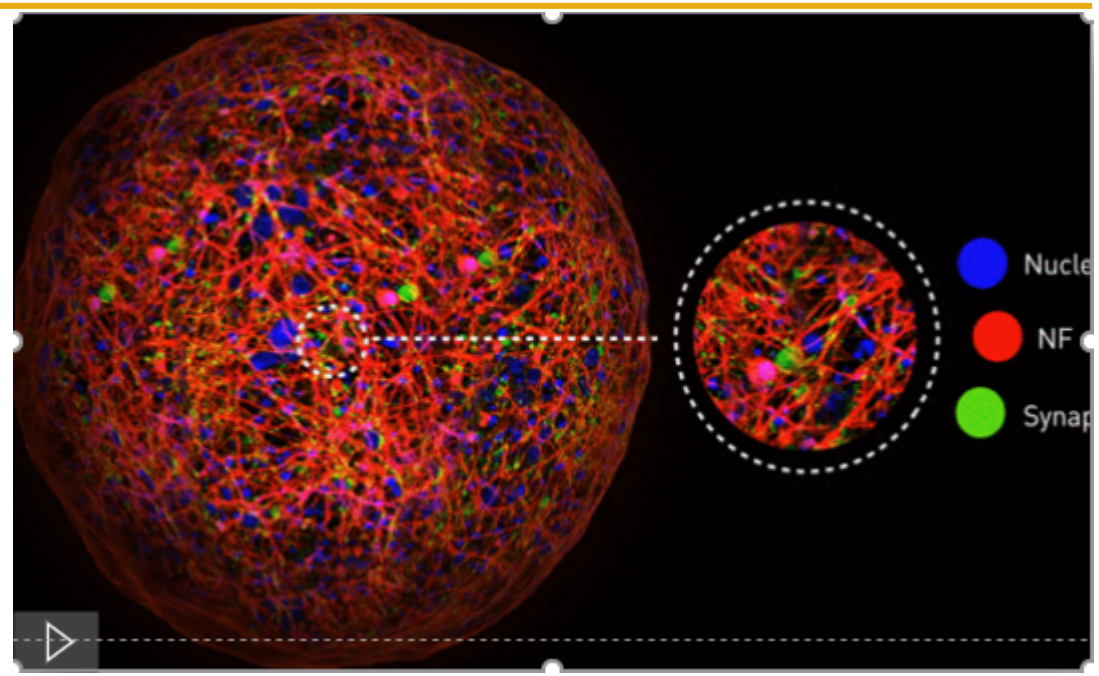
Example of using KEs + underlying mechanistic data in the absence of MIEs

Conclusions Case Study

- Overall, evidence available and the approach taken in this IATA case study allowed to achieve an acceptable level of certainty in prioritization of compounds for further testing
- It also allowed for DNT hazard identification and characterization of the OPFRs which was one of the purposes of the assessment
- The analysis could likely be used by organizations like the CPSC to prioritize compounds for further testing and use the mechanistic data generated here as weight of evidence

Future Directions for the DNT IVB

- Reduce uncertainty levels
  - Anchor data to AOPs
  - Confirm IVIVE models with in vivo data
  - Harmonization of protocols
  - Transferability of assays
  - Understand bioavailability in the different assays



- Explore ways of weighting the different assays
  - How many assays need to be positive for different regulatory purposes?
  - Should assays with higher biological activity be given more weight?
- Develop assays for key events currently missing
  - E.g., Myelination, differentiation and proliferation of astrocytes and microglia, ontogeny of neurotransmitters and receptors

Additional Ongoing and Future Work by DNT-HEI

- Assay development and enhancement *in vitro* and *in vivo*
- Incorporating testing of mixtures in the DNT IVB (collaboration with Combined Exposures and Mixtures Program)
- Further develop the IATA FR case study to build AOPs
- Develop additional case studies in collaboration with EPA
- Support the development of an EFSA data base with *in vivo* DNT studies
- Explore computational tools for:
  - PBPK (placenta model and young children)
  - *IVIVE/IVIVC*
- Prioritize chemicals for further testing
- Population variability and susceptibility

The DNT HEI and NICEATM Groups