Deriving Points-of-Departure for Developmental Neurotoxicity New Approach Methodologies

Katie Paul Friedman

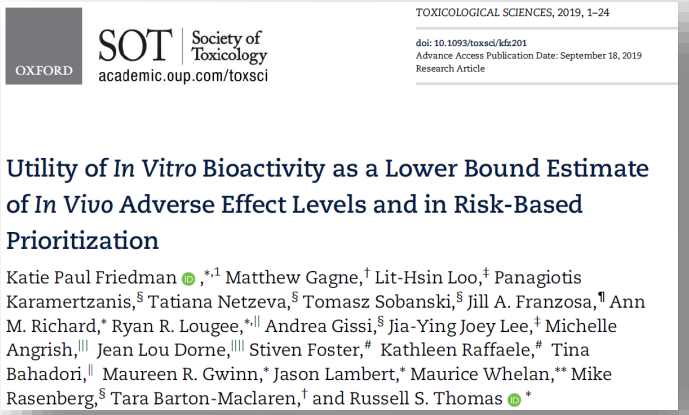
April 27, 2021

Presented to the CSS Monthly Science Webinar

The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA

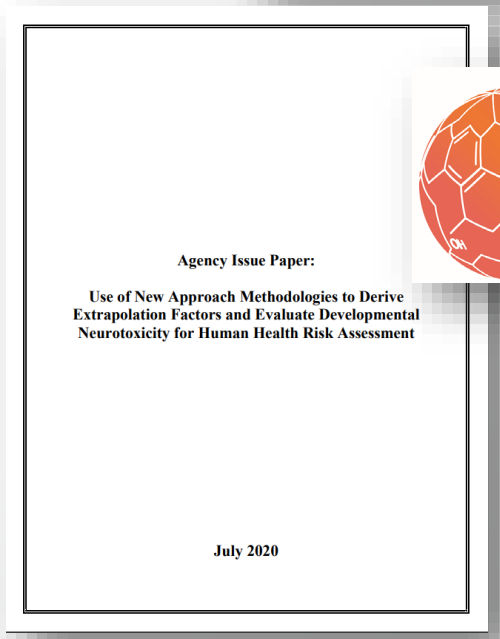
Fit-for-purpose considerations for NAMs in derivation of PODs

- Is the task one that is risk-informed?
  - *Use of a threshold for any bioactivity may be useful.*
  - *Mimics identification of animal-based POD, i.e., a threshold dose at which no effects are anticipated in the animal models employed.*
- Is the task one where specific hazards need to be considered?
  - *Identification of NAMs that are fit-for-purpose regarding the specific hazard may be needed*
  - *Consideration of how to identify "selective" bioactivity from specific NAMs, i.e. a "lead" bioactivity that precedes other bioactivity types.*


OXFORD
SOT Society of Toxicology
academic.oup.com/toxsci
TOXICOLOGICAL SCIENCES, 2019, 1–24
doi: 10.1093/toxsci/kfz201
Advance Access Publication Date: September 18, 2019
Research Article

Utility of *In Vitro* Bioactivity as a Lower Bound Estimate of *In Vivo* Adverse Effect Levels and in Risk-Based Prioritization

Katie Paul Friedman,[*,1] Matthew Gagne,[†] Lit-Hsin Loo,[‡] Panagiotis Karamertzanis,[§] Tatiana Netzeva,[§] Tomasz Sobanski,[§] Jill A. Franzosa,[¶] Ann M. Richard,[*] Ryan R. Lougee,[*,||] Andrea Gissi,[§] Jia-Ying Joey Lee,[‡] Michelle Angrish,[|||] Jean Lou Dorne,[||||] Stiven Foster,[#] Kathleen Raffaele,[#] Tina Bahadori,[||] Maureen R. Gwinn,[*] Jason Lambert,[*] Maurice Whelan,[**] Mike Rasenberg,[§] Tara Barton-Maclaren,[†] and Russell S. Thomas[*]


Agency Issue Paper:

Use of New Approach Methodologies to Derive Extrapolation Factors and Evaluate Developmental Neurotoxicity for Human Health Risk Assessment

July 2020

APCRA
ACCELERATING THE PACE OF CHEMICAL RISK ASSESSMENT

Derivation of PODs from NAMs: IVIVE that employs toxicokinetic extrapolation of dose

High-throughput toxicokinetic (HTTK) approaches make it possible to predict doses corresponding to *in vitro* bioactivity for thousands of chemicals.

TOXICOLOGICAL SCIENCES 125(1), 157–174 (2012)
doi:10.1093/toxsci/kfr254
Advance Access publication September 26, 2011

2012

Integration of Dosimetry, Exposure, and High-Throughput Screening Data in Chemical Toxicity Assessment

Barbara A. Wetmore,* John F. Wambaugh,† Stephen S. Ferguson,‡ Mark A. S
Kimberly Freeman,‡ Harvey J. Clewell, III,* David J. Dix,† Melvin E. Andersen,
Richard S. Judson,† Reetu Singh,* Robert J. Kavlock,† Ann M. Richard,

*The Hamner Institutes for Health Sciences, Research Triangle Park, North Carolina 27709-2137; †United
Research and Development, National Center for Computational Toxicology, Research Triangle Park, North
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OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 142(1), 2014, 210–224
doi: 10.1093/toxsci/kfu169
Advance Access Publication Date: August 21, 2014

2014

Incorporating Population Variability and Susceptible Subpopulations into Dosimetry for High-Throughput

. Clewell, III*,
mond[‡], Mark A. Sochaski*,

North Carolina 27709-2137, [†]United States
nt, National Center for Computational
p Limited (a Certara company), Blades

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opment, National Center for Computational Toxicology,

OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 147(1), 2015, 55–67
doi: 10.1093/toxsci/kfv118
Advance Access Publication Date: June 16, 2015
Research Article

2015

Toxicokinetic Triage for Environmental Chemicals

John F. Wambaugh*,[1], Barbara A. Wetmore[†], Robert Pearce*, Cory Strope*,[‡], Rocky Goldsmith[§], James P. Sluka[¶], Alexander Sedykh[||], Alex Tropsha[||], Sieto Bosgra[||], Imran Shah*, Richard Judson*, Russell S. Thomas*, R. Woodrow Setzer*

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Health Sciences, Research Triangle Park, North Carolina 2770
Education Grantee P.O. Box 117, Oak Ridge, Tennessee 37831-
Indiana University, Bloomington, Indiana 47405-7105; [||]Depar
Chemistry, University of North Carolina, Chapel Hill, North C
Organisation for Applied Scientific Research (TNO), 3700 AJ Ze

[1]To whom correspondence should be addressed at National Center for Computatior
Alexander Dr., Research Triangle Park, North Carolina 27711. Fax: (919) 541-1194. E-m

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Environmental Protection Agency. Reference to commercial products or services doe

FIFRA Scientific Advisory Panel Minutes No. 2014-03

2014

A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding New High Throughput Methods to Estimate Chemical Exposure

July 29-30, 2014
FIFRA Scientific Advisory Panel Meeting
Held at the
EPA Conference Center
Arlington, VA

Environmental Science & Technology

2017

An Intuitive Approach for Predicting
with the Tox21 10k Library

Nisha S. Sipes,*,[†] John F. Wambaugh,[‡] Robert Pearce,[‡]
Jui-Hua Hsieh,[#] Andrew J. Shapiro,[†] Daniel Svoboda,[§] Mi

[†]National Toxicology Program, National Institute of Environmental Heal
Park, North Carolina 27709, United States
[‡]National Center for Computational Toxicology, U.S. Environmental Pro
Triangle Park, North Carolina 27711, United States
[§]Sciome, Research Triangle Park, 2 Davis Drive, North Carolina 27709, United States
[#]Kelly Government Solutions, 111 T.W. Alexander Drive, Research Triangle Park, North Carolina 27709, United States
[||]National Exposure Research Laboratory, U.S. Environmental Protection Agency, 109 T.W. Alexander Drive, Research Triangle Park,
North Carolina 27711, United States

(2017) 44:549–565
-7

2017

CrossMa

Evaluation and calibration of high-throughput predictions of chemical distribution to tissues

Robert G. Pearce[1,2] · R. Woodrow Setzer[1] · Jimena L. Davis[1,3] · John F. Wambaugh[1]

OXFORD SOT Society of Toxicology academic.oup.com/toxsci

TOXICOLOGICAL SCIENCES, 172(2), 2019, 235–251
doi: 10.1093/toxsci/kfz205
Advance Access Publication Date: September 18, 2019
Research Article

2019

Assessing Toxicokinetic Uncertainty and Variability in Risk Prioritization

John F. Wambaugh ,*,[1] Barbara A. Wetmore,[†] Caroline L. Ring ,*,[‡,2] Chantel I. Nicolas,*,[‡,§] Robert G. Pearce,*,[‡] Gregory S. Honda,*,[‡] Roger Dinallo,[¶] Derek Angus,[¶] Jon Gilbert,[¶] Teresa Sierra,[¶] Akshay Badrinarayanan,[¶] Bradley Snodgrass,[¶] Adam Brockman,[¶] Chris Strock,[¶] R. Woodrow Setzer,* and Russell S. Thomas *

*National Center for Computational Toxicology; [†]National Exposure Research Laboratory, Office of Research and Development, U.S. EPA, Research Triangle Park, North Carolina 27711; [‡]Oak Ridge Institute for Science and Education, Oak Ridge, Tennessee 37831; [§]Office of Pollution Prevention and Toxics, U.S. EPA, Washington, District of Columbia 20460; and [¶]Cyprotex US, LLC, Watertown, Massachusetts 02472

[1]To whom correspondence should be addressed at 109 T.W. Alexander Dr./, NC 27711. Fax: (919) 541-1194. E-mail: wambaugh.john@epa.gov.
[2]Present address: ToxStrategies, Austin, TX 78759.

Disclaimer: The views expressed in this publication are those of the authors and do not necessarily represent the views or policies of the U.S. EPA. Reference to commercial products or services does not constitute endorsement.

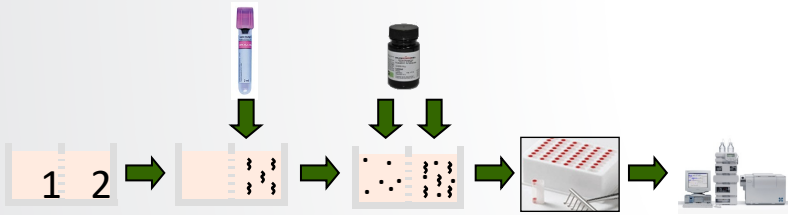
A subset of the papers describing the development of a high-throughput toxicokinetic approach

Reverse dosimetry can be leveraged in IVIVE to estimate the exposure that would produce the plasma concentration corresponding to bioactivity

High throughput toxicokinetics (HTTK)

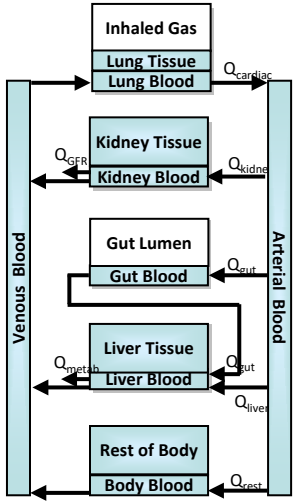
in vitro data

Hepatic clearance from suspended hepatocytes



Plasma protein binding

Generic toxicokinetic models



httk

Some high-level assumptions:

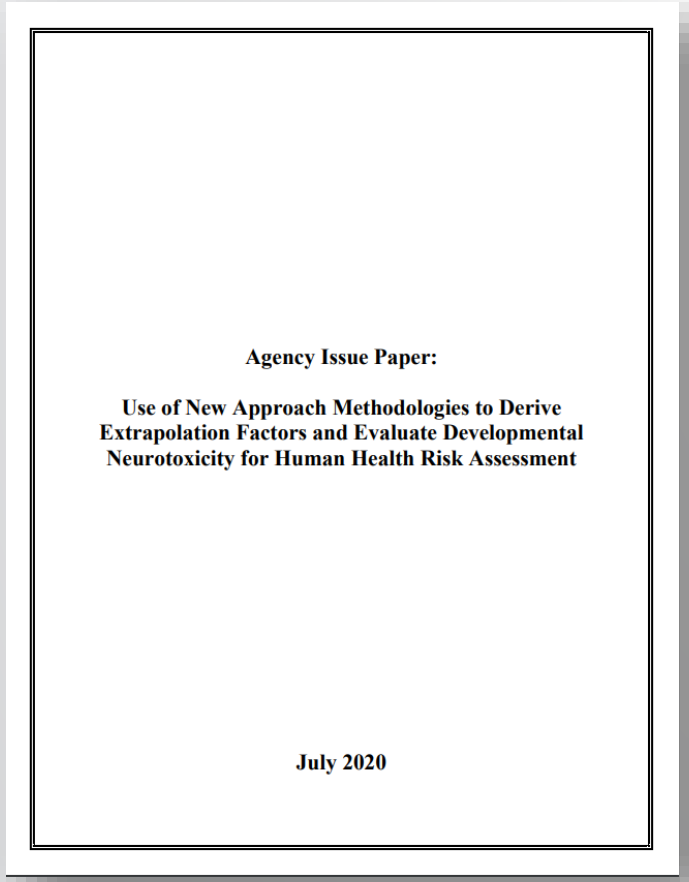
(1) bioactive nominal *in vitro* assay concentration ~ *in vivo* plasma concentration that would correspond to a similar effect;

(2) external exposures (in mg/kg/day units) that may have resulted in that plasma concentration can be constructed using estimates of species-specific physiology and Phase I and Phase II enzyme-driven hepatic clearance; and,

(3) Often, we expect that plasma concentration can be approximated by steady-state kinetics (unless we have enough information to use other dose metrics).

Led by John Wambaugh, Barbara Wetmore, and colleagues

Application of hazard-specific NAMs to specific questions about the potential developmental neurotoxicity



Agency Issue Paper:

Use of New Approach Methodologies to Derive Extrapolation Factors and Evaluate Developmental Neurotoxicity for Human Health Risk Assessment

July 2020

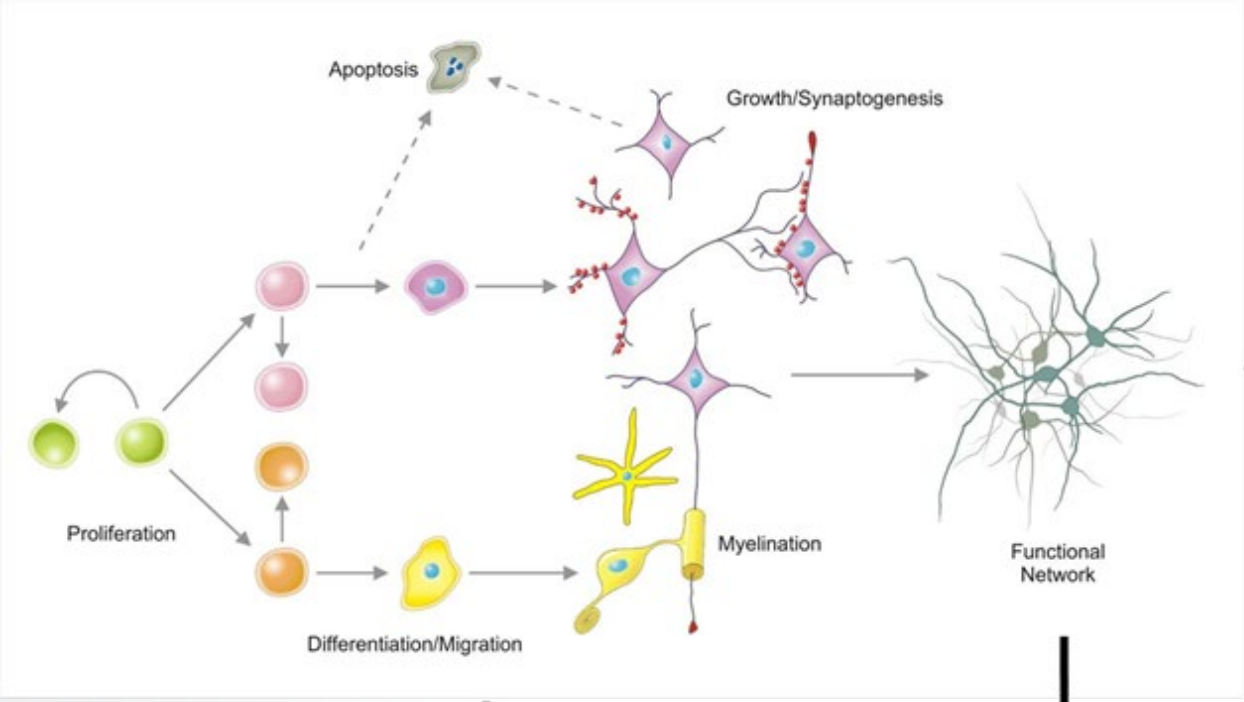
ORD DNT NAMs Team: Josh Harrill, Tim Shafer, Stephanie Padilla, Katie Paul Friedman, John Wambaugh

September 15-18, 2020 Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel met to review this Issue Paper and presentations

https://beta.regulations.gov/document/EPA-HQ-OPP-2020-0263-0006

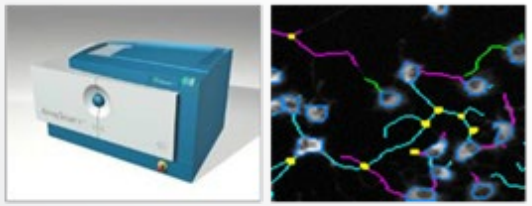
Phenotypic Screening for DNT Hazard

Assays should allow quantitative measurements of key neurodevelopmental events *in vitro*



Cognition
Behavior

Key Neurodevelopmental Events (High Content Imaging)



Network Formation (Multi-Electrode Array)

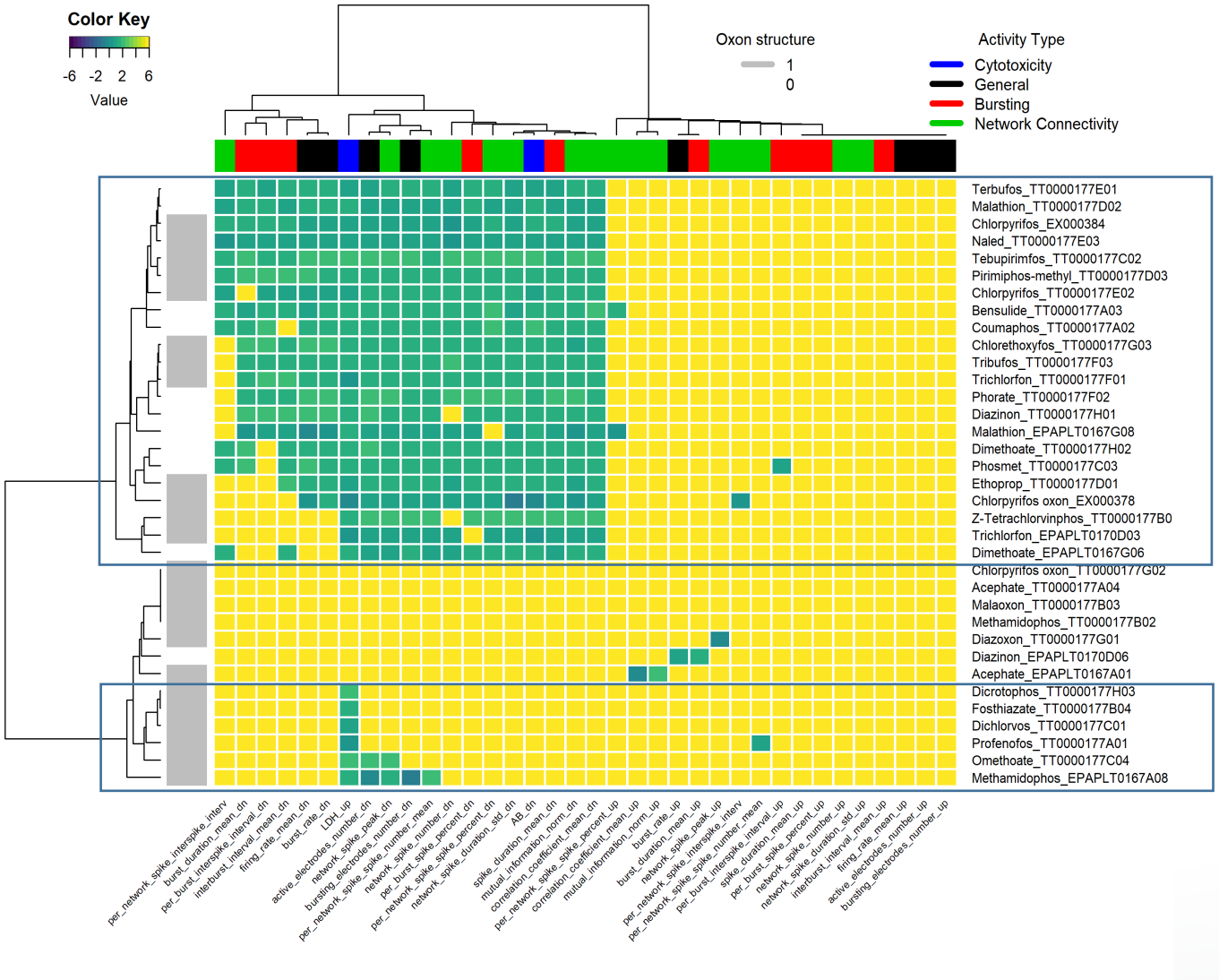
ZF behavior coming soon but not in this work

One of several charge questions addressed derivation of PODs

"In order to compare the relative sensitivity of the MEA NFA and HCI assay results to doses that inhibit acetylcholinesterase in laboratory animals, in vitro to in vivo extrapolation (or IVIVE) approaches were used to approximate NAM administered equivalent doses for a subset of organophosphate pesticides. ***Please comment on the strengths and limitations of this comparison and whether there are alternative approaches for this evaluation****."*

- Underscore the reproducibility of the DNT NAM assays.
- Describe the differential performance of OPs in the DNT NAM assays that are currently available.
- Demonstrate an IVIVE approach to derive doses for comparison to BMD and BMDL values based on rat AChE inhibition.

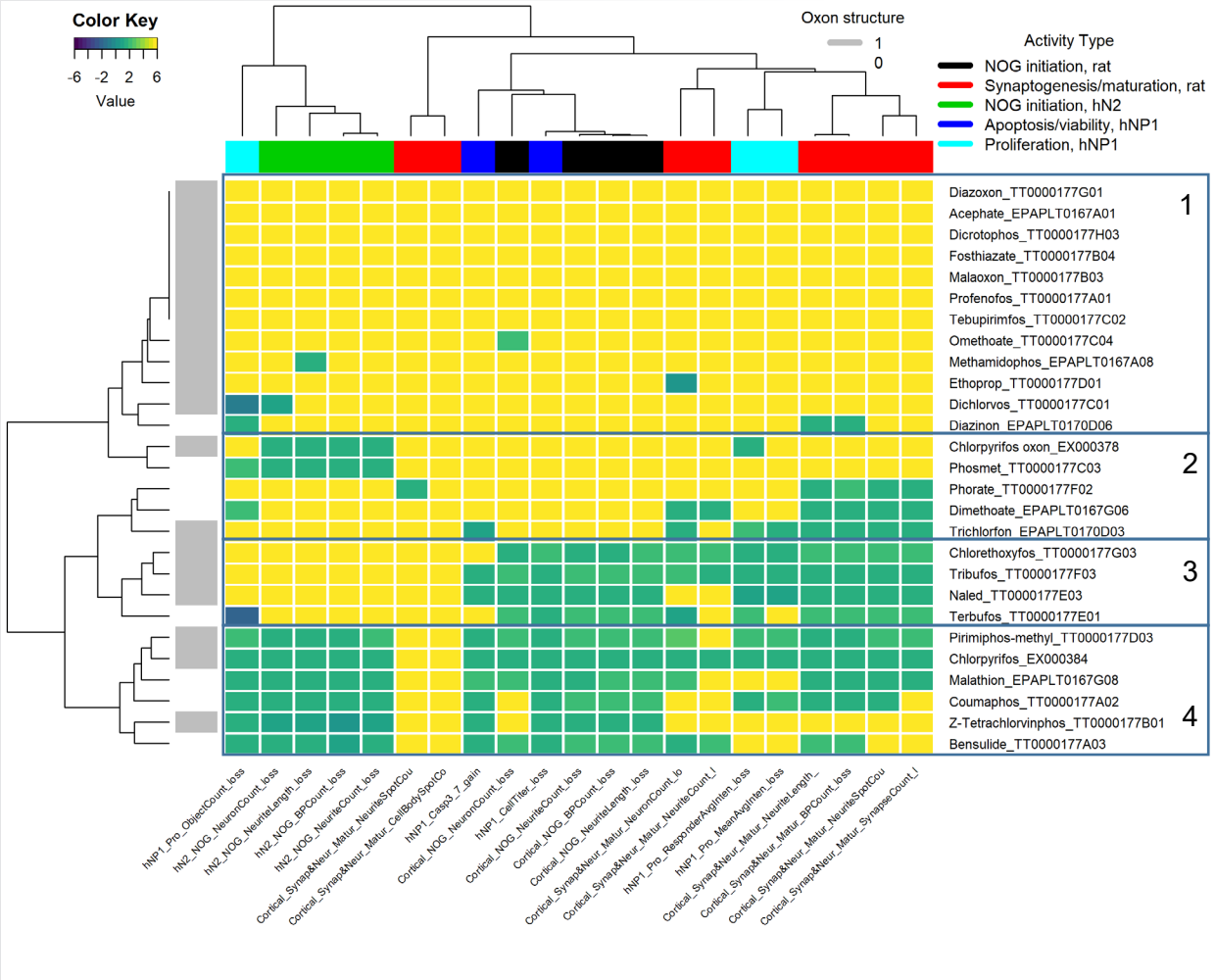
Like the assay controls, some OPs decrease MEA NFA activity types



- Top active cluster of OPs contains oxon and non-oxon structures.
- These OPs, like the assay performance controls, appear to generally decrease all activity types and most assay endpoints.
- Cytotoxicity and activity occur within a narrow concentration range.
- Bottom cluster with minimal actives appears somewhat driven by cytotoxicity in the LDH assay.

Conclusion: while not all OPs are active in the MEA NFA, those that are active appear to behave much like the assay performance controls that inhibit NOG and/or synaptogenesis.

OPs demonstrate differential responses in the HCI assays.



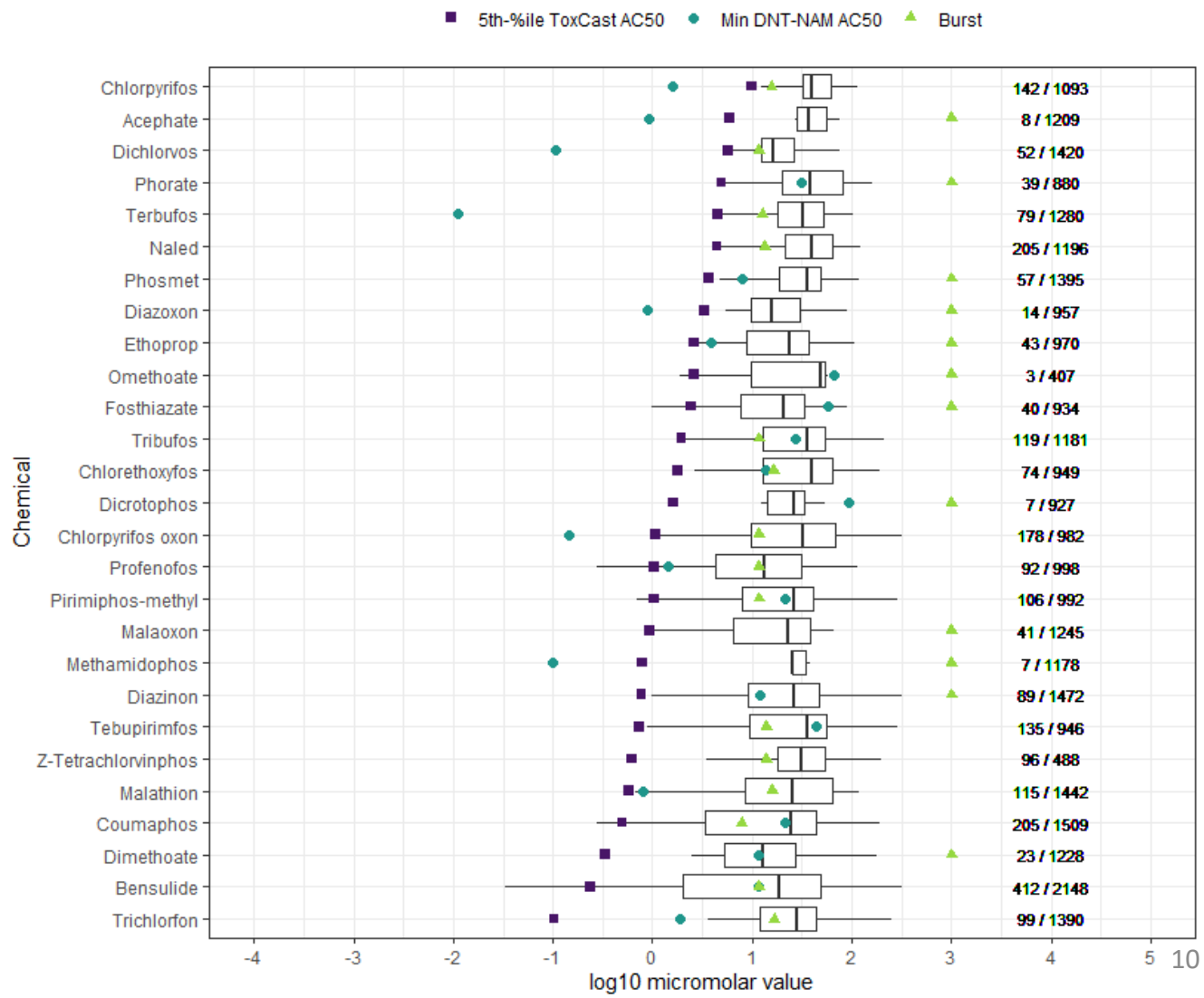
- Cluster 1: negative or with effects in 1-3 endpoints.
- Cluster 2: effects on five or more assay endpoints
- Cluster 3: OP samples with effects on all HCI assay activity types except for NOG initiation in hN2 cells
- Cluster 4: widespread effects across activity types

For some OPs, the minimum DNT-NAM AC50 < an estimate of bioactivity from the rest of ToxCast.

DNT-NAM battery may provide a more potent estimate of bioactivity for substances with minimum DNT-NAM AC50 < 5th percentile of filtered ToxCast AC50 values:

- Chlorpyrifos and chlorpyrifos oxon
- Acephate
- Dichlorvos
- Terbufos
- Diazoxon
- Methamidophos

Suggests that the DNT-NAM battery, in covering some new biology not previously in ToxCast, may yield bioactivity threshold concentrations lower than what is already available for some neuroactive substances in ToxCast.



Simplifying assumptions for the HTTK approach employed here using httk R package

- 100% bioavailability (all of an oral dose is received by the liver through the portal vein);
- No extrahepatic metabolism: the liver is the only source of chemical clearance from the body by metabolism;
- Hepatic metabolism is first order (proportional to concentration) and does not saturate;
- Renal clearance is proportional to fraction unbound in plasma and glomerular filtration rate (i.e., no active transport); and,
- No biliary excretion or enterohepatic recirculation occurs.

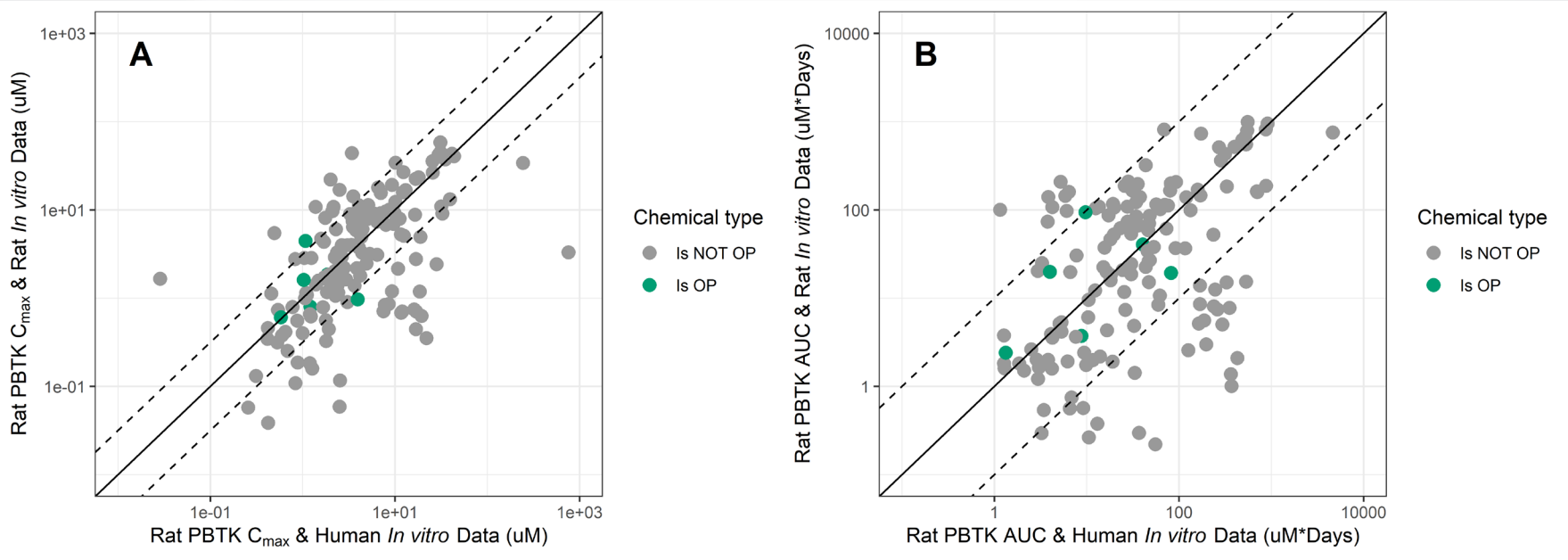
With these assumptions, HTTK models have demonstrated reasonable accuracy in predicting relevant TK endpoints, for example plasma concentrations over time (AUC) (R^2 = 0.62) and maximum plasma concentrations (Cmax) (R^2 = 0.48) (Wambaugh et al., 2018).

AED values in mg/kg/day units were calculated using the following equation (Eq. 2):

$$\textit{Eq.2:}\quad AED_{50}\left(\frac{\frac{mg}{kg}}{day}\right) = AC_{50}(\mu M) * \frac{1\frac{mg}{kg}}{\frac{day}{Css_{50}}}$$

Where the Css (steady-state concentration) values for the median individual based on Monte Carlo simulation of species-specific physiological parameters (Css_{50}) (Pearce et al. 2017) were generated using the 3-compartment steady state model.

To address more of the OPs, we used the "huRat" (gap-filling approach)



Supplemental Appendix Figure 2

- In the absence of hepatic clearance values from rat hepatocytes, rat liver microsomes, or rat liver Phase I enzymes, would the use of human hepatocyte-derived hepatic clearance values be a reasonable substitute?

- In addition to comparing rat-derived AED_{50} values to BMD10 and BMDL10 values from rat studies, we also compared AED values from the "humanized-rat" or the huRat, which used human HTTK data in a model parameterized with rat physiology, to BMD10 and BMDL10 values from rat studies.

General approach to AED50 estimation

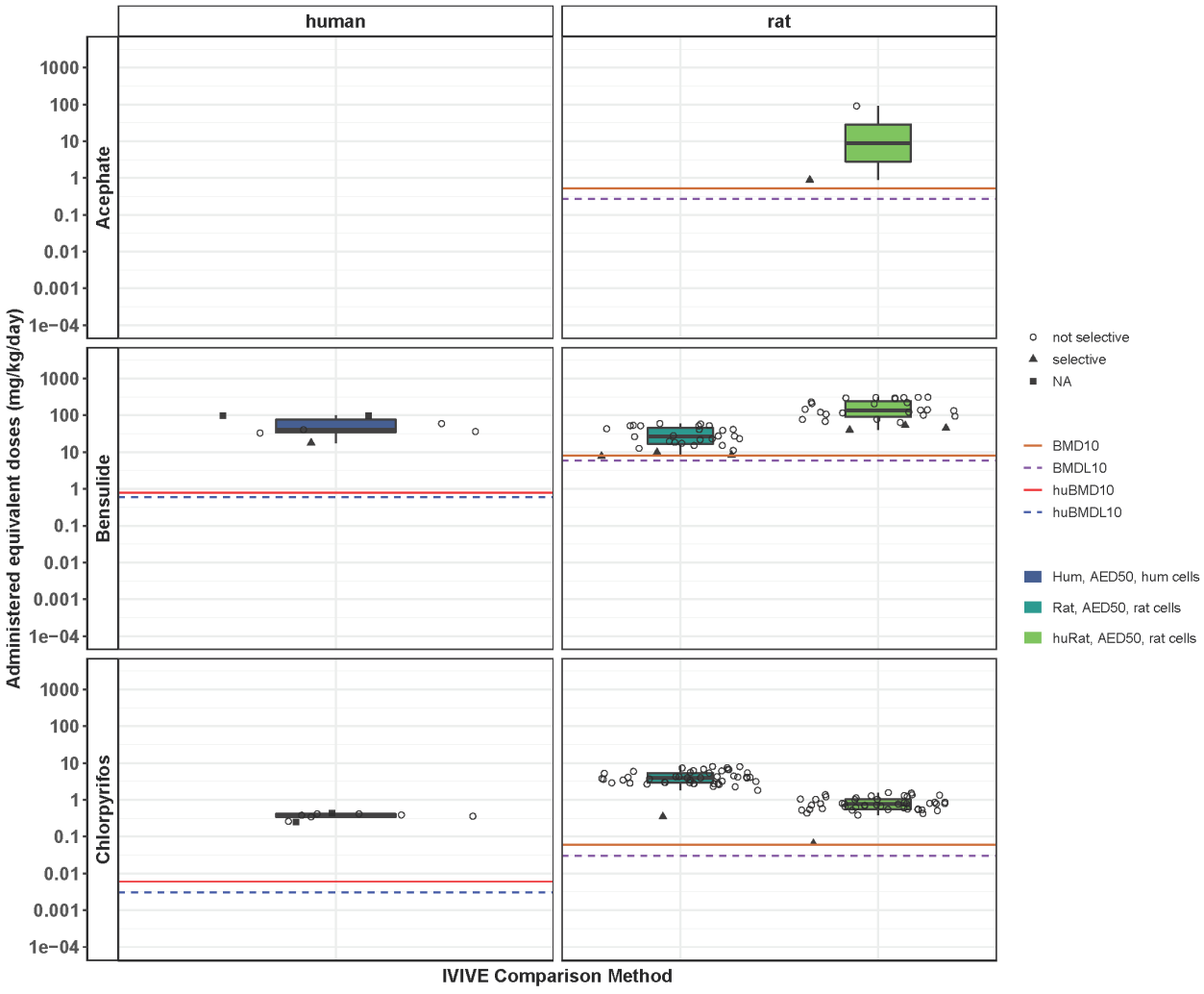
- **Species-specific comparisons** (human *in vitro* assay data to "human"-adjusted benchmark dose values, rat *in vitro* assay data to rat benchmark dose values)
- **Human comparisons:**
  - 9/57 total assay endpoints in the DNT-NAM battery that evaluate neural cell proliferation, apoptosis and viability, and neurite outgrowth initiation were evaluated using human-derived cells
  - human-derived HTTK data and a human-parameterized 3compss model
  - BMD10/BMDL10 values from rat divided by an uncertainty factor of 10 (default interspecies uncertainty factor applied in risk assessments to account for extrapolation from laboratory animals to humans) were compared
  - **Only 17 OP chemicals had positive values in the human assays, and of these 14 had sufficient HTTK data and modeling to calculate human AED_{50} values with the 3 compss model**
- **Rat/huRat comparisons:**
  - 48 of the 57 total assay endpoints in the DNT-NAM battery that evaluate neuronal network formation and function, neurite outgrowth initiation, and neurite maturation and synaptogenesis were evaluated using rat primary cortical cells
  - rat-derived (or for huRat, human derived Clint data) and a rat-parameterized 3 compss model
  - BMD10/BMDL10 values from rat were compared
  - **23/27 OP chemicals have enough data and modeling available to derive huRat AED_{50} values**
  - **9 OP chemicals had enough data and modeling available to derive rat AED_{50} values**
- Three OP chemicals (chlorethoxyfos, naled, Z-tetrachlorvinphos) were excluded from the IVIVE comparisons due to chemical instability in the matrices used for HTTK assays (unpublished, Wetmore 2020)
- Malaoxon was completely negative in all assay endpoints

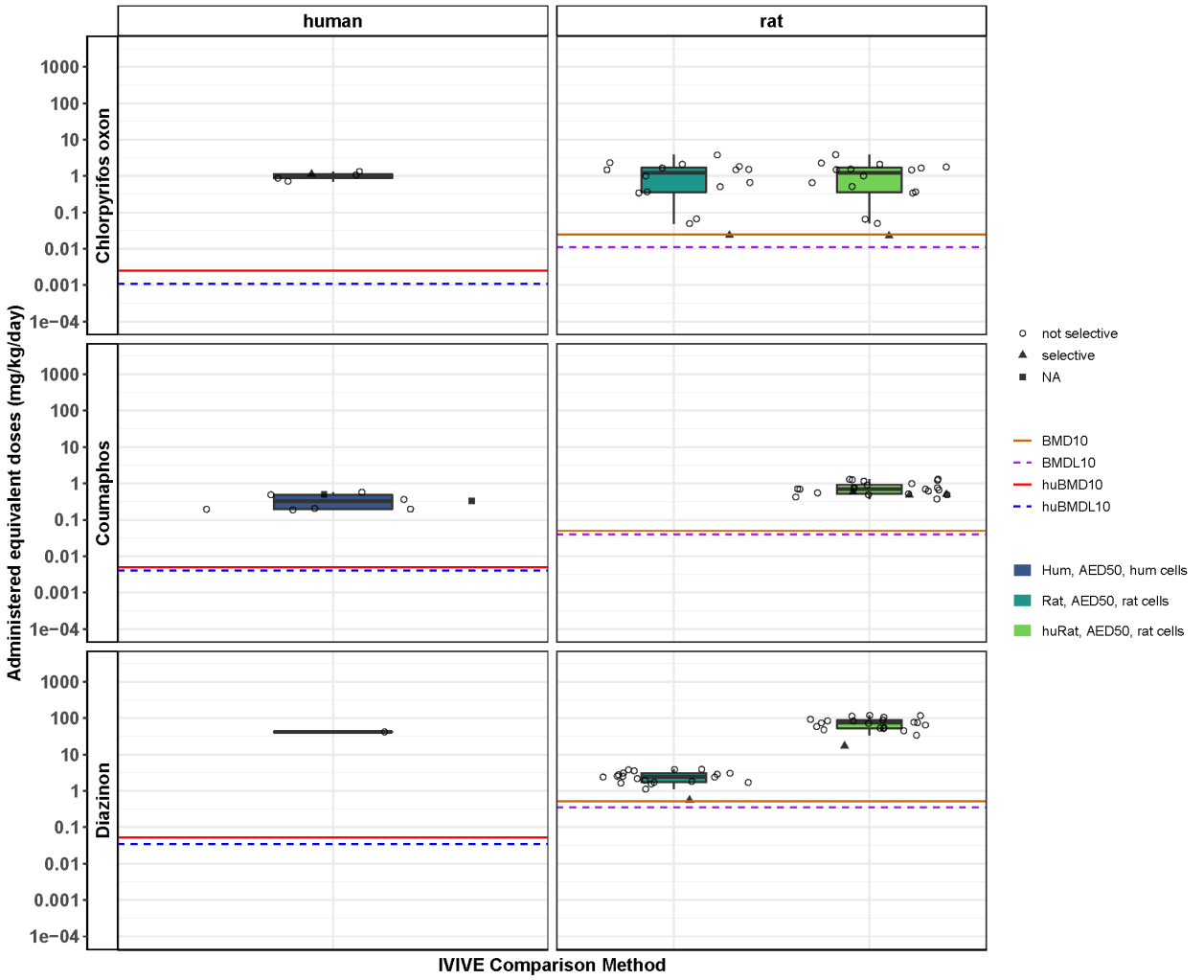
Summary of the AED50 to BMD/BMDL comparison

- Some of the rat and huRat AED_{50} values approached the *in vivo* rat BMD10 and BMDL10 thresholds identified using *in vivo* rat studies of AChE.
- The human IVIVE comparison was more constrained because fewer OP chemicals had positive responses in this smaller subset of the DNT-NAM assay set.
- The human AED_{50} values and huRat AED_{50} values were typically similar; both of these sets of values use the human HTTK data to inform human and rat models, respectively.
  - Deviation between the human and huRat AED_{50} values appears to be impacted by the smaller dataset available for human AED_{50} derivation.
- Chemical-dependent differences between the rat and huRat AED_{50} values are apparent when both are available; though for some chemicals (chlorpyrifos oxon, ethoprop, malathion, omethoate) the values are very similar, for other chemicals (bensulide, chlorpyrifos, diazinon, diazoxon, dimethoate) there may be as much as 1 log10 order of magnitude separation between the median AED_{50} values. There is no uniform direction to these differences, but these differences are expected and consistent with the impact of using human or rat HTTK data to inform a rat physiology-based model.

Overall, these comparisons suggest that the estimated doses required to achieve plasma concentrations (in the median individual in the general population) that demonstrate *in vitro* bioactivity relevant to DNT are higher than or in some cases approaching/equal to the doses that have been associated with significant changes in AChE activity in rats.

Example: AED50 to BMD/BMDL10 comparisons





Overarching conclusions for application of DNT-NAMs

- MEA NFA and HCI assay suite recapitulates key cellular events and processes relevant to DNT, as demonstrated through the use of appropriate assay performance controls;
- the DNT-NAMs presented here represent a major milestone for *in vitro* fit-for-purpose identification of putative DNT-related hazard, though additional methods may be available in the future;
- the MEA NFA and HCI assay suite demonstrates reproducibility in terms of positive responses and potency of these responses;
- the 27 OP chemicals in this set are differentially active in the MEA NFA and HCI assay suite; and,
- application of IVIVE approaches for the *in vitro* bioactivity observed in these DNT-NAMs results in AED_{50} values that are greater than or in some cases approximate the doses that inhibit AChE *in vivo.*

…and where do we go from here now that the SAP is over?

Ongoing international collaboration for DNT NAM development, use, and evaluation

OXFORD SOT Society of Toxicology
www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 167(1), 2019, 45–57
doi: 10.1093/toxsci/kfy211
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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‖

Towards regulatory DNT testing: Alternative methods

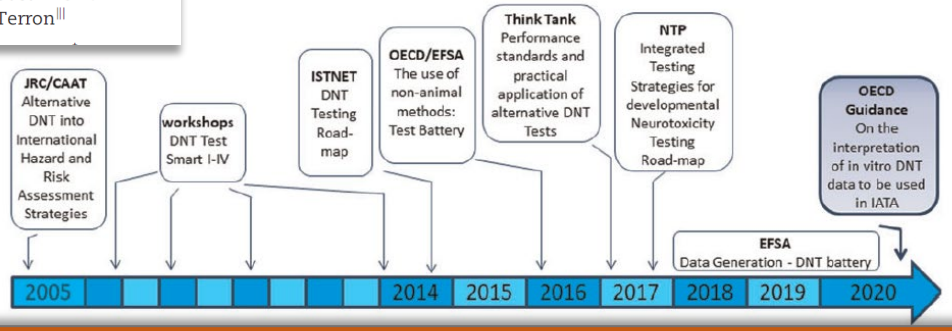


Table 2. Proposed Assays for Evaluation As an *In Vitro* DNT Battery

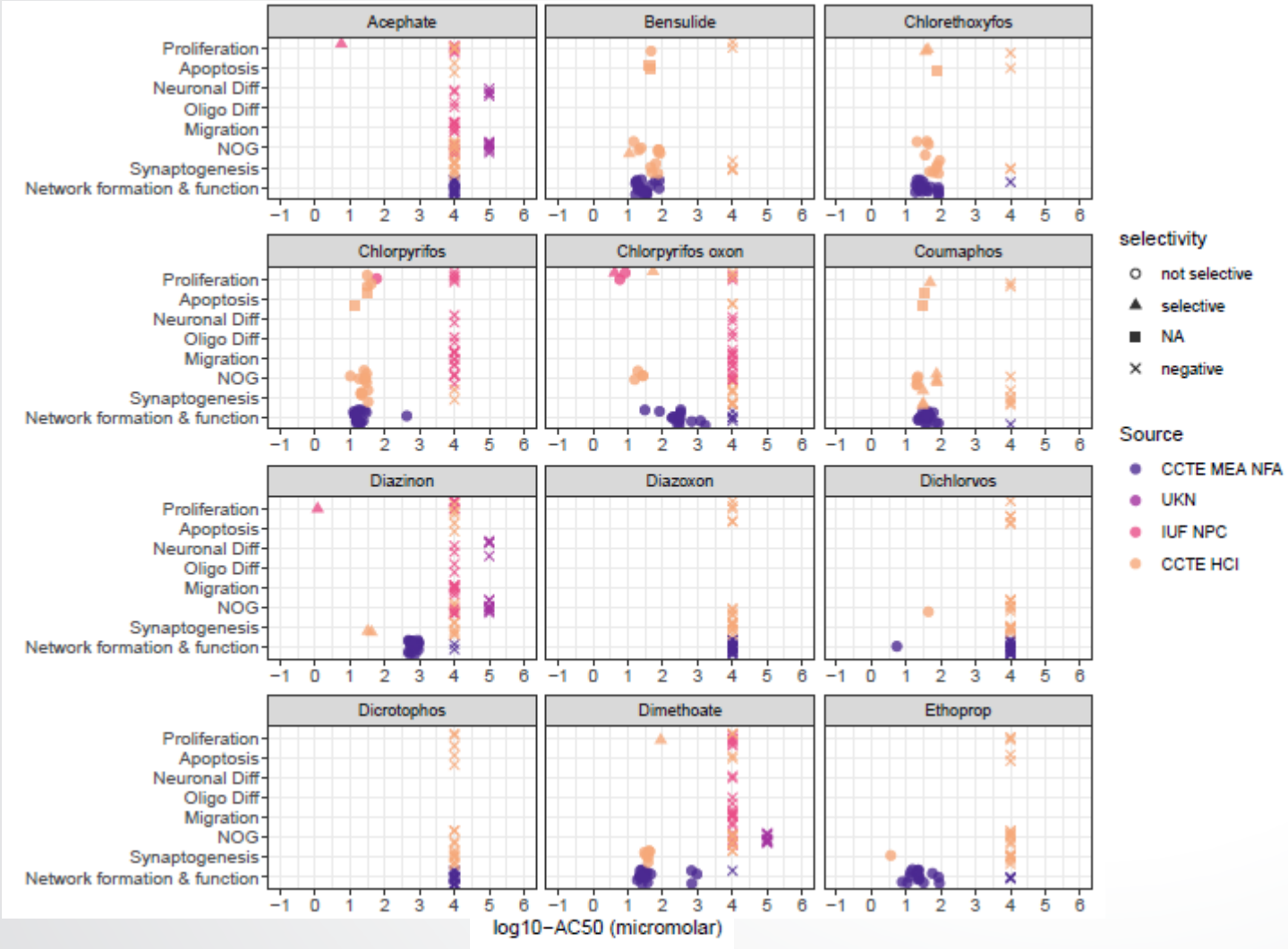
Process	Assays	References
Proliferation	hNP1	Harrill *et al.* (2018)
	NPC1	Baumann *et al.* (2016) and Barenys *et al.* (2017)
	UKN1	Balmer *et al.* (2012)
Apoptosis	hNP1	Harrill *et al.* (2018)
Migration	NPC2	Baumann *et al.* (2016) and Barenys *et al.* (2017)
	UKN2	Nyffeler *et al.* (2017)
Neuron differentiation	NPC3	Baumann *et al.* (2016) and Barenys *et al.* (2017)
Oligodendrocyte differentiation & maturation	NPC5/6	Baumann *et al.* (2016) and Barenys *et al.* (2017)
Neurite outgrowth	~~iCell gluta~~ hN2	Harrill *et al.* (2018)
	UKN 4 & 5	Krug *et al.* (2013)
	NPC4	Baumann *et al.* (2016) and Barenys *et al.* (2017)
Synaptogenesis	Rat primary synaptogenesis	Harrill *et al.* (2018)
Network formation	MEA-NFA	Brown *et al.* (2016) and Frank *et al.* (2018)

Status

- ToxCast Pipelining of UKN and NPC partner data is close to completion, puts all of the data in the same curve-fitting and format.
- Ongoing work will aim to understand the value of the full set of assays.
- EFSA-led collaboration with CCTE to curate and share *in vivo* DNT studies for evaluation of DNT-NAM battery (ToxRef-like).
- CCTE: expert panel to better define putative *in vivo* DNT negatives (Martin et al. *in prep*).
- CCTE: research on interpreting assays for proliferation, apoptosis, neurite outgrowth, and network formation for DNT-relevant bioactivity (Carstens et al. *in prep*)
- ORD Leads: Tim Shafer and Katie Paul Friedman, ORD-CCTE

18

Example: *in vitro* data availability, potency, and selectivity by functional process



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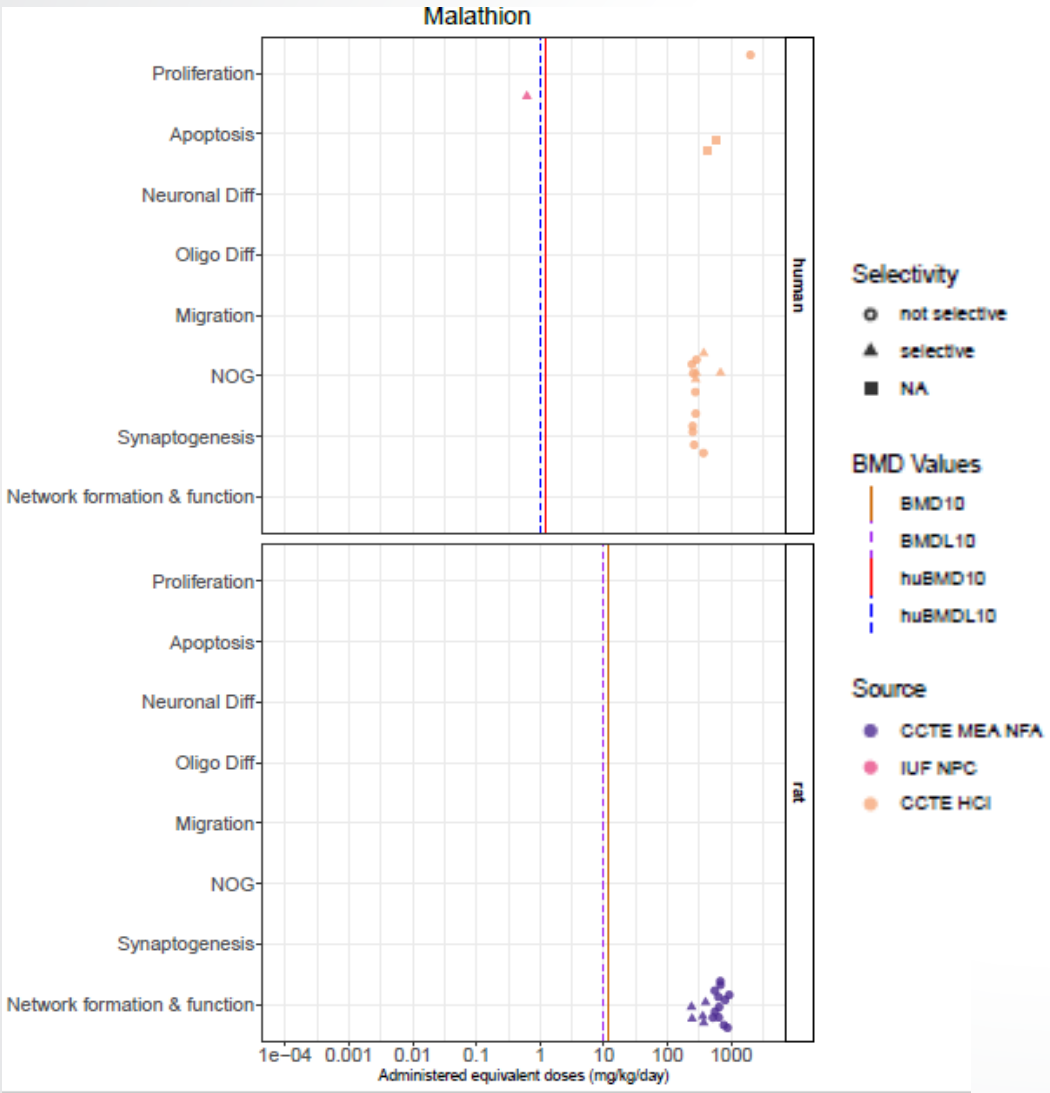
	UKN2	Nyffeler *et al.* (2017)
Neuron differentiation	NPC3	Baumann *et al.* (2016) and Barenys *et al.* (2017)
Oligodendrocyte differentiation & maturation	NPC5/6	Baumann *et al.* (2016) and Barenys *et al.* (2017)
Neurite outgrowth	iCell gluta	Harrill *et al.* (2018)
	UKN 4 & 5	Krug *et al.* (2013)
	NPC4	Baumann *et al.* (2016) and Barenys *et al.* (2017)
Synaptogenesis	Rat primary synaptogenesis	Harrill *et al.* (2018)
Network formation	MEA-NFA	Brown *et al.* (2016) and Frank *et al.* (2018)

Example: updating the AED_{50} comparisons with more processes



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Proliferation	hNP1	Harrill *et al.* (2018)
	NPC1	Baumann *et al.* (2016) and Barenys *et al.* (2017)
	UKN1	Balmer *et al.* (2012)
Apoptosis	hNP1	Harrill *et al.* (2018)
Migration	NPC2	Baumann *et al.* (2016) and Barenys *et al.* (2017)
	UKN2	Nyffeler *et al.* (2017)
Neuron differentiation	NPC3	Baumann *et al.* (2016) and Barenys *et al.* (2017)
Oligodendrocyte differentiation & maturation	NPC5/6	Baumann *et al.* (2016) and Barenys *et al.* (2017)
Neurite outgrowth	iCell gluta	Harrill *et al.* (2018)
	UKN 4 & 5	Krug *et al.* (2013)
	NPC4	Baumann *et al.* (2016) and Barenys *et al.* (2017)
Synaptogenesis	Rat primary synaptogenesis	Harrill *et al.* (2018)
Network formation	MEA-NFA	Brown *et al.* (2016) and Frank *et al.* (2018)

The dataset is still a relatively small matrix across functional processes, approaching ~90 chemicals with all data streams in the ToxCast database.

Many more substances have been screened in the MEA NFA than any other technology

- It may be challenging to build a systems biology model because we do not have a complete matrix across all technologies/processes.
- For the substances where we do have screening across all functional processes, we would like to understand the outcomes for DNT.
- Anticipate ongoing collaborative work with UKN, IUF partners.

How to blend bioactivity-exposure ratios (BERs) and specific NAM-informed hazard indications?

Challenges	Opportunities
NAM-based hazard indications may be “semi-quantitative” or relative to the screened set	Use comparisons to known reference chemicals to inform uncertainties on the relative potency, efficacy, or phenotype for a given new chemical
NAM-based hazard indications may not cover all of biology	Use broader screening platforms to try to cover more breadth and use “Tier 2” and “Tier 3” platforms to further target specific hazards of interest
Many NAM-based hazards may appear to occur at similar concentration ranges	Qualitatively flagging possible hazards and also quantitatively identifying a “lead” hazard may be informative. Seeking “selectivity” may be useful.

Thomas et al. 2019 further evolves a tiered screening strategy that adds in broader biological coverage but also suggests that we can strive to do more than BER in some cases.

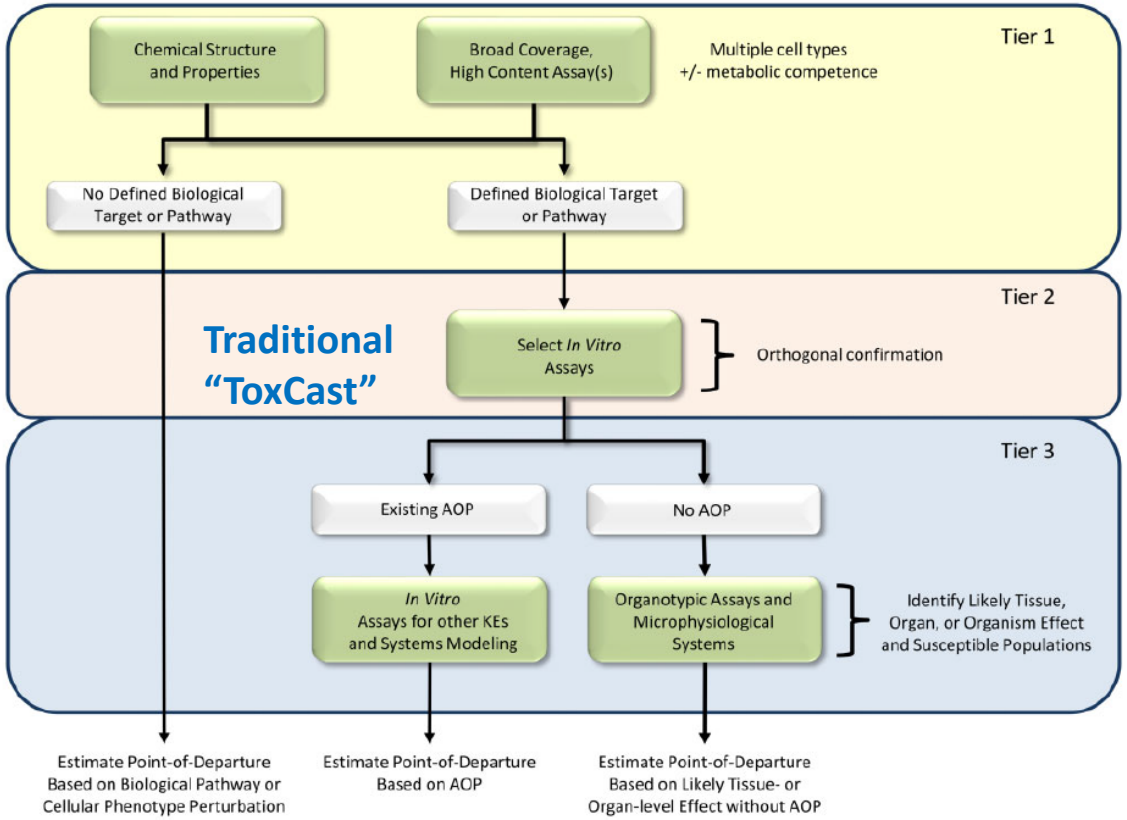
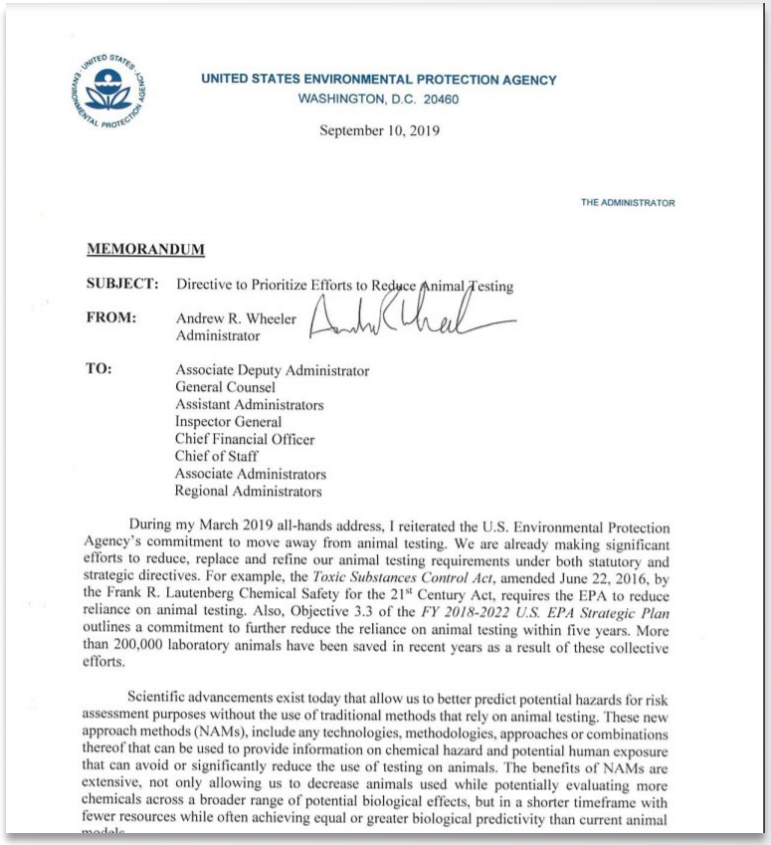


Figure 2. Tiered testing framework for hazard characterization. Tier 1 uses both chemical structure and broad coverage, high content assays across multiple cell types for comprehensively evaluating the potential effects of chemicals and grouping them based on similarity in potential hazards. For chemicals from Tier 1 without a defined biological target / pathway, a quantitative point-of-departure for hazard is estimated based on the absence of biological pathway or cellular phenotype perturbation. Chemicals from Tier 1 with a predicted biological target or pathway are evaluated Tier 2 using targeted follow-up assays. In Tier 3, the likely tissue, organ, or organism-level effects are considered based on either existing adverse outcome pathways (AOP) or more complex culture systems. Quantitative points-of-departure for hazard are estimated based on the AOP or responses in the complex culture system.

Employing NAMs for derivation of PODs

EPA New Approach Methods Work Plan: Reducing Use of Animals in Chemical Testing

https://www.epa.gov/chemical-research/epa-new-approach-methods-work-plan-reducing-use-animals-chemical-testing



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

September 10, 2019

THE ADMINISTRATOR

MEMORANDUM

SUBJECT: Directive to Prioritize Efforts to Reduce Animal Testing

FROM: Andrew R. Wheeler
Administrator

TO: Associate Deputy Administrator
General Counsel
Assistant Administrators
Inspector General
Chief Financial Officer
Chief of Staff
Associate Administrators
Regional Administrators

During my March 2019 all-hands address, I reiterated the U.S. Environmental Protection Agency's commitment to move away from animal testing. We are already making significant efforts to reduce, replace and refine our animal testing requirements under both statutory and strategic directives. For example, the *Toxic Substances Control Act*, amended June 22, 2016, by the Frank R. Lautenberg Chemical Safety for the 21st Century Act, requires the EPA to reduce reliance on animal testing. Also, Objective 3.3 of the *FY 2018-2022 U.S. EPA Strategic Plan* outlines a commitment to further reduce the reliance on animal testing within five years. More than 200,000 laboratory animals have been saved in recent years as a result of these collective efforts.

Scientific advancements exist today that allow us to better predict potential hazards for risk assessment purposes without the use of traditional methods that rely on animal testing. These new approach methods (NAMs), include any technologies, methodologies, approaches or combinations thereof that can be used to provide information on chemical hazard and potential human exposure that can avoid or significantly reduce the use of testing on animals. The benefits of NAMs are extensive, not only allowing us to decrease animals used while potentially evaluating more chemicals across a broader range of potential biological effects, but in a shorter timeframe with fewer resources while often achieving equal or greater biological predictivity than current animal models.

https://www.epa.gov/research/administrator-memo-prioritizing-efforts-reduce-animal-testing-september-10-2019

- How much uncertainty can be tolerated?
- Can BER be informative for the problem?
- Are there specific hazards of interest?
- How should toxicokinetic modeling be tuned?

There is a lot more work to do, and case studies will help build confidence and identify gaps to fill.

Acknowledgements

DNT-NAMs team:

Josh Harrill, Tim Shafer, Stephanie Padilla, and John Wambaugh

EPA ORD trainees working on related DNT-NAM work:

Kelly Carstens, PhD now working on extending this work to the full chemical set to understand MEA NFA and HCI performance for DNT assessment; Melissa Martin, PhD revising the reference chemical set; Amy Carpenter, BS pre-processing and data analysis

ORD Management engaged:

Maureen Gwinn, John Cowden, Kimberly Slentz-Kessler, Rusty Thomas, Jeff Frithsen

OPP Program Office Partners:

Monique Perron, Cecilia Tan, Liz Mendez, Anna Lowit, and others

Appendix slides

Many works apply HTTK to prioritization and assessment case studies

Chemical Research in Toxicology — PERSPECTIVE — pubs.acs.org/crt

2011

Estimating Toxicity-Related Biological Pathway Altering Doses for High-Throughput Chemical Risk Assessment

Richard S. Judson,*,† Robert J. Kavlock,† R. Woodrow Setzer,† Elaine A. Cohen Hubal,† Matthew T. Martin,† Thomas B. Knudsen,† Keith A. Houck,† Russell S. Thomas,‡ Barbara A. Wetmore,‡ and David J. Dix†

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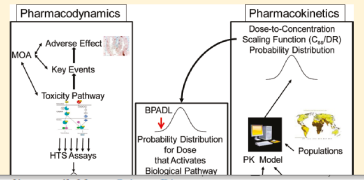
‡The Hamner Institutes for Health Sciences, Research Triangle Park, North Carolina 27709, United States

ABSTRACT: We describe a framework for estimating the human dose at which a chemical significantly alters a biological pathway *in vivo*, making use of *in vitro* assay data and an *in vitro*-derived pharmacokinetic model, coupled with estimates of population variability and uncertainty. The quantity we calculate, the biological pathway altering dose (BPAD), is analogous to current risk assessment metrics in that it combines dose–response data with analysis of uncertainty and population variability to arrive at conservative exposure limits. The analogy is closest when perturbation of a pathway is a key event in the mode of action (MOA) leading to a specified adverse outcome.



OXFORD — SOT Society of Toxicology — www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 148(1), 2015, 121–136

doi: 10.1093/toxsci/kfv171
Advance Access Publication Date: August 6, 2015
Research Article

2015

Incorporating High-Throughput Exposure Predictions With Dosimetry-Adjusted *In Vitro* Bioactivity to Inform Chemical Toxicity Testing

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Toxicology in Vitro 47 (2018) 213–227

Contents lists available at ScienceDirect

2018

Toxicology in Vitro

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

Review

In vitro to in vivo extrapolation for high throughput prioritization and decision making

Shannon M. Bell[a], Xiaoqing Chang[a], John F. Wambaugh[b], David G. Allen[a], Mike Bartels[c,1], Kim L.R. Brouwer[d], Warren M. Casey[e], Neepa Choksi[a], Stephen S. Ferguson[f], Grazyna Fraczkiewicz[g], Annie M. Jarabek[b], Alice Ke[h], Annie Lumen[i], Scott G. Lynn[j], Alicia Paini[k], Paul S. Price[b], Caroline Ring[l,2], Ted W. Simon[m], Nisha S. Sipes[f], Catherine S. Sprankle[a], Judy Strickland[a], John Troutman[n], Barbara A. Wetmore[o,3], Nicole C. Kleinstreuer[c,*]

Contents lists available at ScienceDirect

2019

Food and Chemical Toxicology

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

Profiling 58 compounds including cosmetic-relevant chemicals using ToxRefDB and ToxCast

Ly L. Pham[a,b], Lisa Truong[a,b,c], Gladys Ouedraogo[d], Sophie Loisel-Joubert[e], Matthew T. Martin[a,f], Katie Paul Friedman[a,*]

Environment International 137 (2020) 105470

Contents lists available at ScienceDirect

2020

Environment International

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

High-throughput screening tools facilitate calculation of a combined exposure-bioactivity index for chemicals with endocrine activity

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OXFORD — SOT Society of Toxicology — academic.oup.com/toxsci

2019

TOXICOLOGICAL SCIENCES, 2019, 1–24

doi: 10.1093/toxsci/kfz201
Advance Access Publication Date: September 18, 2019
Research Article

Utility of *In Vitro* Bioactivity as a Lower Bound Estimate of *In Vivo* Adverse Effect Levels and in Risk-Based Prioritization

Katie Paul Friedman,*,1 Matthew Gagne,† Lit-Hsin Loo,‡ Panagiotis Karamertzanis,§ Tatiana Netzeva,§ Tomasz Schenki,§ Jill A. Franzosa,¶ Ann M. Richa[illegible] Angrish,[illegible] Bahadori[illegible] Rasenbe[illegible]

Toxicology and Applied Pharmacology 387 (2020) 114774

Contents lists available at ScienceDirect

2020

Toxicology and Applied Pharmacology

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

The role of fit-for-purpose assays within tiered testing approaches: A case study evaluating prioritized estrogen-active compounds in an in vitro human uterotrophic assay

Tyler Beames[a,*,1], Marjory Moreau[a,1], L. Avery Roberts[b], Kamel Mansouri[b], Saad Haider[a], Marci Smeltz[a], Chantel I. Nicolas[b], Daniel Doheny[b], Martin B. Phillips[a], Miyoung Yoon[b,2], Richard A. Becker[c], Patrick D. McMullen[a], Melvin E. Andersen[a], Rebecca A. Clewell[b,3], Jessica K. Hartman[a,*]

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PLOS ONE

2020

RESEARCH ARTICLE

Using the concordance of *in vitro* and *in vivo* data to evaluate extrapolation assumptions

Gregory S. Honda[1,2], Robert G. Pearce[1,2], Ly L. Pham[1,2], R. W. Setzer[1], Barbara A. Wetmore[3], Nisha S. Sipes[4], Jon Gilbert[5], Briana Franz[5], Russell S. Thomas[1], John F. Wambaugh[1]*

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A subset of the papers describing the application of a high-throughput toxicokinetic approach – too many to fit

Selecting an HTTK model: 3 compartment steady state model

Models:	3-compartment steady state (3compss)	PBTK
Chemical-specific parameters	Clint only	Clint, Fup, logP, pKa
Model inputs	A single oral dose	A single oral dose
Model outputs	Steady-state blood concentrations	Time course of blood concentrations; estimate Cmax, AUC (24 hr), Cmean (AUC/time) from time course simulations
Human interindividual variability	Human physiological parameters (first order hepatic metabolic clearance; plasma protein binding; liver volume, blood flow, and cell density; and glomerular filtration rate) can be varied in a Monte Carlo simulation to estimate the dose required to achieve equivalent blood concentrations for the most to least sensitive individuals. In this Issue Paper, the median individual is used.	
Rat interindividual variability	Rat physiological parameters (rat liver volume and glomerular filtration rate) can be varied in a Monte Carlo simulation to estimate the dose required to achieve equivalent blood concentrations for the most to least sensitive individuals. In this Issue Paper, the median individual is used.	

- Many substances in this set lacked sufficient rat-specific (fraction unbound in plasma and hepatic intrinsic clearance) to inform HTTK PBTK models
- Because the fraction unbound in plasma (Fup) assay fails for highly bound chemicals (Wambaugh et al., 2015), the steady state model is advantageous because it can be used with the assumption that plasma protein binding is simply "small," i.e., typically 0.5% (Wetmore et al., 2012)

To provide the most complete view of a potency comparison between AEDs based on DNT-NAMs and BMD10 and BMDL10 values based on observations of in vivo rat AChE inhibition, and to present an approach that would require the minimum amount of data using the simplest modeling approach, AED values in this Issue Paper were calculated using the 3-compartment steady state model.

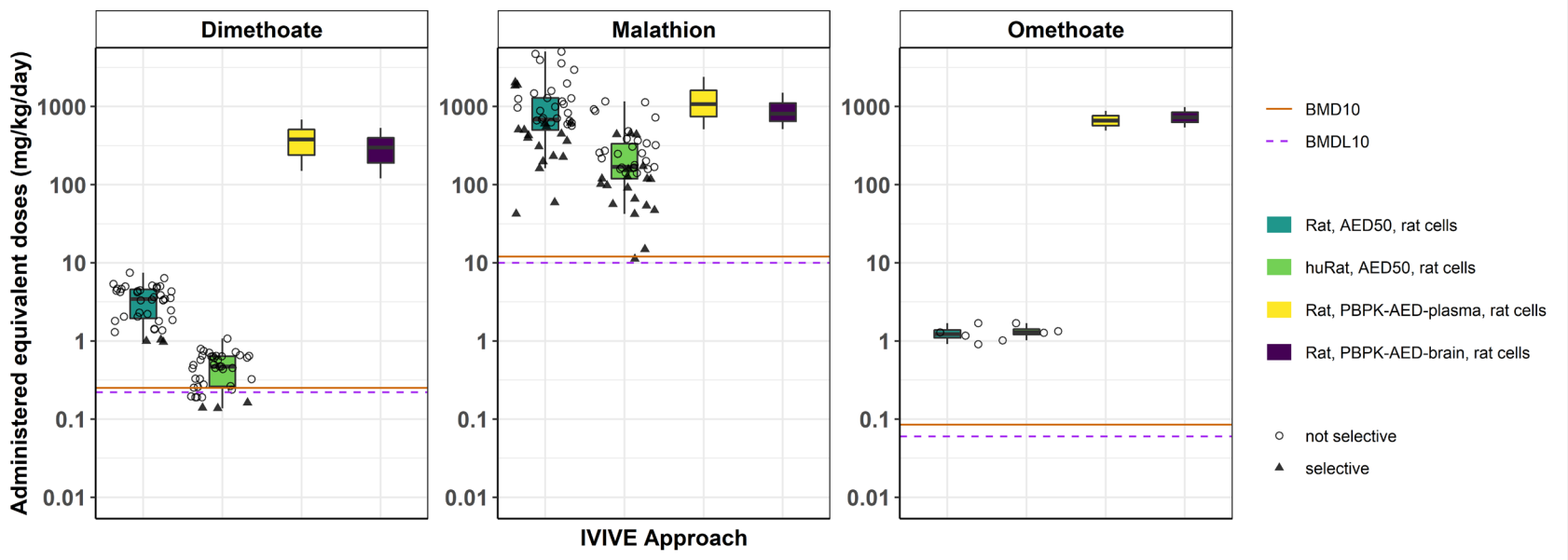
Summary of the AED50 to BMD/BMDL comparison

	Chemicals with AED50 values >>> BMD/BMDL comparator	Chemicals with lowest AED50 within 1 log10 order of magnitude of BMD/BMDL comparator	Chemicals with lowest AED50 approaching BMD/BMDL comparator	Missing in vitro data for comparison
Rat/HuRat	Coumaphos, diazoxon, dicrotophos, ethoprop, fosthiazate, omethoate	acephate, bensulide, chlorpyrifos, chlorpyrifos oxon, diazinon, dimethoate, malathion, methamidophos, and phorate	lower quartile of huRat AED_{50} values for dimethoate and methamidophos (these AED_{50} values appear to have included selective assay endpoints). The huRat AED_{50} value for dichlorvos (only one positive rat assay endpoint) overlaps with the BMDL10 value, and it was not based on selective bioactivity in the DNT-NAM battery. The lowest huRat AED_{50} values (selective) for malathion also approach the BMD/BMDL10 values.	Malaoxon was negative in all assays.
Human	bensulide, chlorpyrifos, chlorpyrifos oxon, coumaphos, diazinon, dimethoate, malathion, methamidophos, phosmet, pirimiphos-methyl, tribufos, and trichlorfon		For dichlorvos, only two AED_{50} values are available for comparison, and these values are centered around the BMD10/10 and BMDL10/10 values. Neither of these AED_{50} values appear selective because the bioactivity was observed in assay endpoints relevant to cell viability. Similarly, for terbufos, only 3 human AED_{50} values are available for comparison, and the lowest one of these values approaches the BMD10/10 value. This lowest AED_{50} value for terbufos does not appear selective because it is derived from a cell viability related assay endpoint (object count in the HCI hNP1 proliferation assay endpoint).	Acephate, diazoxon, dicrotophos, ethoprop, fosthiazate, omethoate, phorate, profenofos, and tebupirimfos had positive rat assay data but lacked positive responses in the human cell-based assays. Malaoxon was negative in all assays.

Comparing HTTK to PBPK-PD models

- Physiologically-based pharmacokinetic (PBPK)-pharmacodynamic (PD) models were available for: dimethoate, omethoate, and malathion *based on a chlorpyrifos model that is no longer available*.
- Though the HTTK model employed and the PBPK-PD models all assumed 100% bioavailability, the HTTK model accounts for hepatic Clint whereas PBPK-PD models incorporate additional metabolism sites in plasma, brain, and kidneys.

HTTK may provide more rapid results that are similar to or more conservative than PBPK-PD models



- Dimethoate and omethoate: PBPK-AED values using plasma and brain AUC were more than two orders of magnitude greater than the HTTK-derived AEDs
- Malathion, the PBPK-AED values were similar to the range of HTTK-derived AED_{50} values for rat