New Approach Methodology for Developmental Neurotoxicity: Past, Present and Future

Timothy J Shafer, PhD
Center for Computational Toxicology and Exposure
Biomolecular and Computational Toxicology Division
Rapid Assay Development Branch
Shafer.tim@epa.gov

Disclosure:
This work has been funded by the US. Environmental Protection Agency. I have no conflicts to declare.
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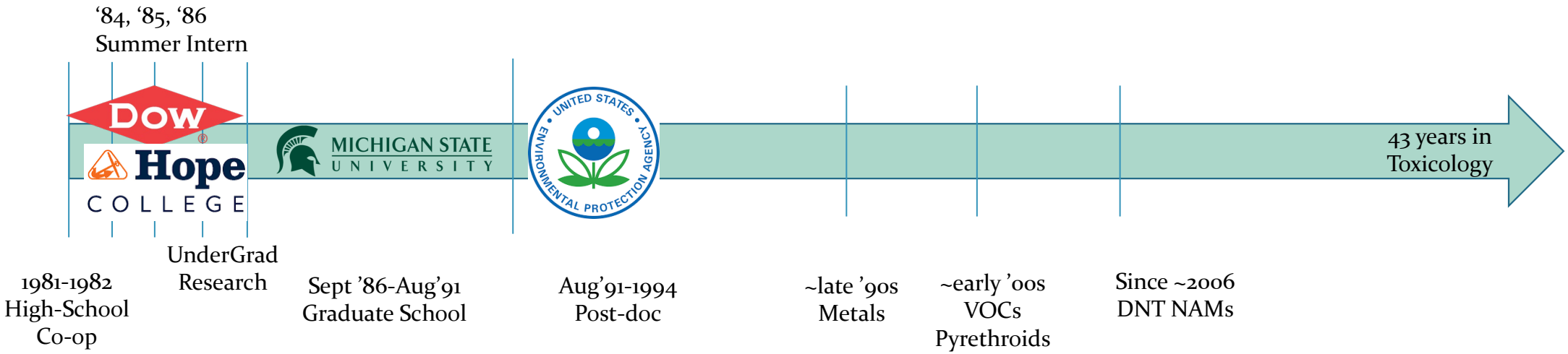
Overview

I. My Career Path
II. Brief History of the EPA and Regulatory Statutes
III. The Need for Alternative Approaches for Neurotoxicity and Developmental Neurotoxicity Hazard Assessment
IV. A Brief History of NAMs for DNT
V. Examples of How DNT NAMs are being utilized for decision-making
VI. Informing AOP Development with Omics Data
VII. Current projects
VIII. Questions

My Career Path



Roles I've had:

- Branch Chief (3 months)
- Division Director (15 months)
- Associate Laboratory Director (10 months)

- NIH Study Section (4 years)
- SOT
  - Sec/Treas NTSS
  - Program Committee
  - Collaborative Conference Committee

What I've "survived" at EPA:

- 6 Presidents (3 Democrats, 3 Republicans)
- 4 Government Shutdowns totaling 69 days (Always got paid).
- 1 Government Furlough (~50 hrs)- went fishing (Didn't get paid).
- 9 Division Directors (not including me) and counting.
- 4 Reorganizations.
- 1 Laboratory move
- ~13 Months of WFH during COVID
  - 5 months of lab shutdown

Some approaches over the years…

CRITICAL EVALUATION OF THE FATHEAD MINNOW 7-DAY STATIC RENEWAL TEST

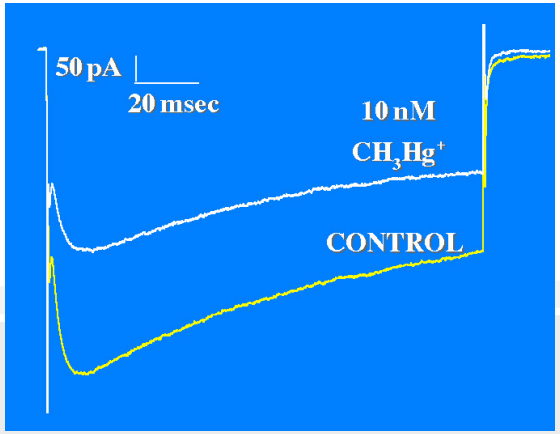
M. A. Mayes*, T. J. Shafer, and M. G. Barron

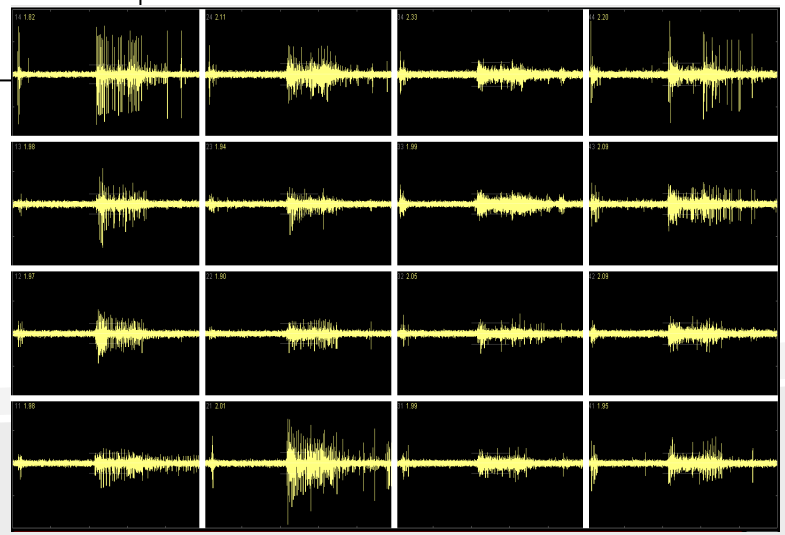
Bull. Environ. Contam. Toxicol. (1990) 44:729–736

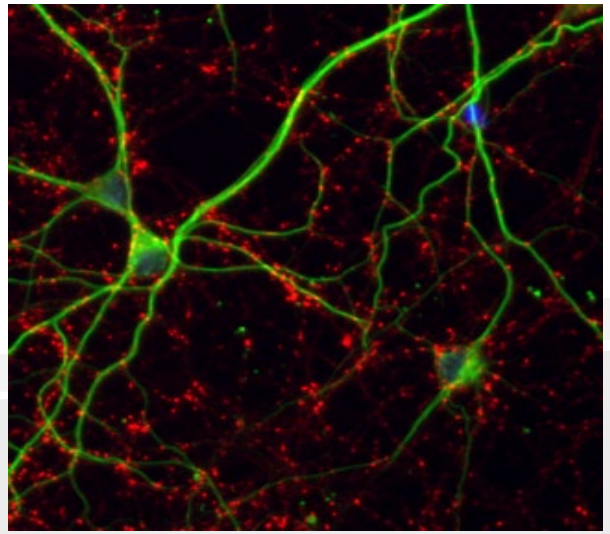
Bulletin of Environmental Contamination and Toxicology

Activation of Chemical Promutagens by *Selenastrum capricornutum* in the Plant Cell/Microbe Coincubation Assay

J. M. Gentile, M. Lippert, P. Johnson, and T. Shafer







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History of the EPA

- Republican President Richard Nixon established the EPA in 1972
  - Unambiguous pollution issues
  - Pb in gasoline; smog; water pollution; failure of raptor nesting

EPA has made a difference…

The EPA is still needed

PFAS contamination continue to surface at Van Etten Lake (Oscoda County, MI)

EPA's mission:

to protect human health and the environment

What does EPA do to accomplish this mission?:

- Sets standards (limits) for chemicals in the environment.
- Registers chemicals (develop guidelines).
- Develops pollution prevention technology.
- Conducts risk assessments (based on sound science).
- Informs and educates the public.
- **Conducts research to provide a solid scientific basis for all of the above activities**.

EPA's Research is Centered Around Regulatory Needs

Legislation	Acronym	Primary EPA Program Office	ORD Research Program
Clean Air Act	CAA	OAR	Air, Climate, & Energy (ACE)
Clean Water Act	CWA	OW	Safe and Sustainable Water Resources (SSWR)
Comprehensive Environmental Response, Compensation, and Liability Act	CERCLA	OLEM	Safe and Healthy Communities (SHC) & Homeland Security (HS)
Federal Food, Drug, and Cosmetic Act	FFDCA	OCSPP/OPP	
Federal Insecticide, Fungicide, and Rodenticide Act	FIFRA	OCSPP/OPP	Chemical Safety for Sustainability (CSS)
Food Quality Protection Act	FQPA	OCSPP/OPP/OW	CSS
National Environmental Policy Act	NEPA		
Resource Conservation and Recovery Act	RCRA	OLEM	SHC
Safe Drinking Water Act	SDWA	OW	SSWR & HS
Toxic Substances Control Act	**TSCA**	**OCSPP/OPPT**	CSS

The Differences between TSCA and FIFRA

Toxic Substances Control Act (TSCA)

All New Chemicals
>60-80K "Grandfathered" Chemicals ("existing" chemicals)

Available Data
90 Day Premanufacture Notice

"Data Poor"- little or nothing may be known about toxicity hazard

Intended to Kill Something →

Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)

All "Pesticides"

Required Guideline Studies
Health and Environmental Effects

Data Rich- Toxicity hazard is well characterized

Lautenberg Chemical Safety Act 2016

- **Must consider risks to susceptible and highly exposed populations**
- **Directs EPA to utilize alternatives to animals**

Food Quality Protection Act of 1996

- **Mandates an extra 10x safety factor for children/infants**

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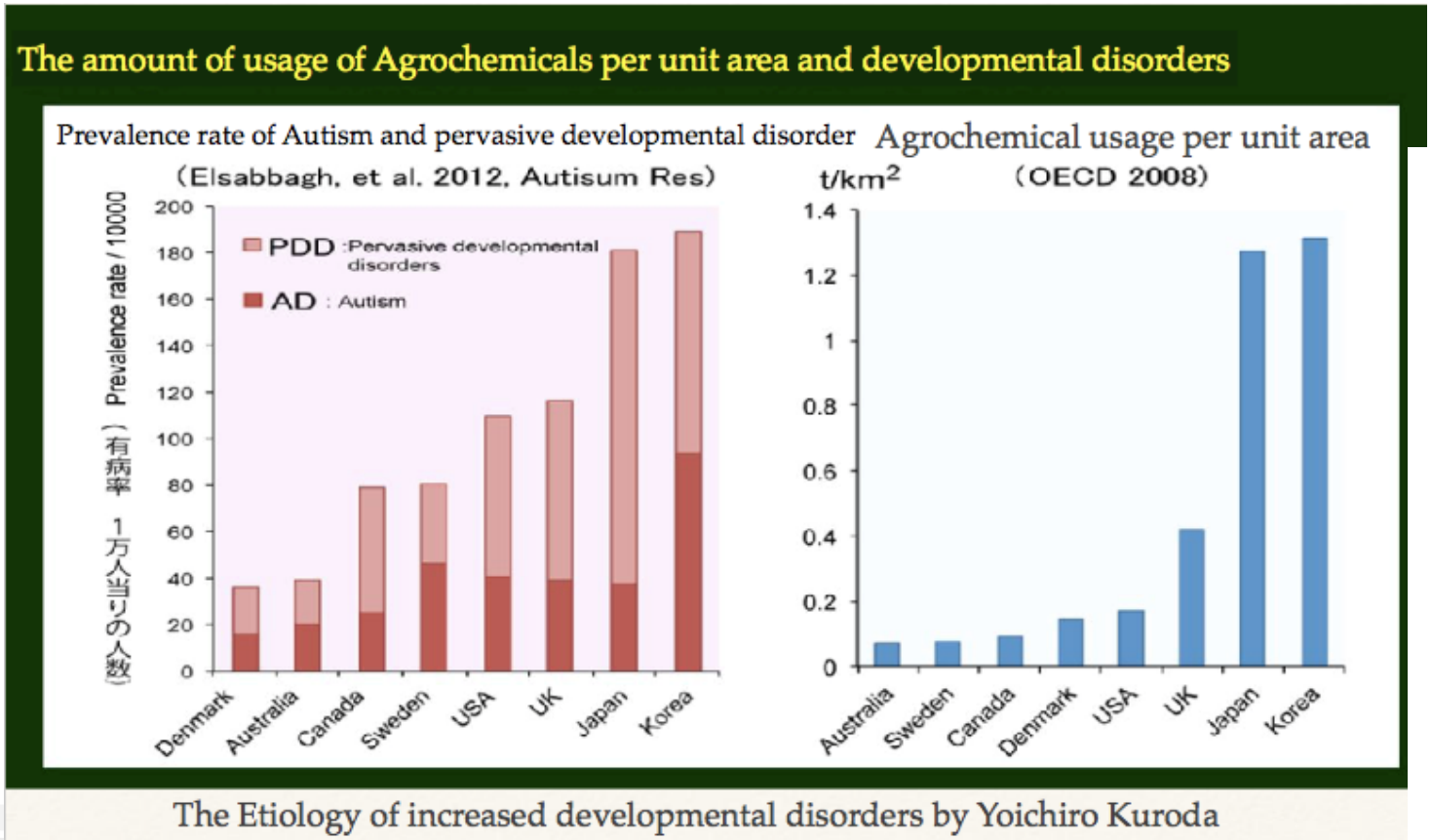
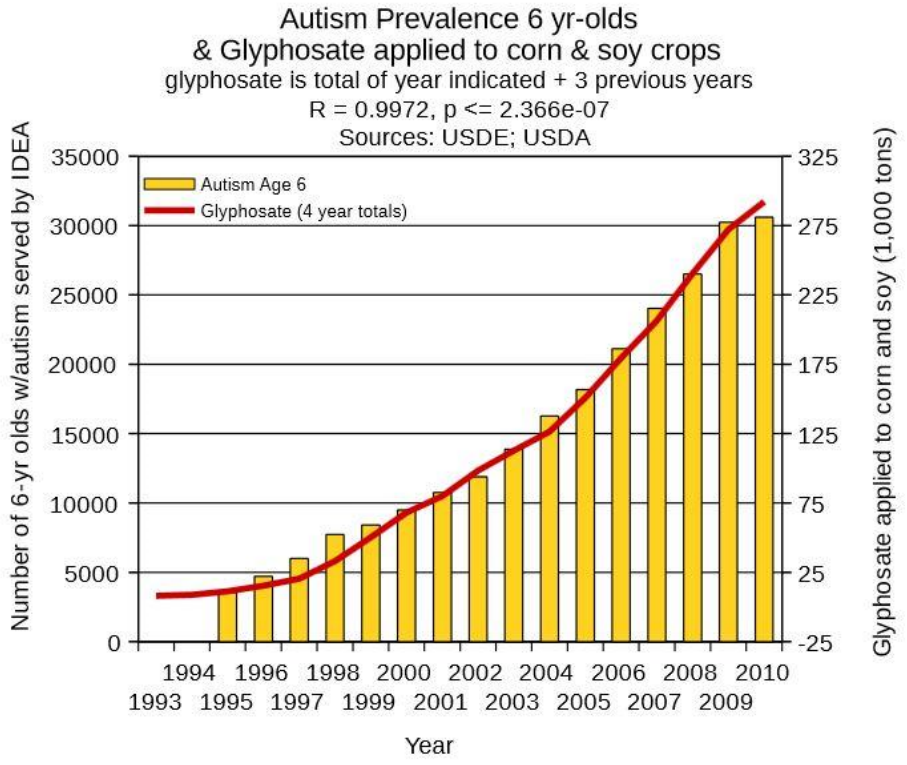
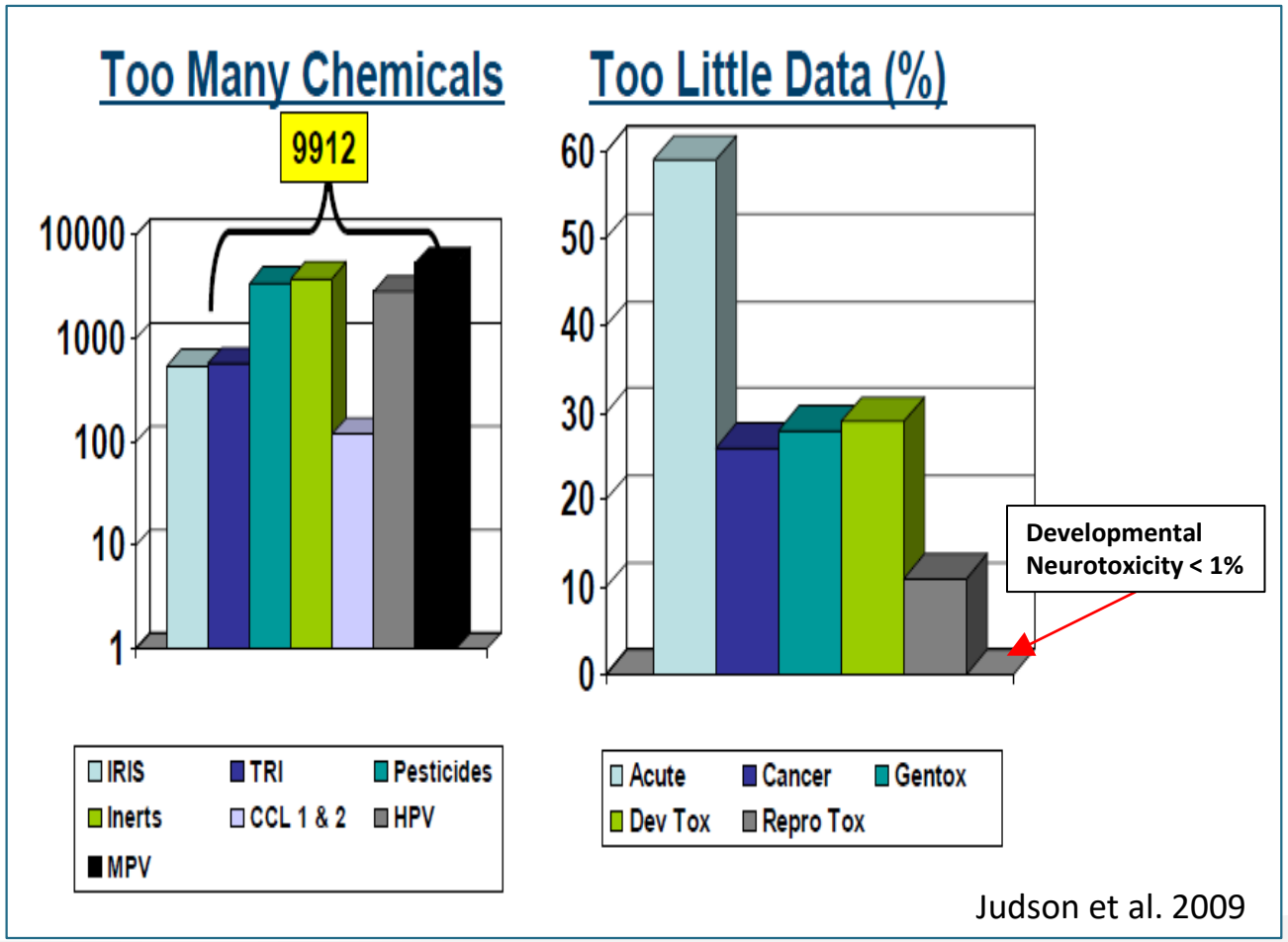
Developmental neurotoxicity is a public concern





Why Developmental Neurotoxicity (DNT) is a problem



Judson et al. 2009

Public Concern

Reports of the potential involvement of environmental chemicals in increased rates of neurodevelopmental disease contributed to increasing public concern about DNT hazard of chemicals

Current testing is too slow; "Guideline" DNT:

- triggered for pesticides, not required for other chemicals
- 1 chemical= $1M cost; 2 yr; 1000 animals
- At current pace, ~200 chemicals in 25 yrs
- Only about ~25% of DNTs used as POD's for risk assessment*

The absence of DNT hazard data on chemicals impedes consideration of this adverse outcome in environmental decision-making.

*Raffaele et al. The use of **developmental neurotoxicity** data in pesticide risk assessments. Neurotoxicol Teratol. 2010 Sep-Oct;32(5):563-72.

Requirements of Guideline DNT Studies

EPA 870.6300 / (OECD TG 426/443)

- 6 Pregnant female rats/dose (20 litters/dose recommended)
- 10 pups/litter (5 male/5 female)
- Minimum 3 doses + control
- Dosing period GD6-PND10
- Assessments on PND 4, 11, 21, 35, 45, 60
- Signs of Maternal Toxicity
- Developmental landmarks
- Brain/body weights (4, 11, 17, 21 PND)
- Motor activity (13, 17, 21, 60 PND)
- Auditory Startle (weaning, PND 60)
- Learning and memory (weaning, PND 60)
- Neuropathology (PND 11 and termination)
  - Major brain regions

https://beta.regulations.gov/document/EPA-HQ-OPPT-2009-0156-0042
https://www.oecd-ilibrary.org/environment/test-no-426-developmental-neurotoxicity-study_9789264067394-en
https://www.oecd.org/chemicalsafety/test-no-443-extended-one-generation-reproductive-toxicity-study-9789264185371-en.htm

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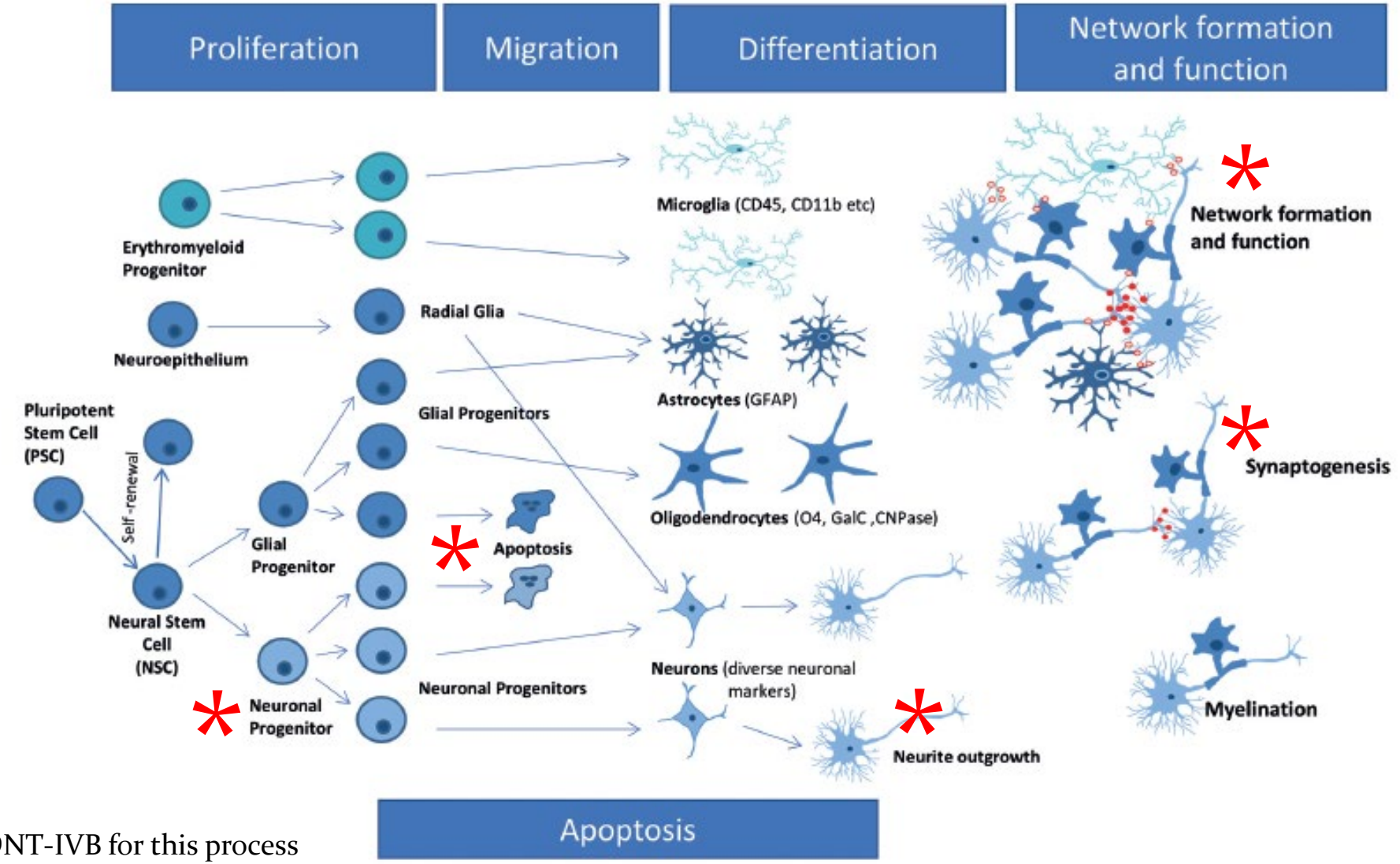
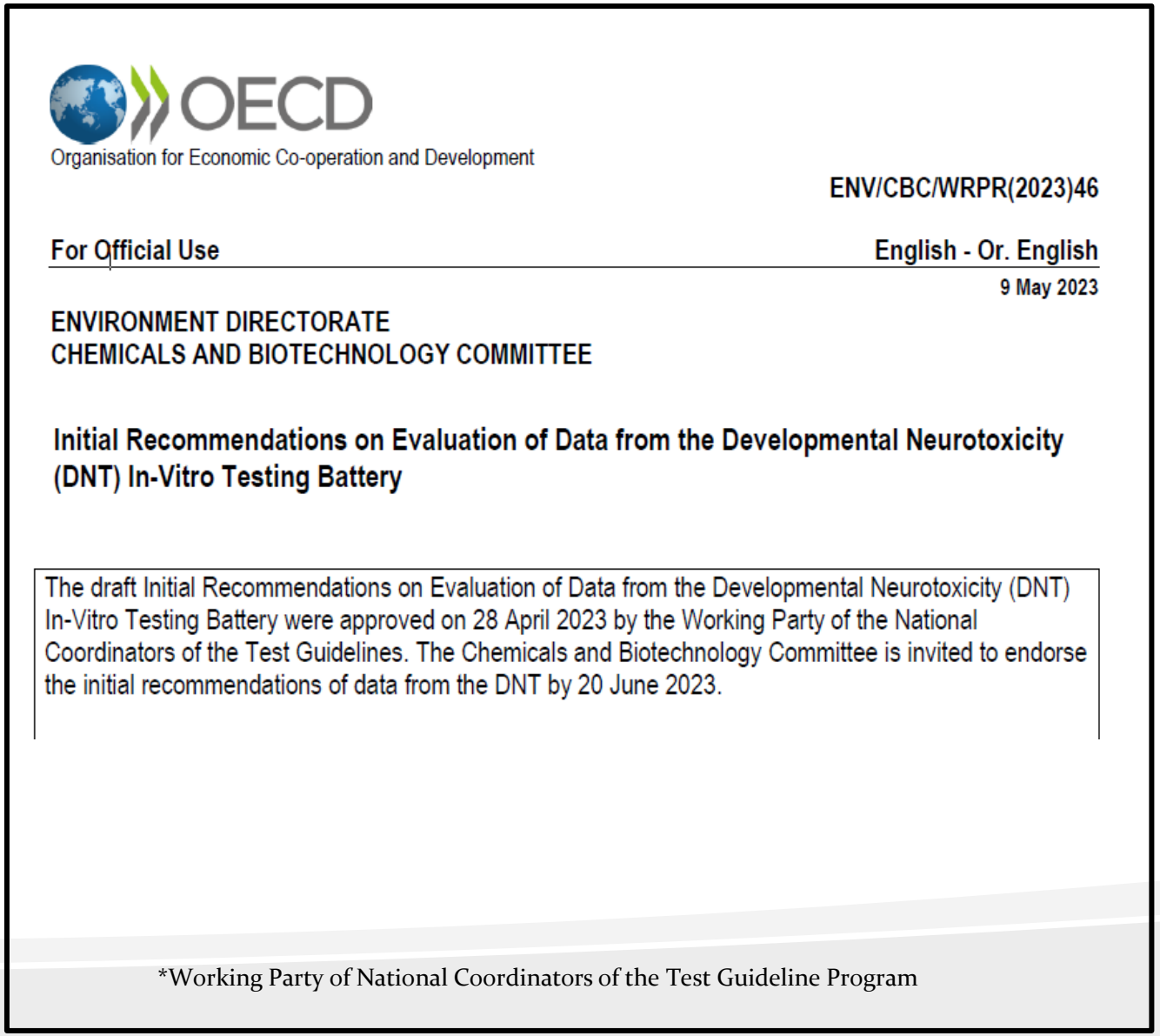
The Developmental Neurotoxicity *In Vitro* Battery (DNT-IVB) targets *Key Neurodevelopmental Processes*



*Assay(s) in DNT-IVB for this process

From Bal-Price et al. 2018 ALTEX. 2018;35(3):306-352. doi: 10.14573/altex.1712081

On April 28, 2023, the OECD WNT approved the following document:



Organisation for Economic Co-operation and Development

ENV/CBC/WRPR(2023)46

For Official Use — English - Or. English

9 May 2023

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In-Vitro Testing Battery

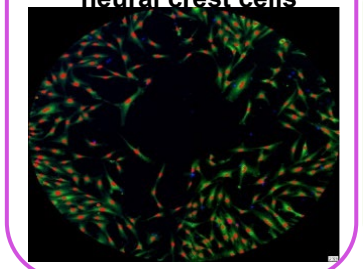
The draft Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In-Vitro Testing Battery were approved on 28 April 2023 by the Working Party of the National Coordinators of the Test Guidelines. The Chemicals and Biotechnology Committee is invited to endorse the initial recommendations of data from the DNT by 20 June 2023.

*Working Party of National Coordinators of the Test Guideline Program

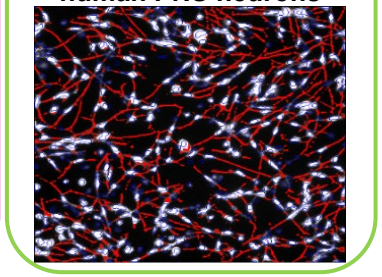
In Vitro Battery of Developmental Neurotoxicity Assays (DNT-IVB)

DNT compounds

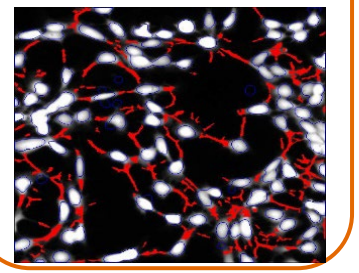
Migration in human neural crest cells



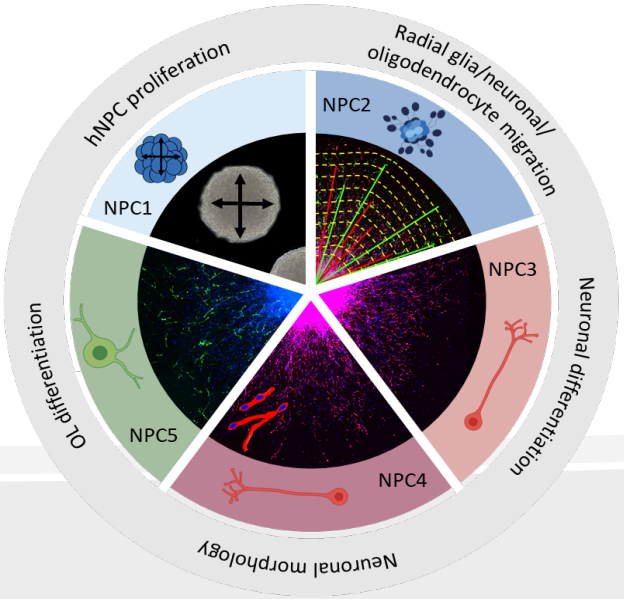
Neurite outgrowth in human PNS neurons



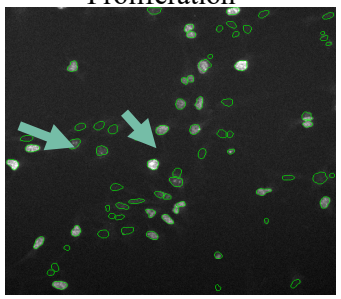
Neurite outgrowth in human CNS neurons



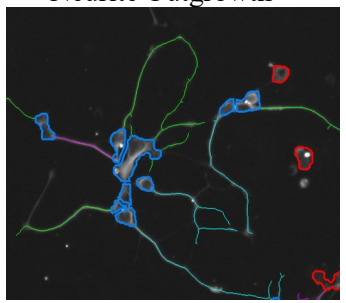
Human Neurospheres



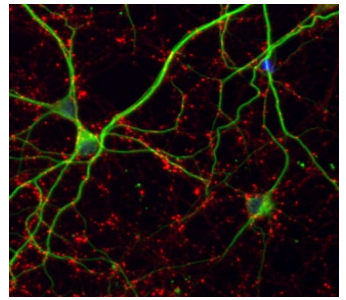
Human Neuroprogenitor Proliferation



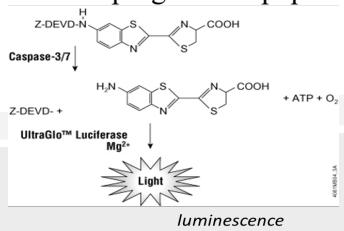
Human and Rat Neuron Neurite Outgrowth



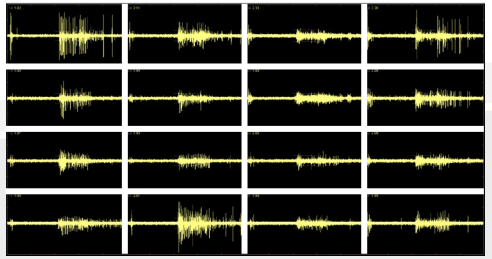
Synaptogenesis in Rat Cortex Neurons



Human Neuroprogenitor Apoptosis



Rat Neural Network Formation

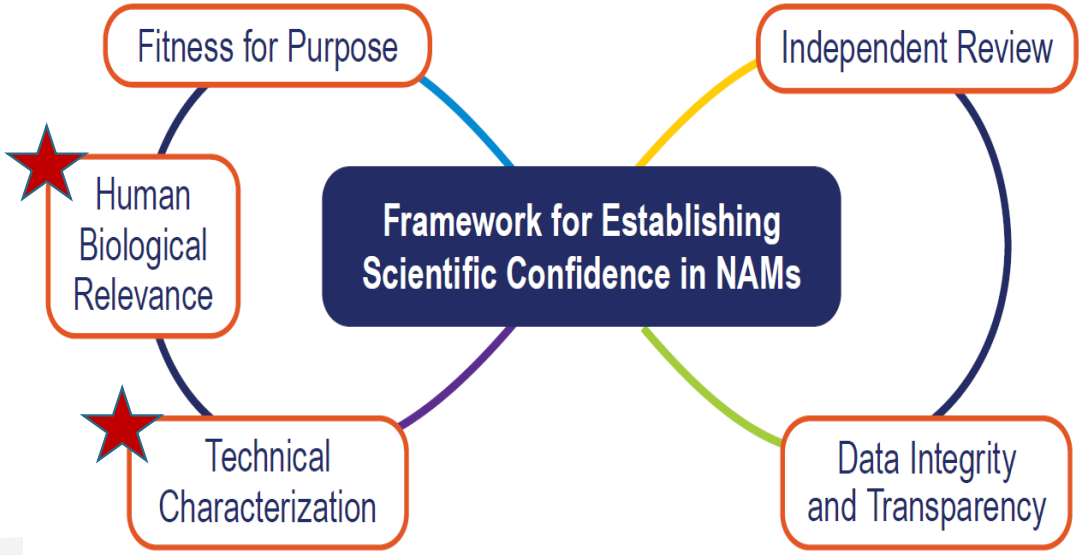


Figures courtesy of Drs Marcel Leist, and Ellen Fritsche

Establishing Confidence in the Assays

Assay Inclusion in the Battery:

- Deemed ready for use in screening and prioritization (Fritsche et al. 2017; Bal-Price et al. 2018; Sachana et al. 2019)
- Tested a common set of chemicals
- Analyzed using the USEPA's ToxCast Pipeline (TCPL)
- Detailed methodological descriptions in the ToxTemp format (Krebs et al. 2019)



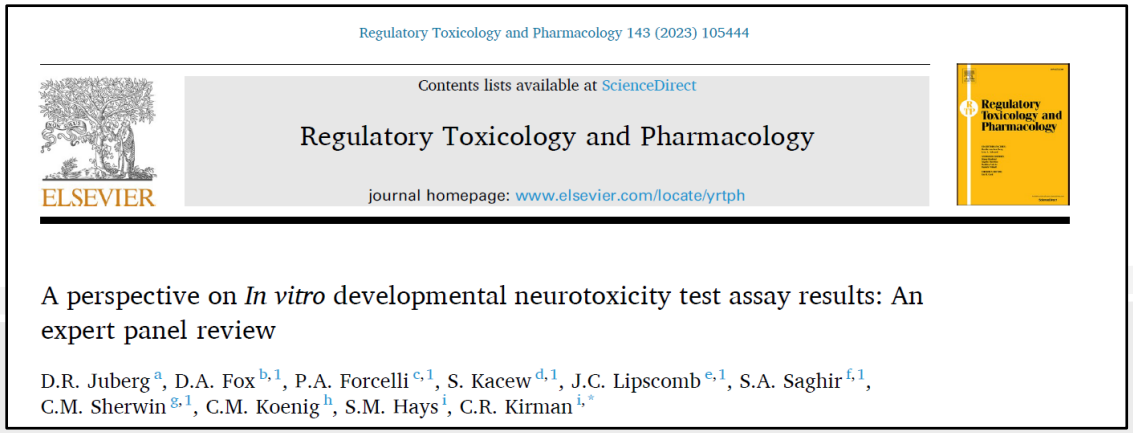
from Van der Zalm, et al., Arch Toxicol. 2022 Nov;96(11):2865-2879. doi: 10.1007/s00204-022-03365-4

Establishing Confidence in the Assays: Fit for Purpose

Consensus from SAP, OECD, Juberg et al.

All three reviews of the DNT-IVB agreed that it could be used for:

- Screening and Prioritization
- Weight of Evidence Decision-Making


Regulatory Toxicology and Pharmacology 143 (2023) 105444

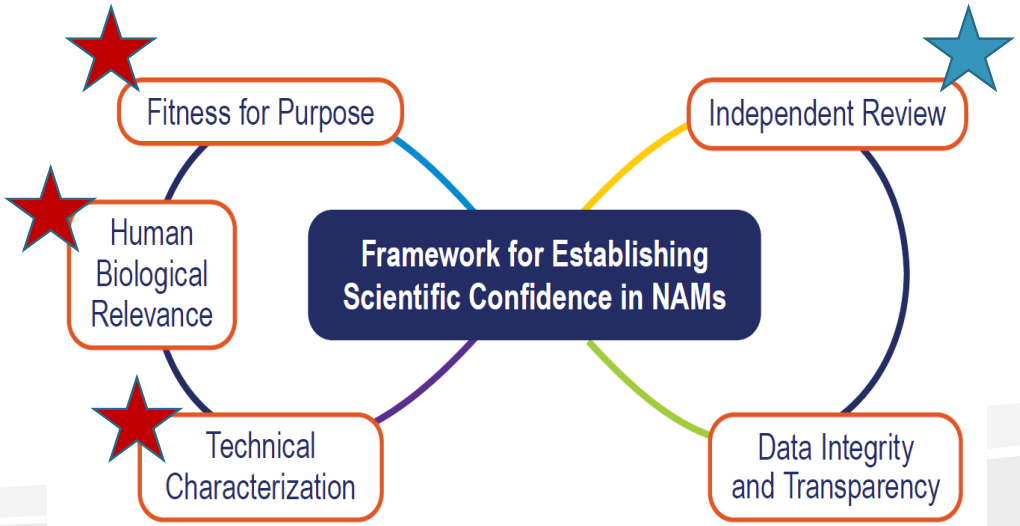
Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

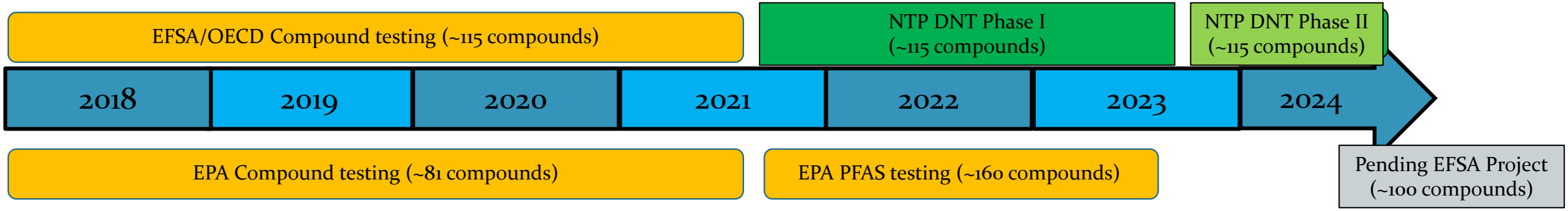
A perspective on *In vitro* developmental neurotoxicity test assay results: An expert panel review

D.R. Juberg [a], D.A. Fox [b,1], P.A. Forcelli [c,1], S. Kacew [d,1], J.C. Lipscomb [e,1], S.A. Saghir [f,1], C.M. Sherwin [g,1], C.M. Koenig [h], S.M. Hays [i], C.R. Kirman [i,*]



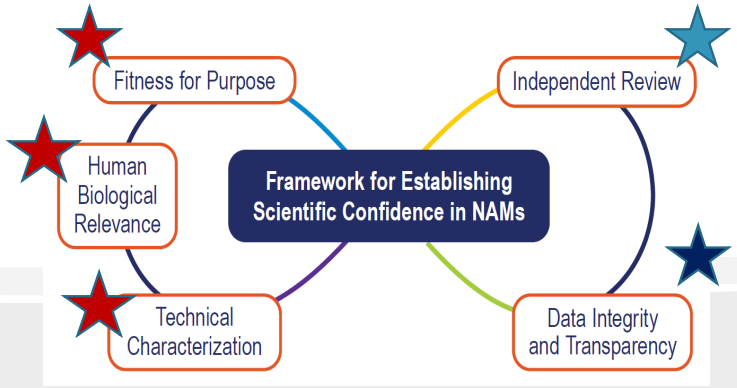
Van der Zalm, et al., doi: 10.1007/s00204-022-03365-4

Establishing Confidence in the Assays: Data Integrity & Transparency



Testing has focused on:

- DNT Reference positive and negative chemicals
- Chemicals with *in vivo* DNT Guideline studies
- Chemicals with specific programmatic interest (PFAS; OPs; botanicals, cannabinoids)



- Tested, data available in ToxCast
- Tested, data pending in ToxCast
- Testing initiated
- Testing in planning process

https://www.epa.gov/comptox-tools/comptox-chemicals-dashboard-resource-hub

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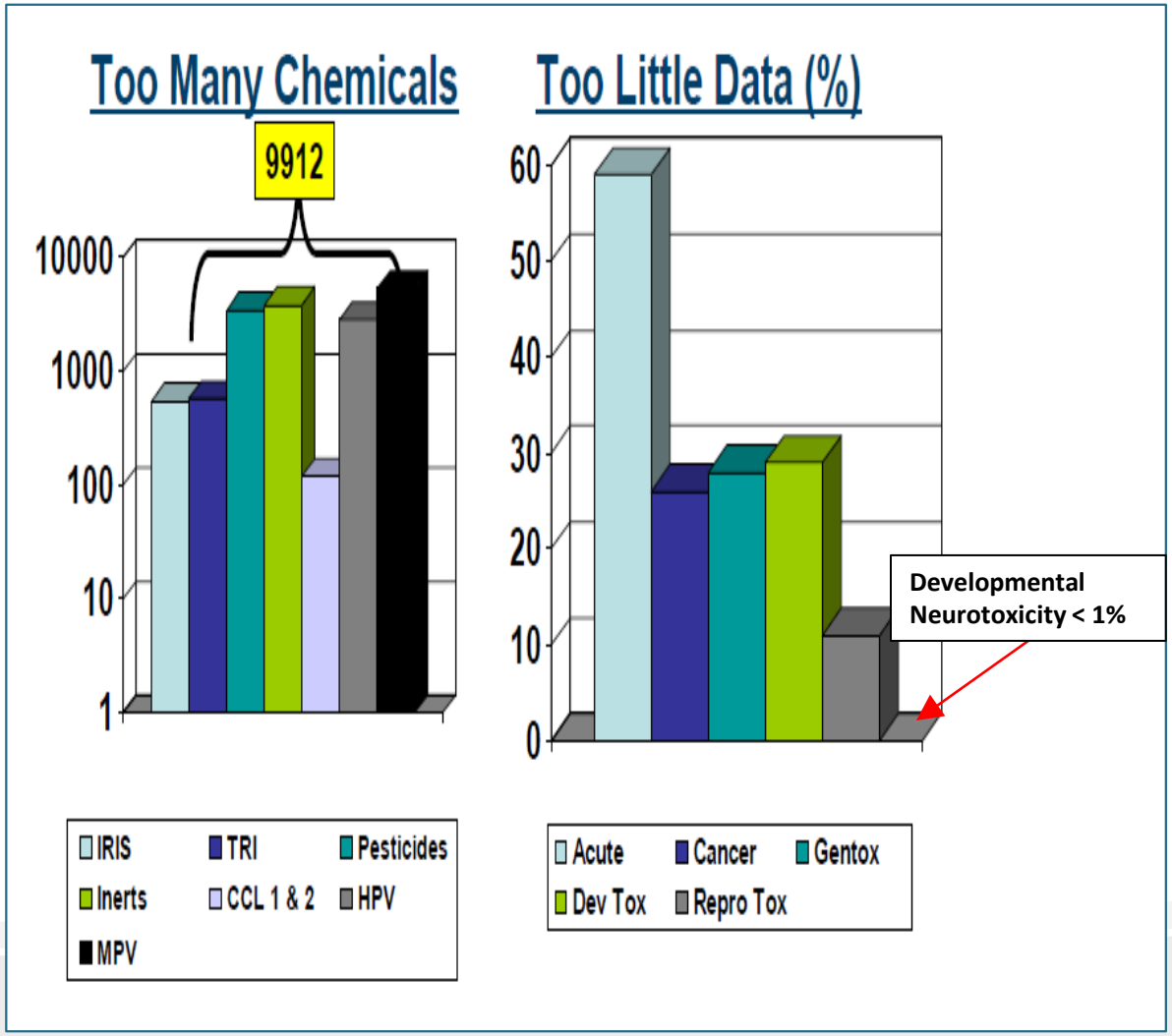

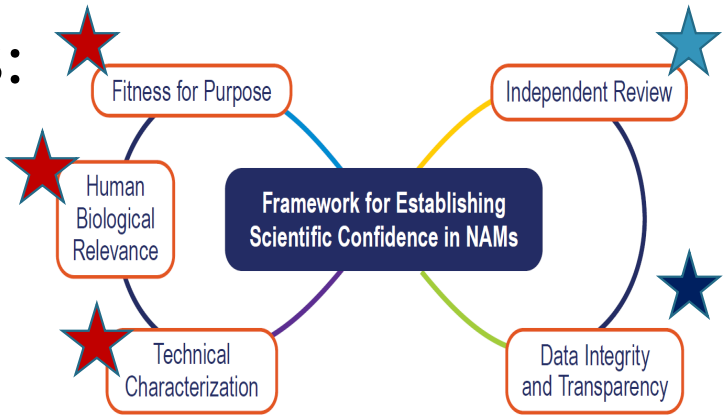

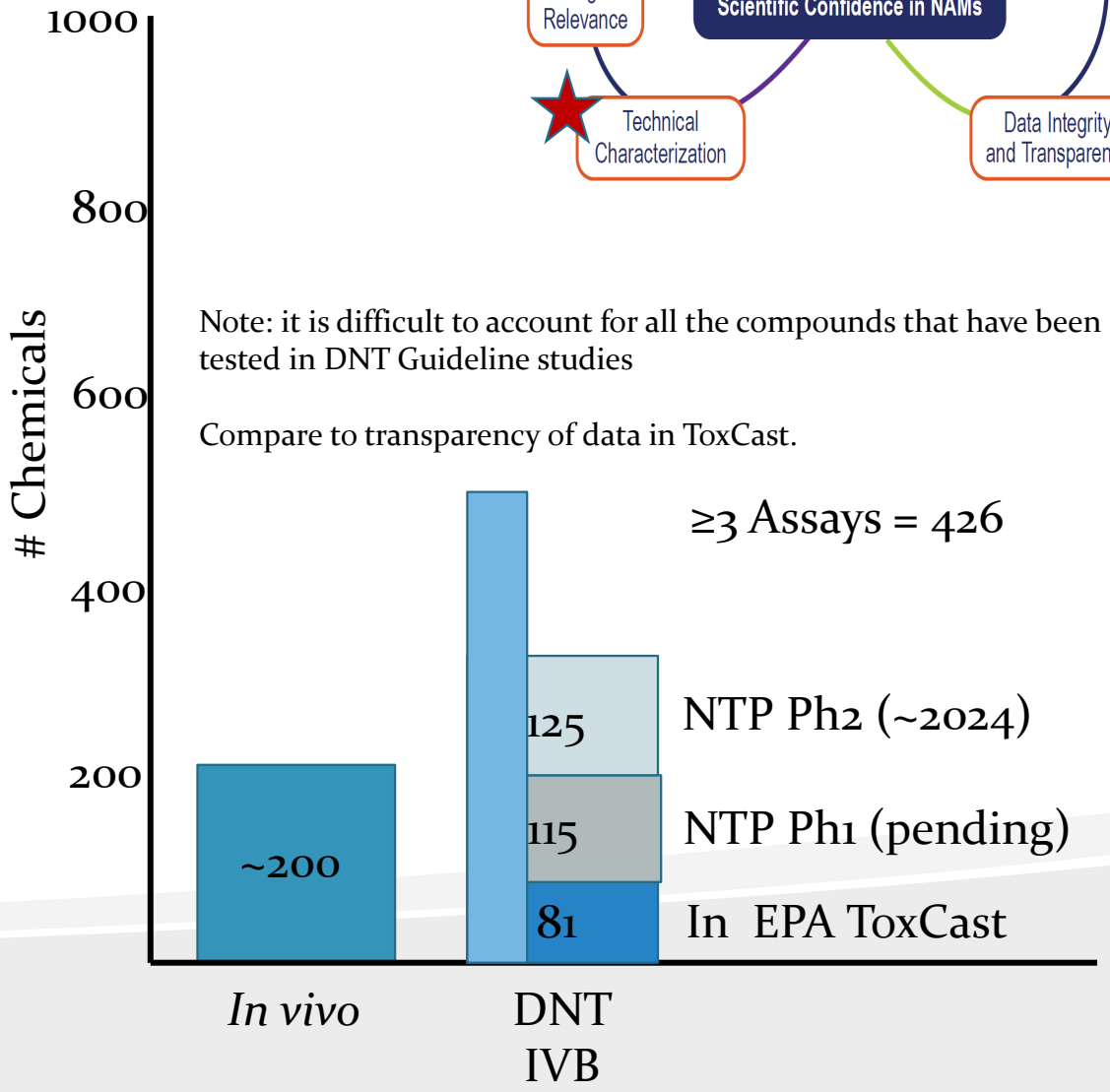

Note: it is difficult to account for all the compounds that have been tested in DNT Guideline studies

Compare to transparency of data in ToxCast.

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Screening Level Information for PFAS Compounds

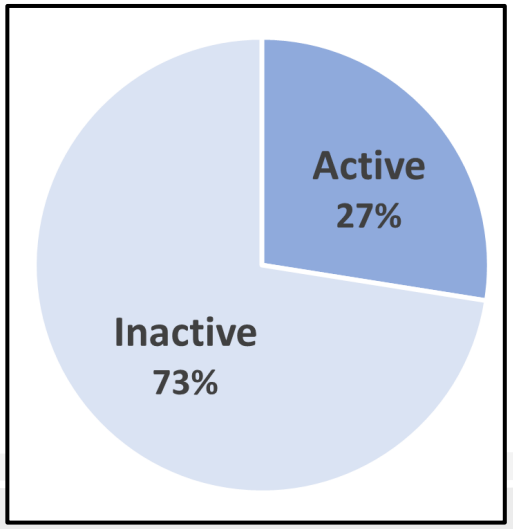
- Structurally diverse group of chemicals
- Little *in vivo* toxicological information on DNT
- DNT evidence is conflicting
  - epidemiological studies are equivocal
  - neurodevelopmental effects associated with exposure to PFAS in rodent and other animal studies

Chemical Research in Toxicology

pubs.acs.org/crt Article

Evaluation of Per- and Polyfluoroalkyl Substances (PFAS) *In Vitro* Toxicity Testing for Developmental Neurotoxicity

Kelly E. Carstens,* Theresa Freudenrich, Kathleen Wallace, Seline Choo, Amy Carpenter, Marci Smeltz, Matthew S. Clifton, W. Matthew Henderson, Ann M. Richard, Grace Patlewicz, Barbara A. Wetmore, Katie Paul Friedman, and Timothy Shafer



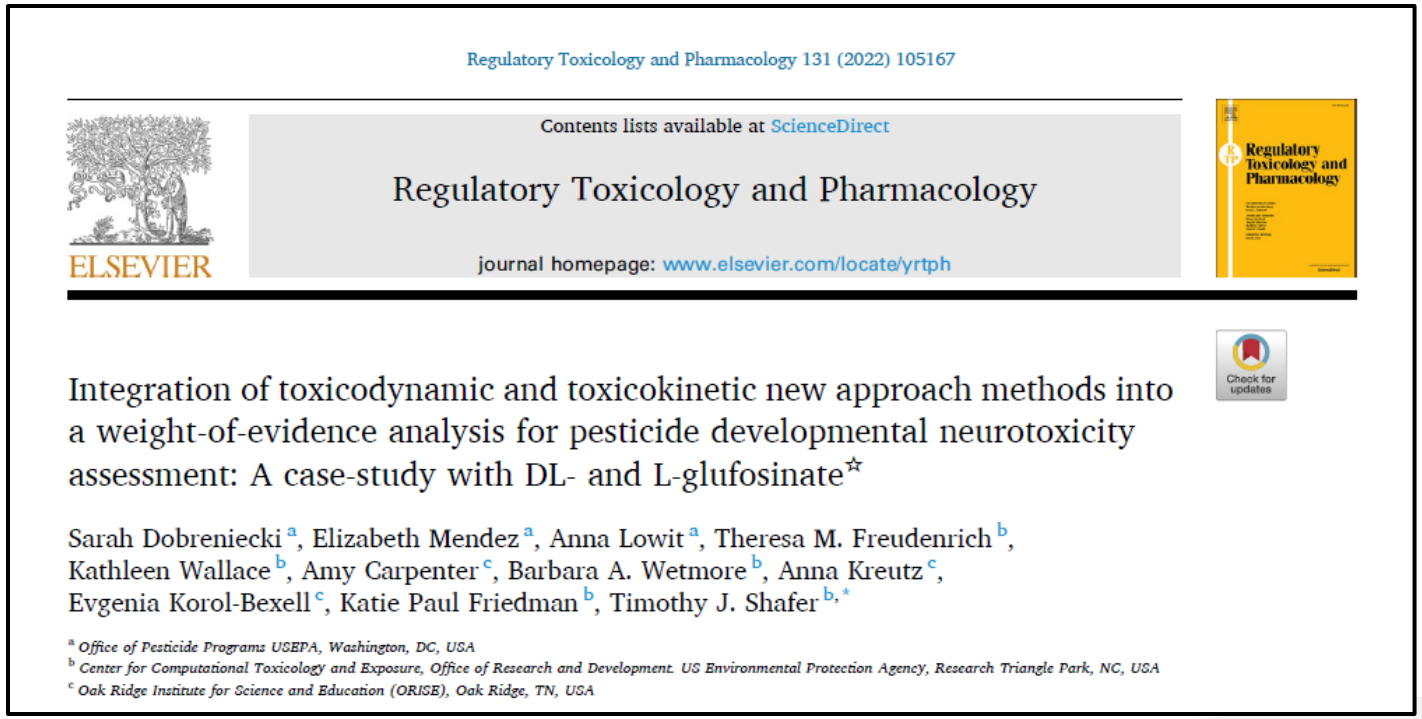
- Out of a set of **160 PFAS**, 118 were inactive, leaving **42** active PFAS that decreased measures of neural network formation, neurite outgrowth, proliferation, or apoptosis
- **24** PFAS demonstrate moderate or low selective activity

These data can now guide future decisions about hazard identification for PFAS compounds

Figure 1 from Carstens et al, 2023, Chem Res Toxicol. 2023 Mar 20;36(3):402-419. doi: 10.1021/acs.chemrestox.2c00344

Waiver Evaluation for Glufosinate based on Weight-of-Evidence

- EPA's Office of Pesticide Programs (OPP) received notification that different parties intended to register L-glufosinate ammonium and L-glufosinate acid as pesticides (herbicides)
- DL-glufosinate ammonium was already registered as a pesticide, and a Guideline DNT study had been submitted to OPP
  - Decreased pup weight, morphometry changes in hippocampus, motor activity changes were reported
- DL-glufosinate also has acute neurotoxicity
- *In vitro*, literature report of altered network activity following acute exposure (Lantz et al., 2014)
- *Question: Is the Guideline DNT for DL-glufosinate sufficient to inform decisions for L-glufosinate isomers?*
- Need: Comparative bioactivity data for DL- vs L-Glufosinate isomers



Regulatory Toxicology and Pharmacology 131 (2022) 105167

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

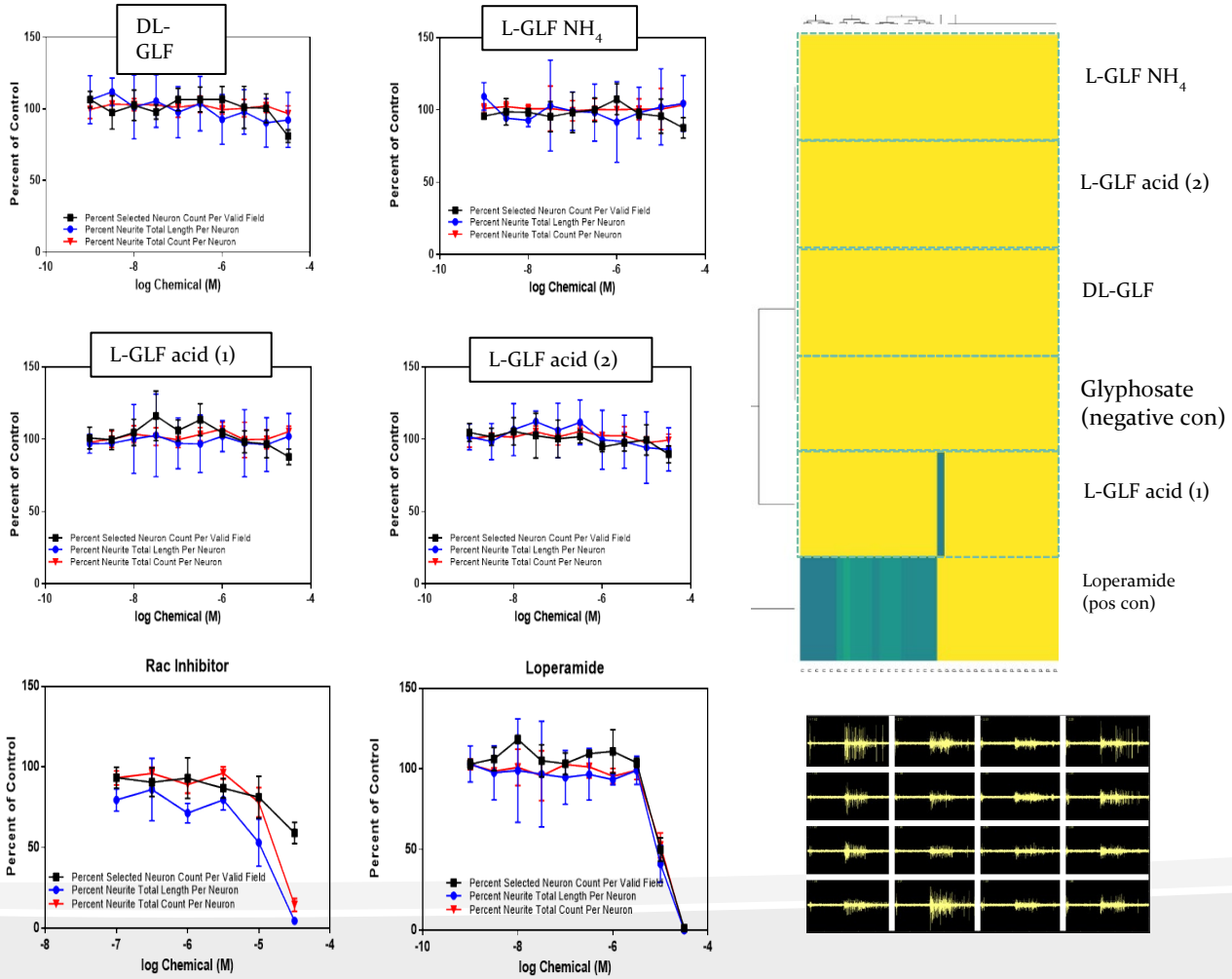
journal homepage: www.elsevier.com/locate/yrtph

Integration of toxicodynamic and toxicokinetic new approach methods into a weight-of-evidence analysis for pesticide developmental neurotoxicity assessment: A case-study with DL- and L-glufosinate☆

Sarah Dobreniecki [a], Elizabeth Mendez [a], Anna Lowit [a], Theresa M. Freudenrich [b], Kathleen Wallace [b], Amy Carpenter [c], Barbara A. Wetmore [b], Anna Kreutz [c], Evgenia Korol-Bexell [c], Katie Paul Friedman [b], Timothy J. Shafer [b,*]

[a] Office of Pesticide Programs USEPA, Washington, DC, USA
[b] Center for Computational Toxicology and Exposure, Office of Research and Development. US Environmental Protection Agency, Research Triangle Park, NC, USA
[c] Oak Ridge Institute for Science and Education (ORISE), Oak Ridge, TN, USA

Using WoE and DNT NAMs for Guideline DNT Waiver



In vitro evidence

- Lack of effect on neurite outgrowth in human cells
- Lack of effect on network formation in rat cortical networks
- **Positive effects on acute network activity** demonstrate biological activity and add confidence to the lack of effects in DNT-related assays (neurite outgrowth and network formation)
- *Similar effects of DL- and L-isoforms in all in vitro assays*

In vitro to *in vivo* extrapolation (IVIVE)

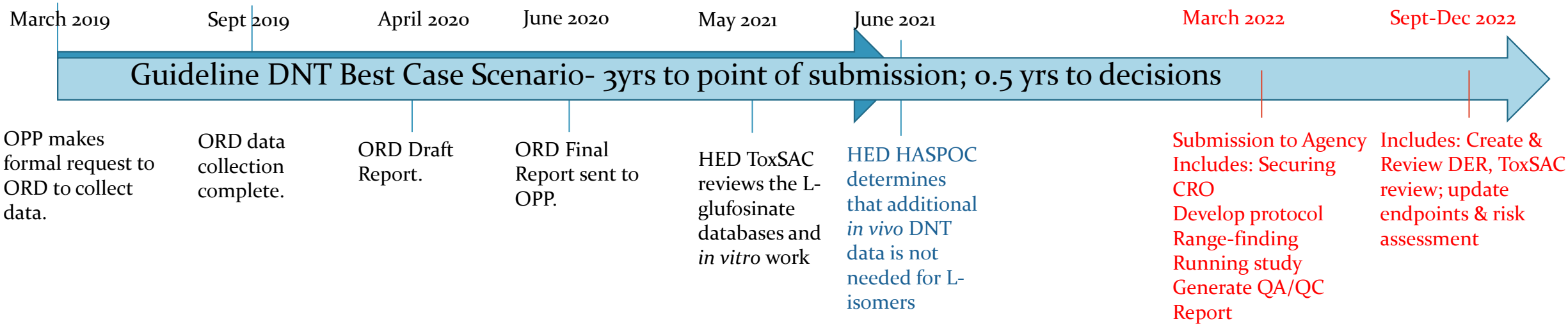
- Tested concentrations *in vitro* > PODs selected for L-glufosinate risk assessment

In vivo evidence

- Existing guideline DNT study for DL-glufosinate showing effects on morphometry, motor activity and pup weight
- Non-guideline DNT for L-glufosinate showing increased motor activity, decreased body wt in pups (morphometrics not conducted)
- *Comparable toxicity profiles for both DL- and L-glufosinate.*

from Dobreniecki et al., Reg Toxicol Pharmacol 2022 Jun;131:105167. doi: 10.1016/j.yrtph.2022.105167

Impacts of DNT NAMs



Animals Used:

- *In vitro* study- 3 Pregnant Dams (~12-15pups)
- Guideline study- 160 Pregnant Dams (2 compounds X 3 doses + control @20/dose (recommended))
  - ~1600 pups

Cost:

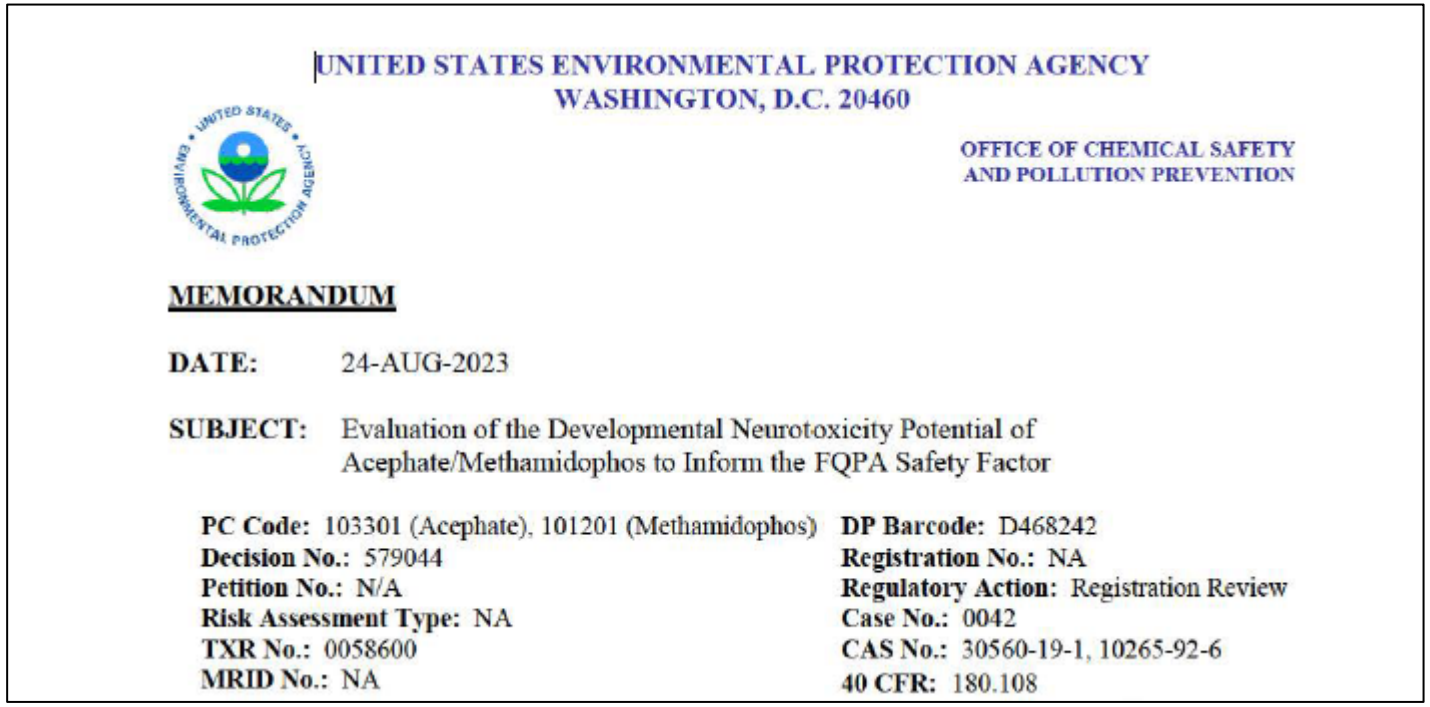
- *In vitro* study- $1000 for Assays + $96,000 labor = $97,000
- Guideline study- $2,000,000 (2 compounds x $1M each)

PFAS:

$160M for Guideline studies
160,000 animals

Other Uses of DNT NAMs

Organophosphate re-assessments



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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: 24-AUG-2023

SUBJECT: Evaluation of the Developmental Neurotoxicity Potential of Acephate/Methamidophos to Inform the FQPA Safety Factor

PC Code: 103301 (Acephate), 101201 (Methamidophos)
Decision No.: 579044
Petition No.: N/A
Risk Assessment Type: NA
TXR No.: 0058600
MRID No.: NA

DP Barcode: D468242
Registration No.: NA
Regulatory Action: Registration Review
Case No.: 0042
CAS No.: 30560-19-1, 10265-92-6
40 CFR: 180.108

OECD Case-Studies

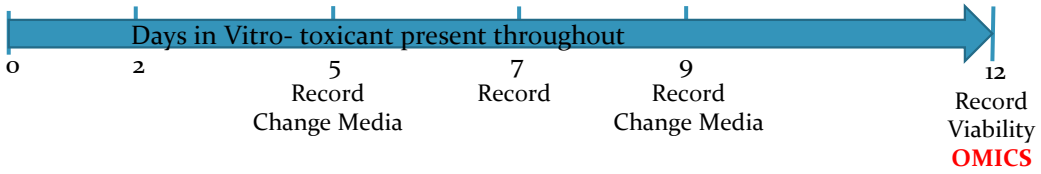
https://www.oecd.org/chemicalsafety/risk-assessment/iata/
(click on "In vitro Battery" checkbox)

2021	1	Case study for the integration of in vitro data in the developmental neurotoxicity hazard identification and characterisation using deltamethrin as a prototype chemical
2021	2	Case study for the integration of in vitro data in the developmental neurotoxicity hazard identification and characterisation using flufenacet
2021	3	Case study on the use of Integrated Approaches for Testing and Assessment for DNT to prioritize a class of Organophosphorus flame retardants
2021	4	Case Study on the use of Integrated Approaches for Testing and Assessment for developmental neurotoxicity hazard characterisation of acetamiprid
2021	5	Case Study on the use of Integrated Approaches for Testing and Assessment for developmental neurotoxicity hazard characterisation of imidacloprid and the metabolite desnitro-imidacloprid

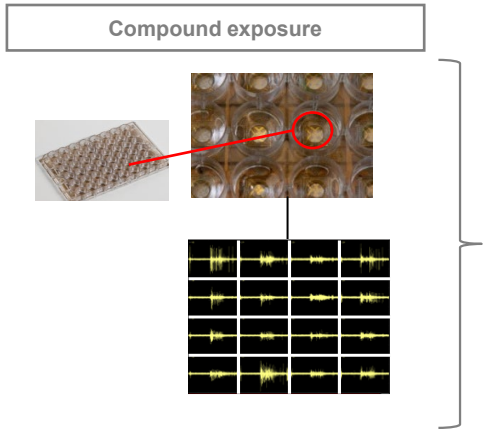
Overview

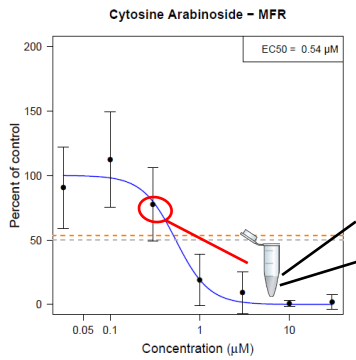
I. My Career Path
II. Brief History of the EPA and Regulatory Statutes
III. The Need for Alternative Approaches for Neurotoxicity and Developmental Neurotoxicity Hazard Assessment
IV. A Brief History of NAMs for DNT
V. Examples of How DNT NAMs are being utilized for decision-making
VI. Informing AOP Development Omics Data
VII. Current projects
VIII. Questions

Proof-of-Concept for collecting –omics information from the rat network formation assay



Step 1: Chemical Dose Identification with Functional Assay

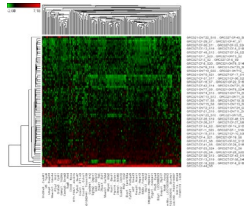




Selected Treatments

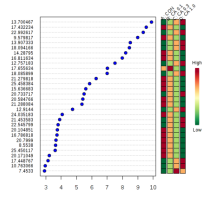
Step 2: Identifying Key Molecular Events Involved in Neurodevelopment

Cell Pellet
media



Transcriptomics

+



Metabolomics

3: Integrated Core Analysis

Transcriptomic level

Disease Analysis (NDD)
Molecular/Cellular
Function Categories

Metabolomic level

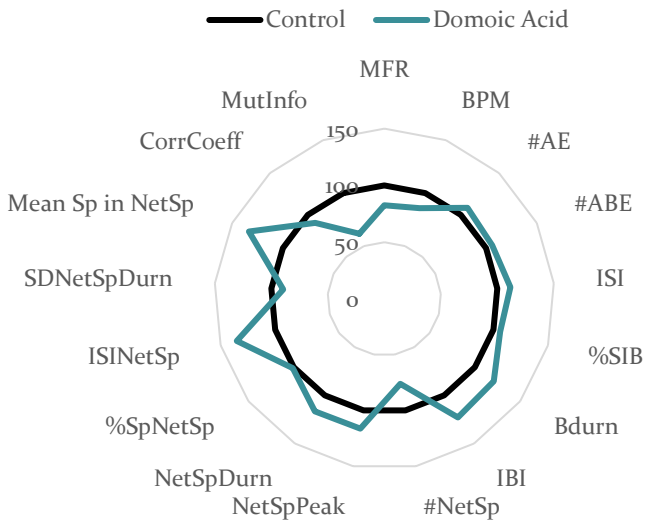
Disease Analysis (NDD)
Molecular/Cellular
Function Categories

Combined Analysis

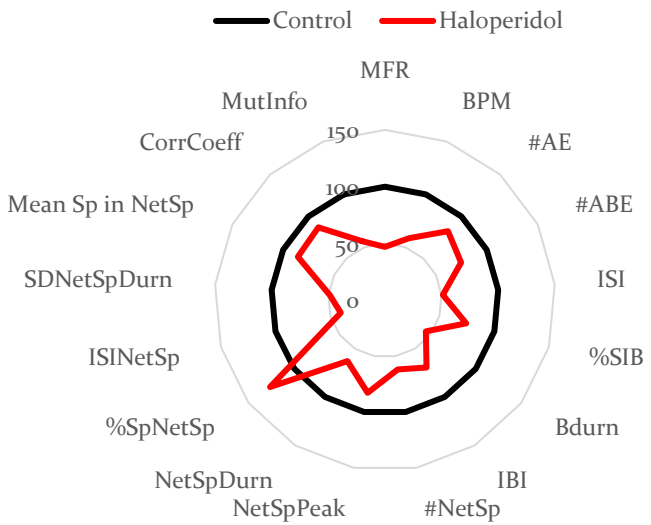
Disease Analysis (NDD)
Molecular/Cellular
Function Categories

Marable et al., Toxicol Sci. 2022 Feb 28;186(1):118-133. doi: 10.1093/toxsci/kfab151

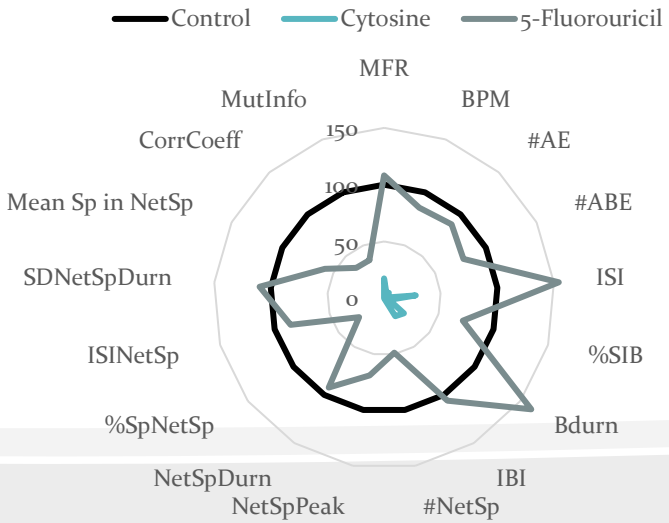
Domoic Acid



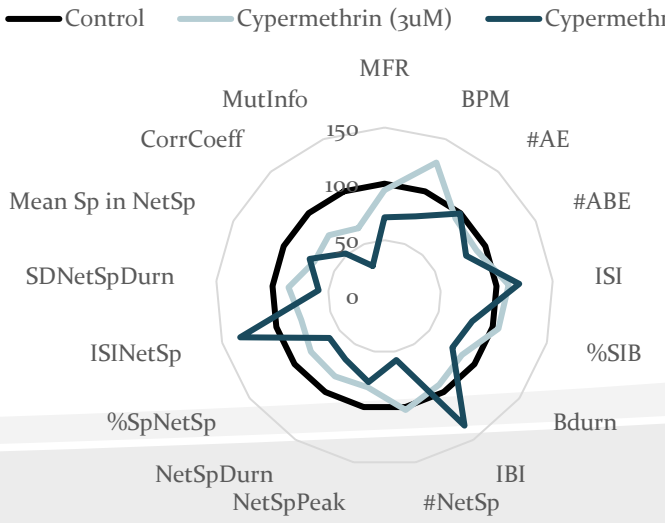
Haloperidol



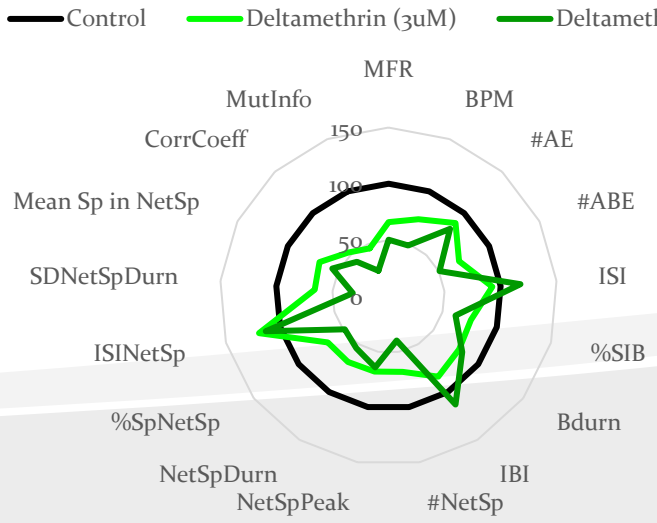
DNA Synthesis Inhibitors



Cypermethrin



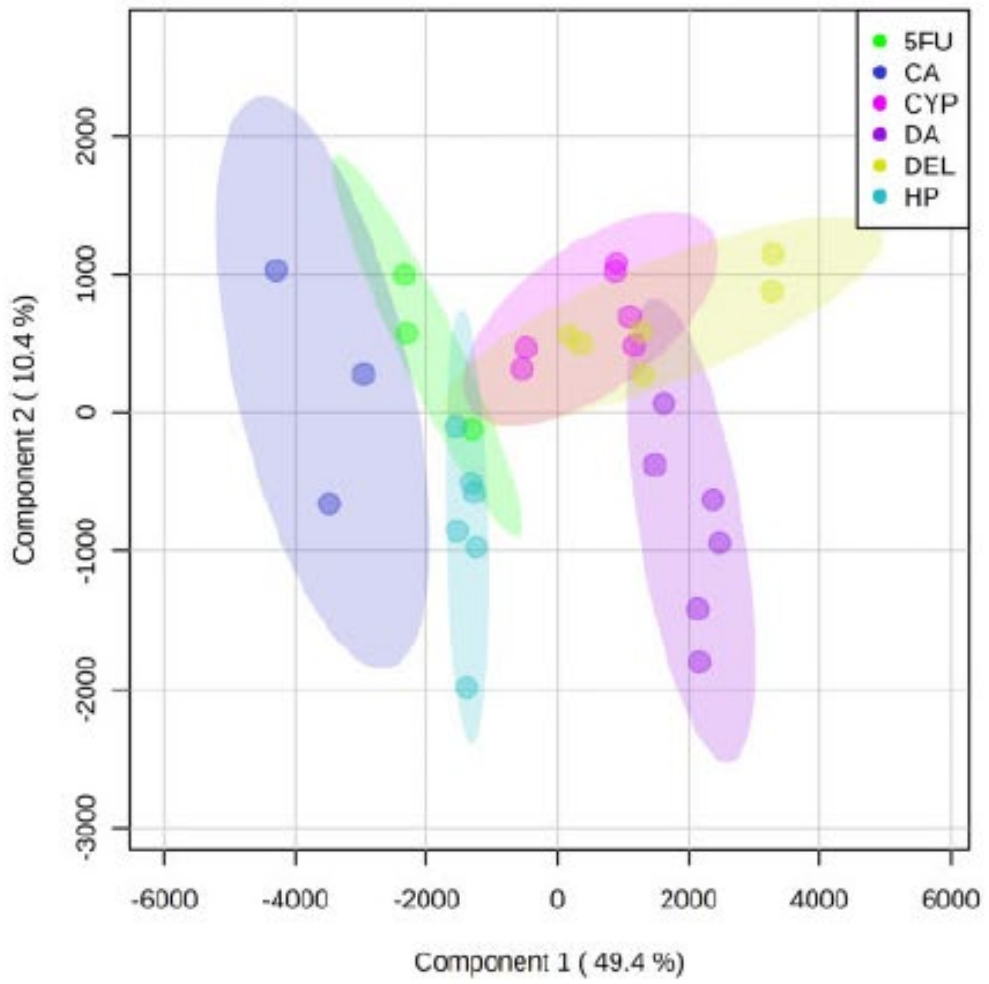
Deltamethrin



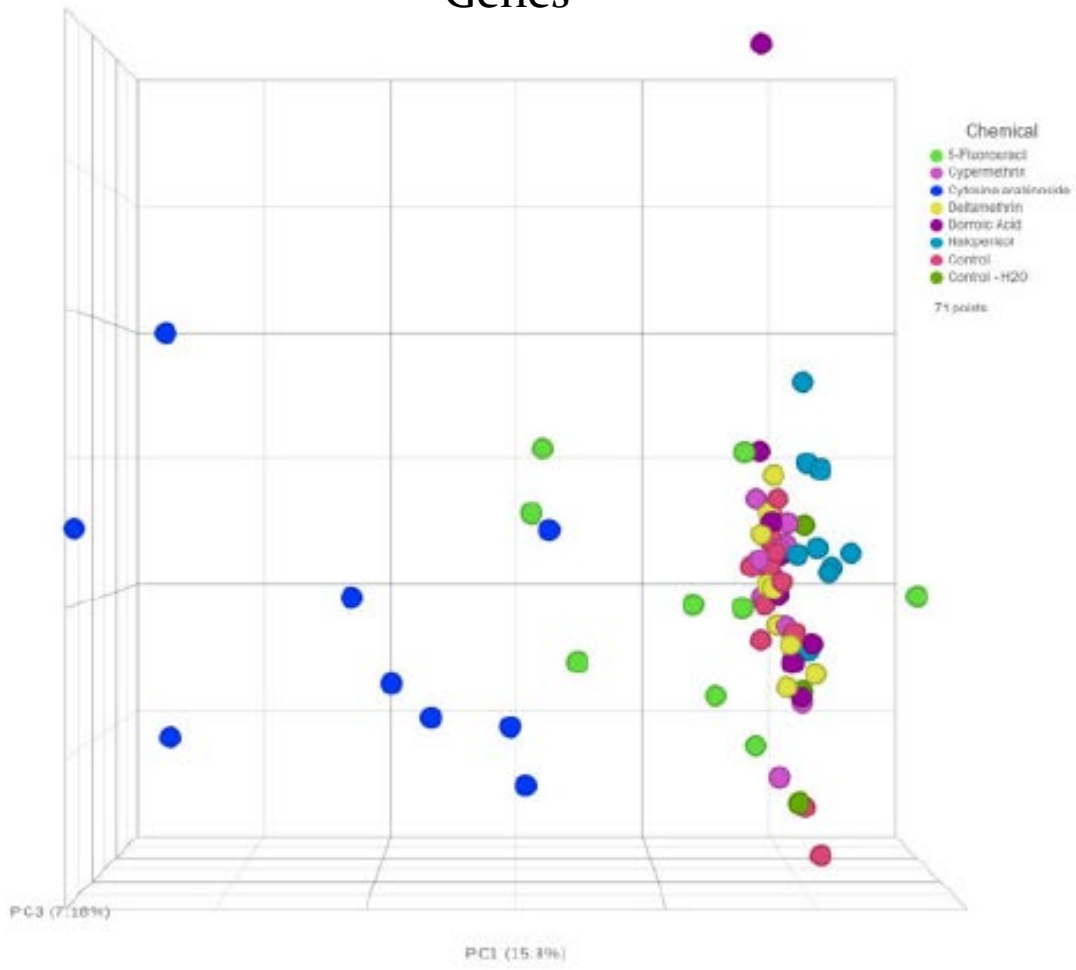
Marable et al., Toxicol Sci. 2022 Feb 28;186(1):118-133. doi: 10.1093/toxsci/kfab151

Principal Components Analysis for Differentially Expressed Genes and Metabolites indicate treatment effects

Metabolites



Genes



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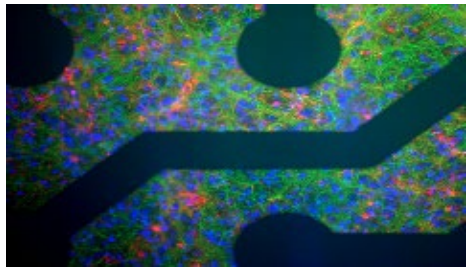

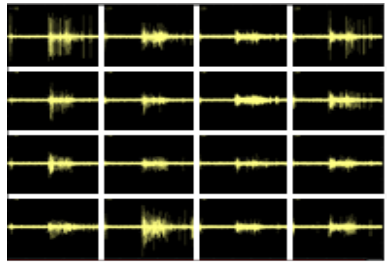

Step 4: Investigate Molecular Relationships between MEA and Omic Identified Events to support future AOP development

Molecular Initiating Event (MIE)

Cytosine Arabinoside

Inhibition of DNA Synthesis

Increased Oxidative stress in Neurons

Key Event #1

↓ Proliferation

↑ Apoptosis

Key Event #2

Decreased glial support (postulated)

Neuronal Cell Death

Key Event #3

Decreased neurite outgrowth

Key Event #4

Neuronal Network Dysfunction

Neurodevelopmentally Relevant Adverse Outcome (AO)

Altered Neurophysiology (Elmer et al., 2004)

Cell Growth & Proliferation

Cell Function & Maintenance

Cell Death & Survival

Cell-to-cell Signaling & Interaction

Human Embryonic Stem Cell Plurapotency

Axonal Guidance Signaling

CREB Signaling in Neurons

Neurological Disease
Psychological Disorders

Marable et al., Toxicol Sci. 2022 Feb 28;186(1):118-133. doi: 10.1093/toxsci/kfab151

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VI. Informing AOP Development Omics Data
VII. Current projects
- Hottest data off the press! (prelimary)

VIII. Questions

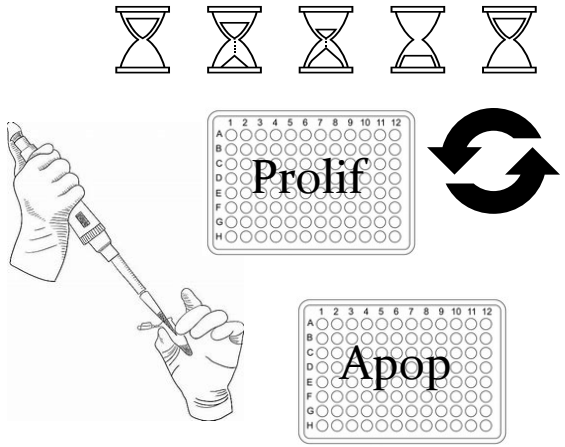
Increasing Throughput to Accelerate Testing

Development of Increased Throughput DNT NAMs to facilitate chemical screening

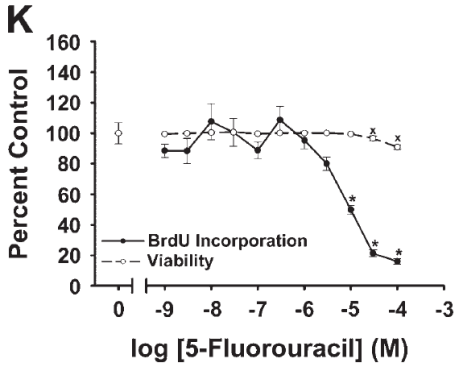
96-well → 384-well

Manual pipetting → Laboratory automation

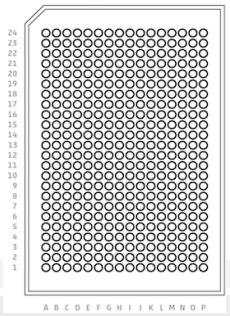
Updated imaging equipment

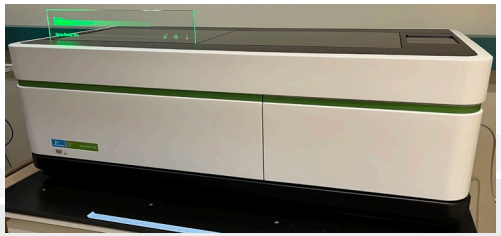


Outdated imaging platform



Scale up of proliferation/apoptosis in hNP1 Cells to 384 wells





Current imaging platform

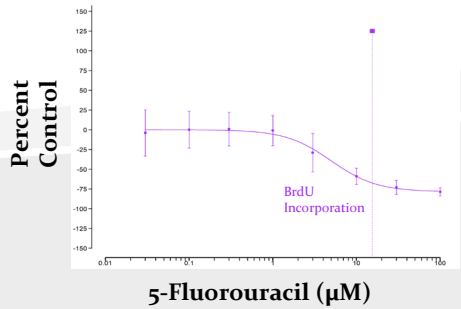
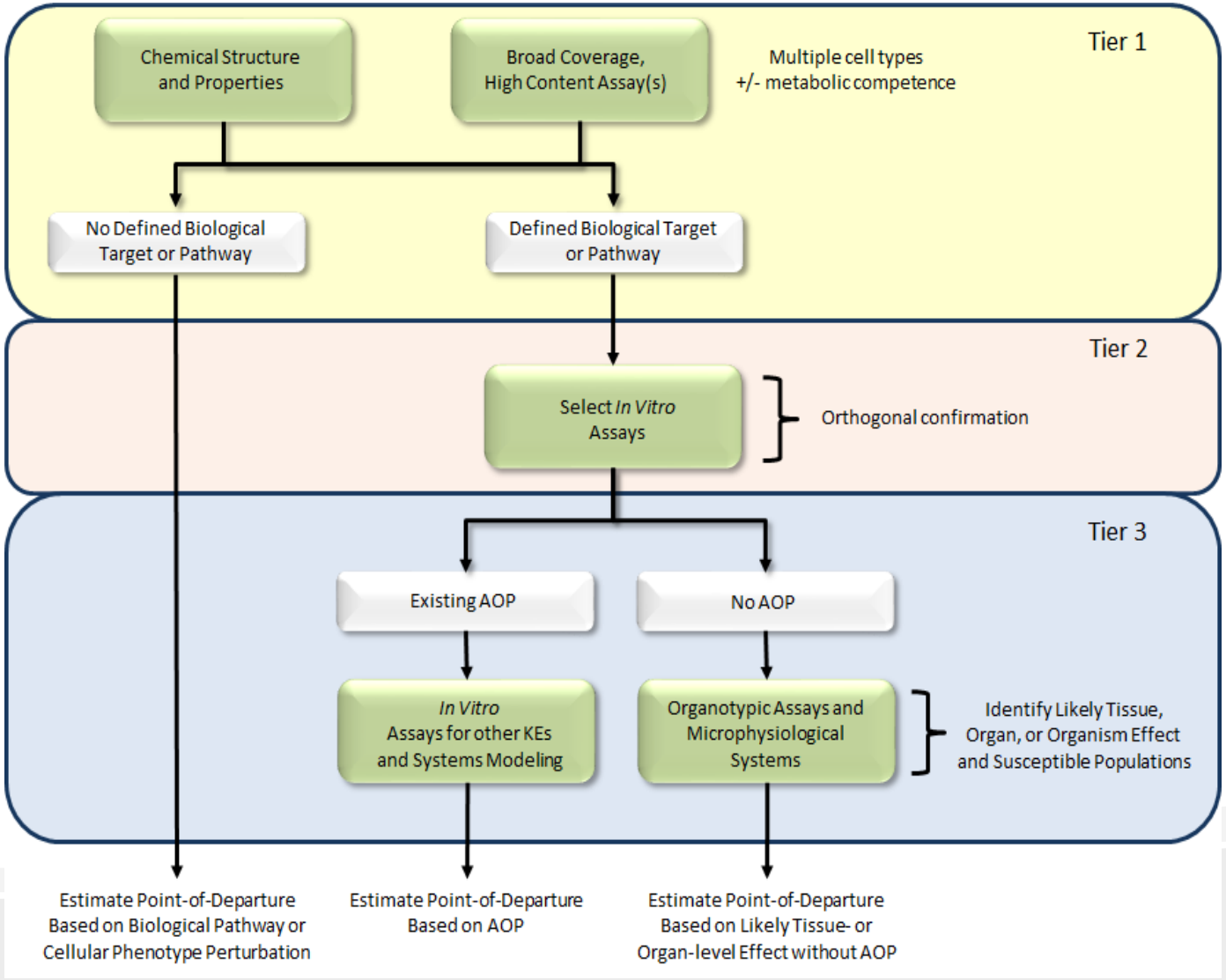


Figure courtesy of Gabby Byrd

NAMs-Based Tiered Hazard Evaluation Approach

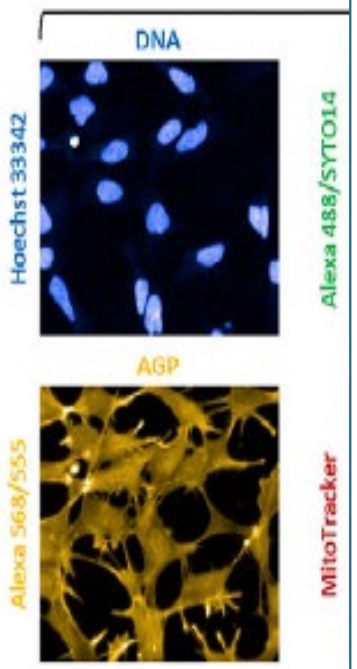


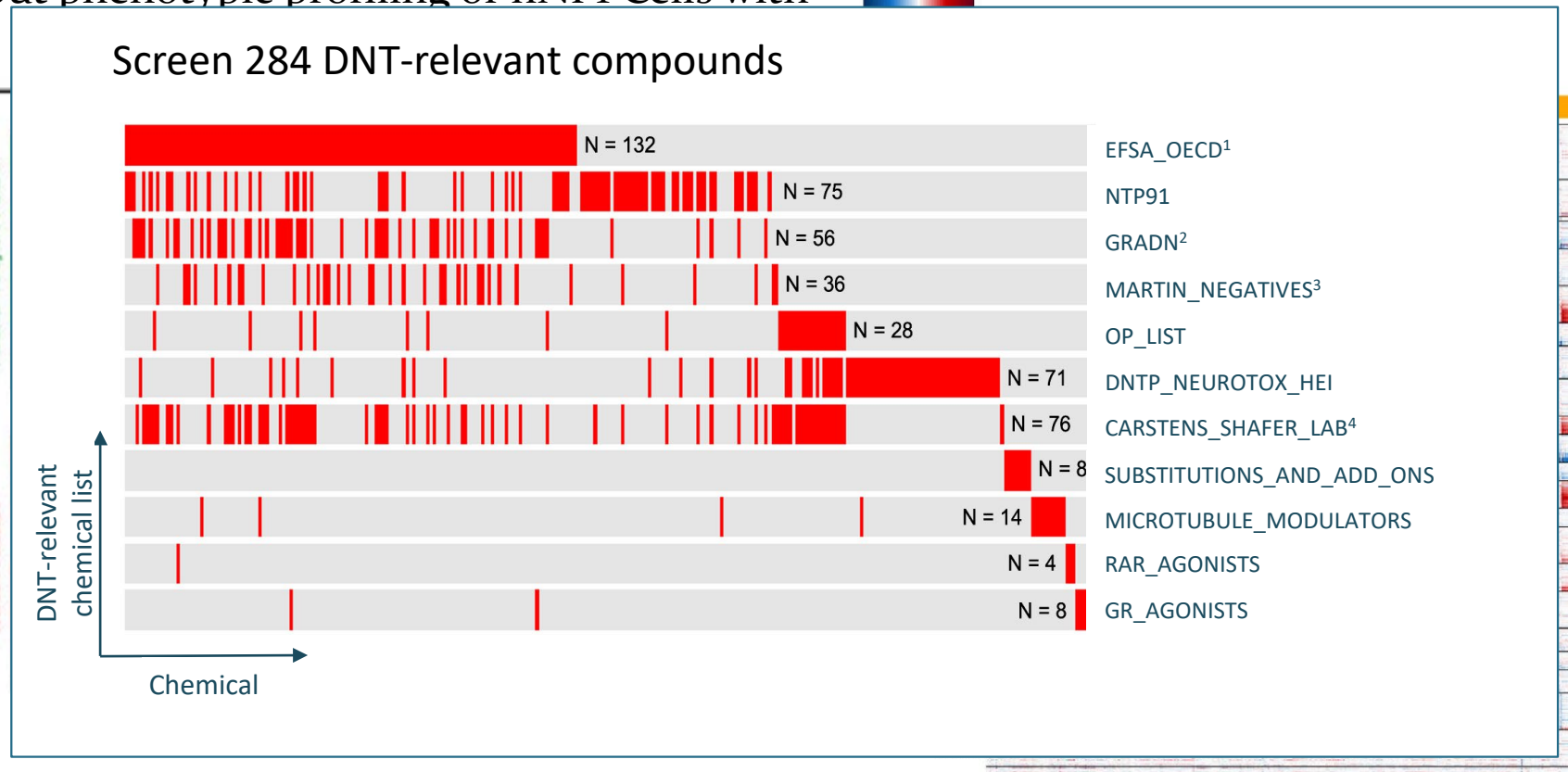
DNT-IVB

from Thomas et al., Tox. Sci. 2019; 169(2):317-322 Jun 1;169(2):317-332. doi: 10.1093/toxsci/kfz058.

Tiered-testing: Development of Tier 1 Assays

High-throughput phenotypic profiling of hNP1 Cells with





Culbreth et al., Front. Toxicol. 2022 Feb 16;3:803987. doi: 10.3389/ftox.2021.803987

Development of organotypic culture models for DNT

Axion Maestro

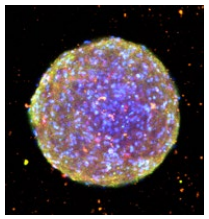
6, 24 &48 wells/plates
Au/Pt based electrodes
768 electrodes/plate
16 electrodes/well @ 48 wells
200 microns between electrodes

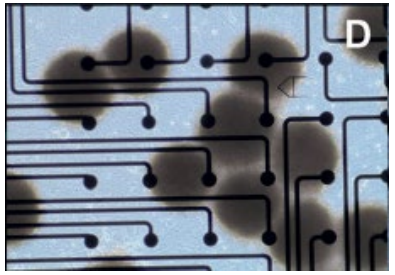
Maxwell MaxTwo HD-MEA

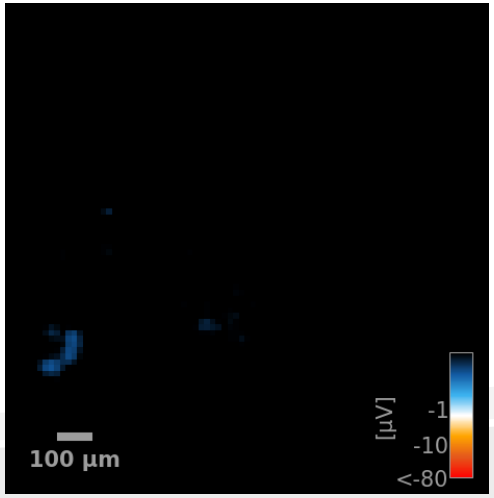
6&24 wells/plate
CMOS based electrodes
26,400 electrodes/well
17.5 microns electrode pitch

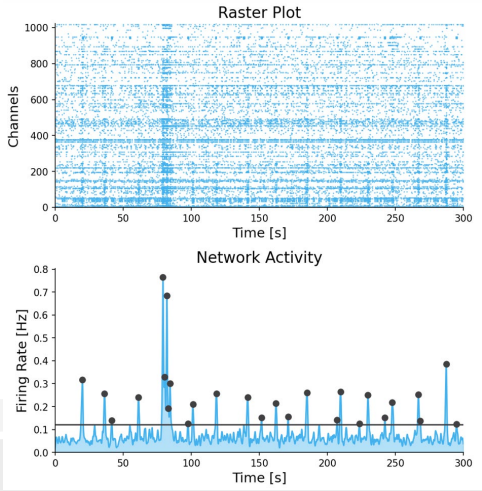
Johns Hopkins University- Neurospheres

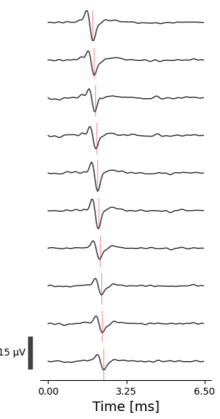
- 8-10 weeks
- 300-600 microns
- 70% TUJI Neurons
  - >40% myelinated axons @ 8 weeks

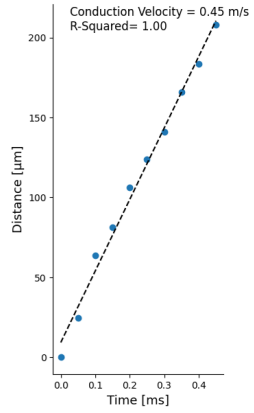




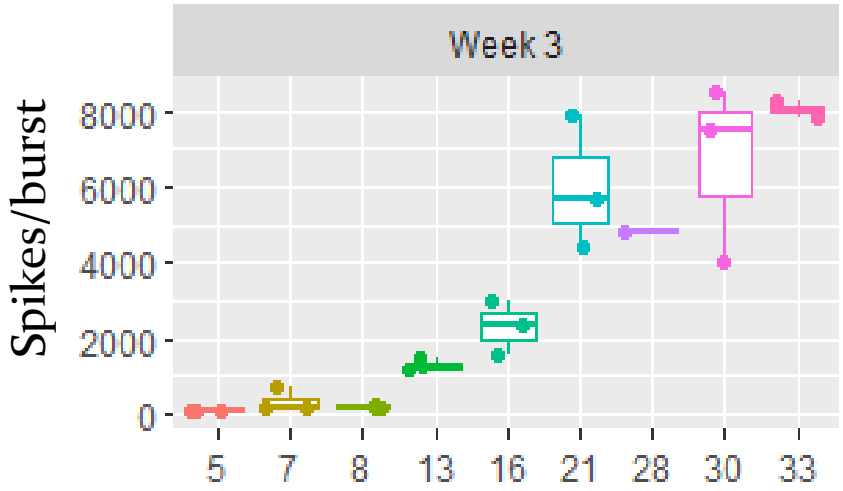


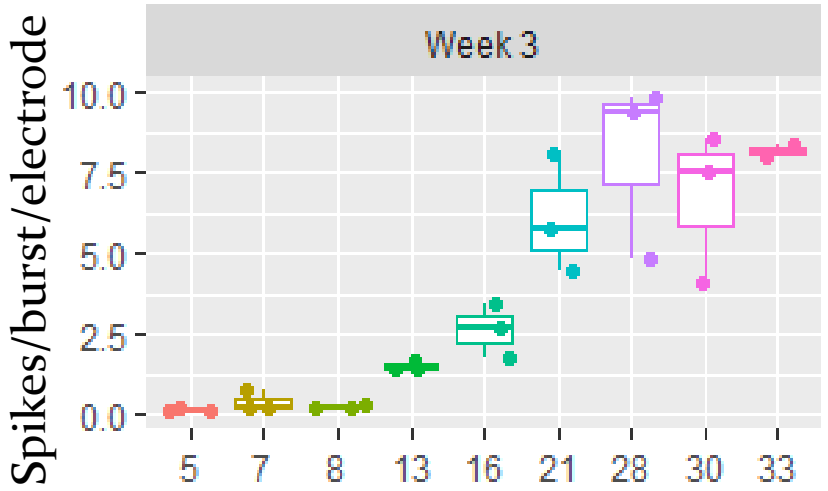


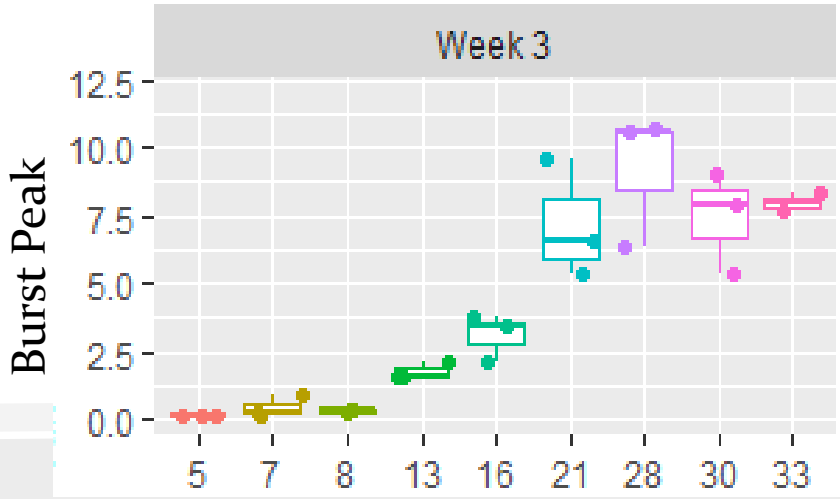


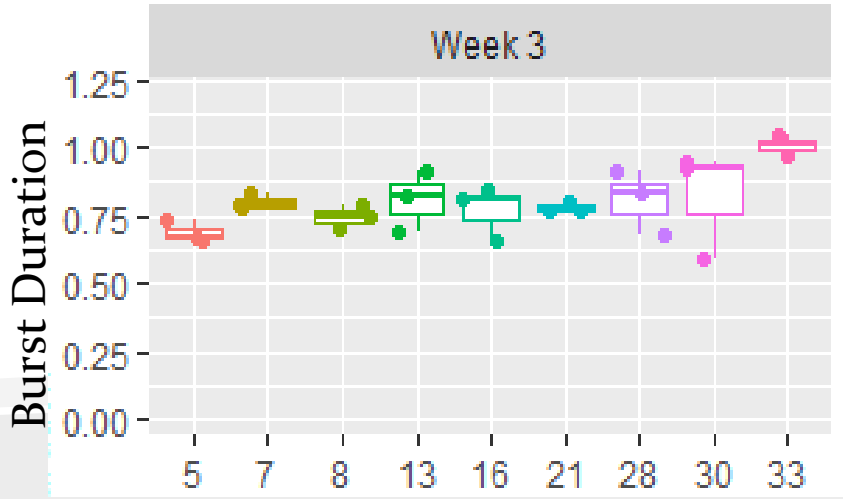


Ontogeny data from neurospheres

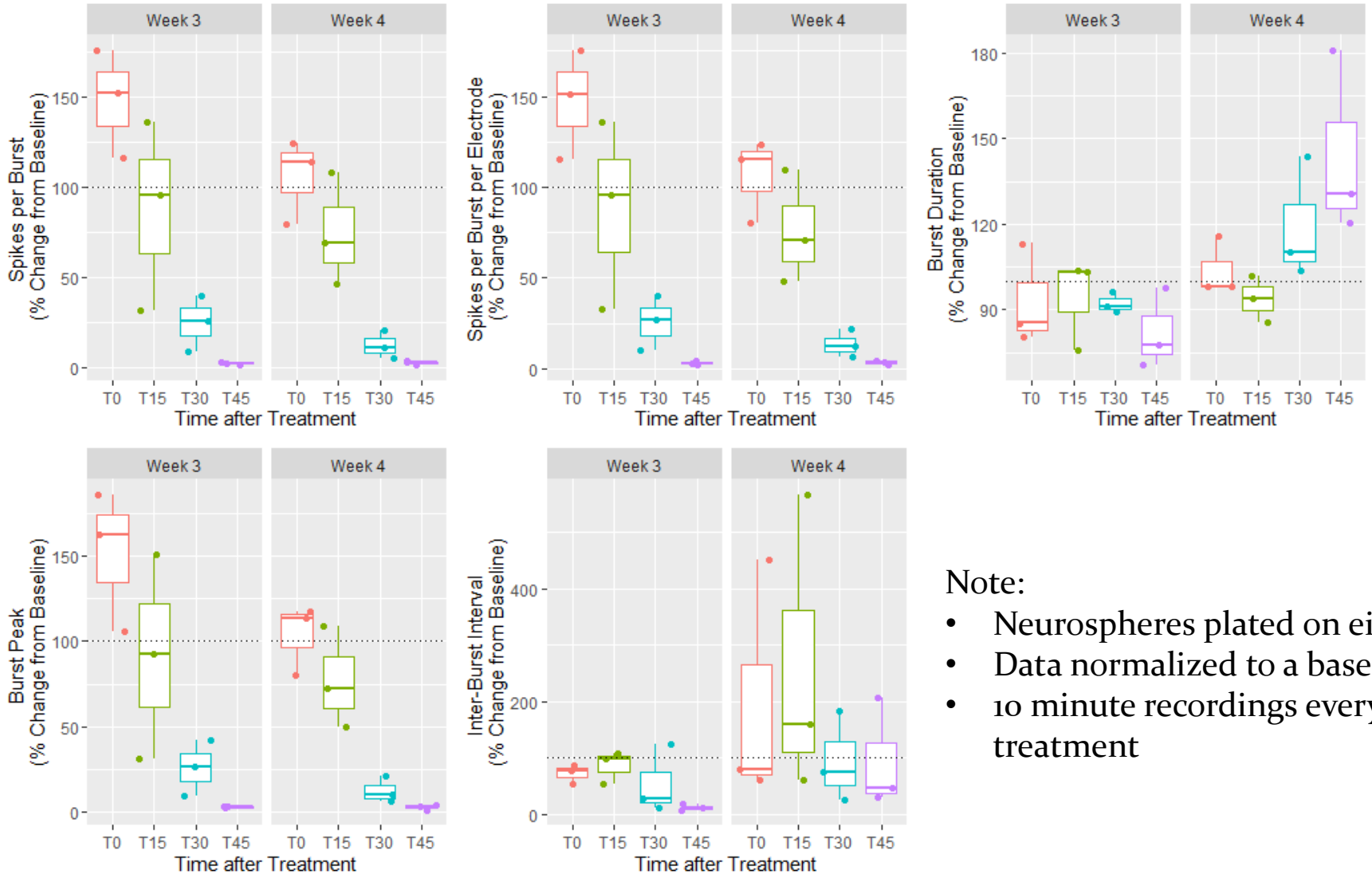








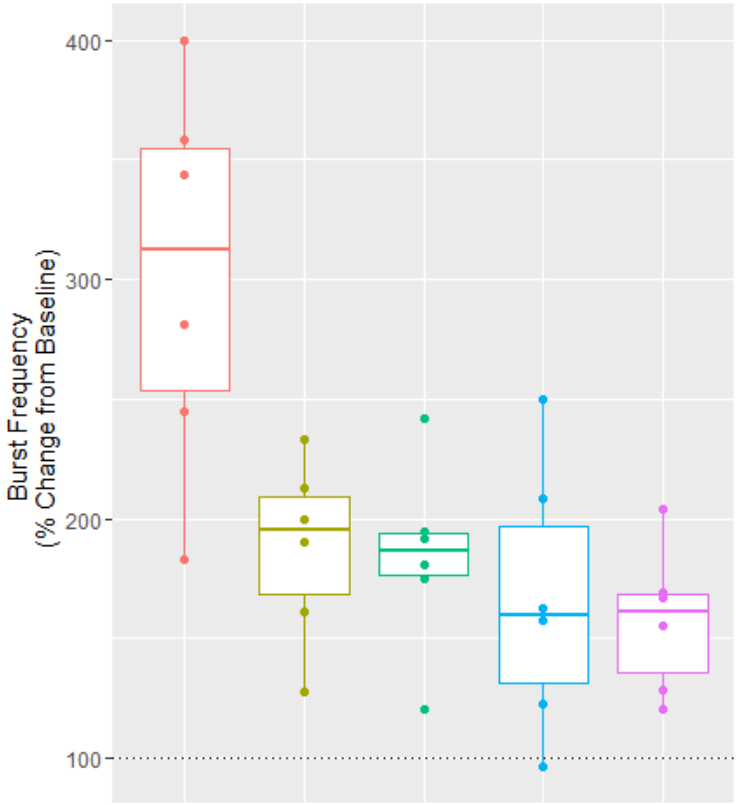
Tetrodotoxin blocks activity in neurospheres

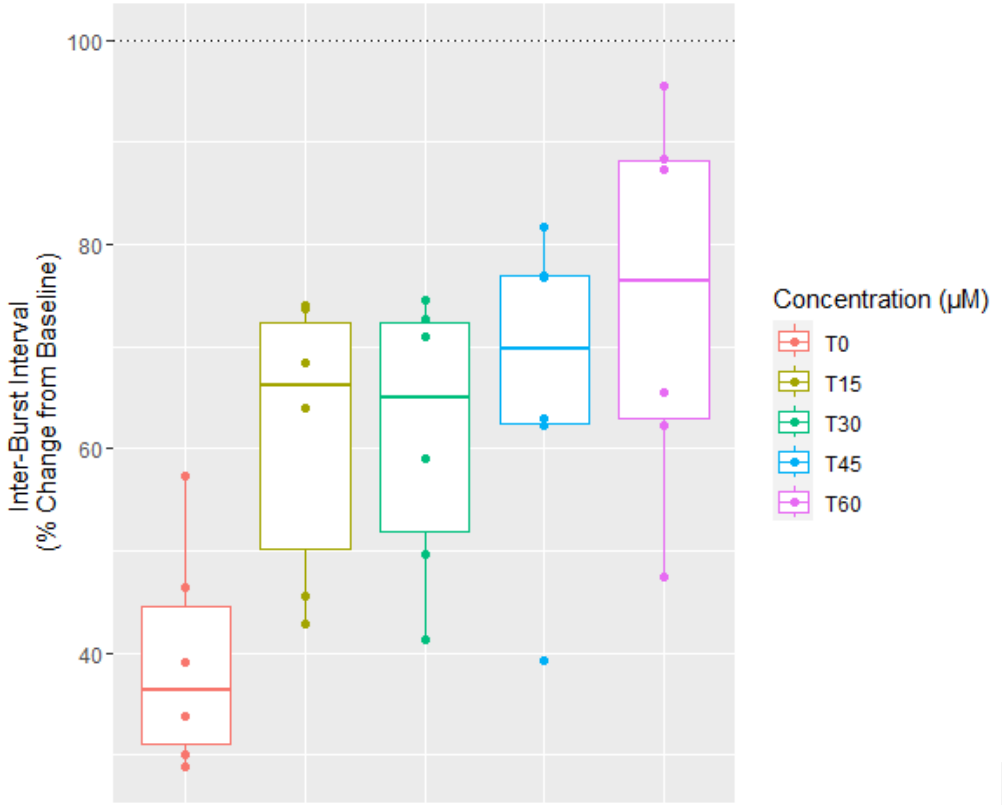


Note:

- Neurospheres plated on either week 3 or 4
- Data normalized to a baseline recording
- 10 minute recordings every 15 minutes after TTX treatment

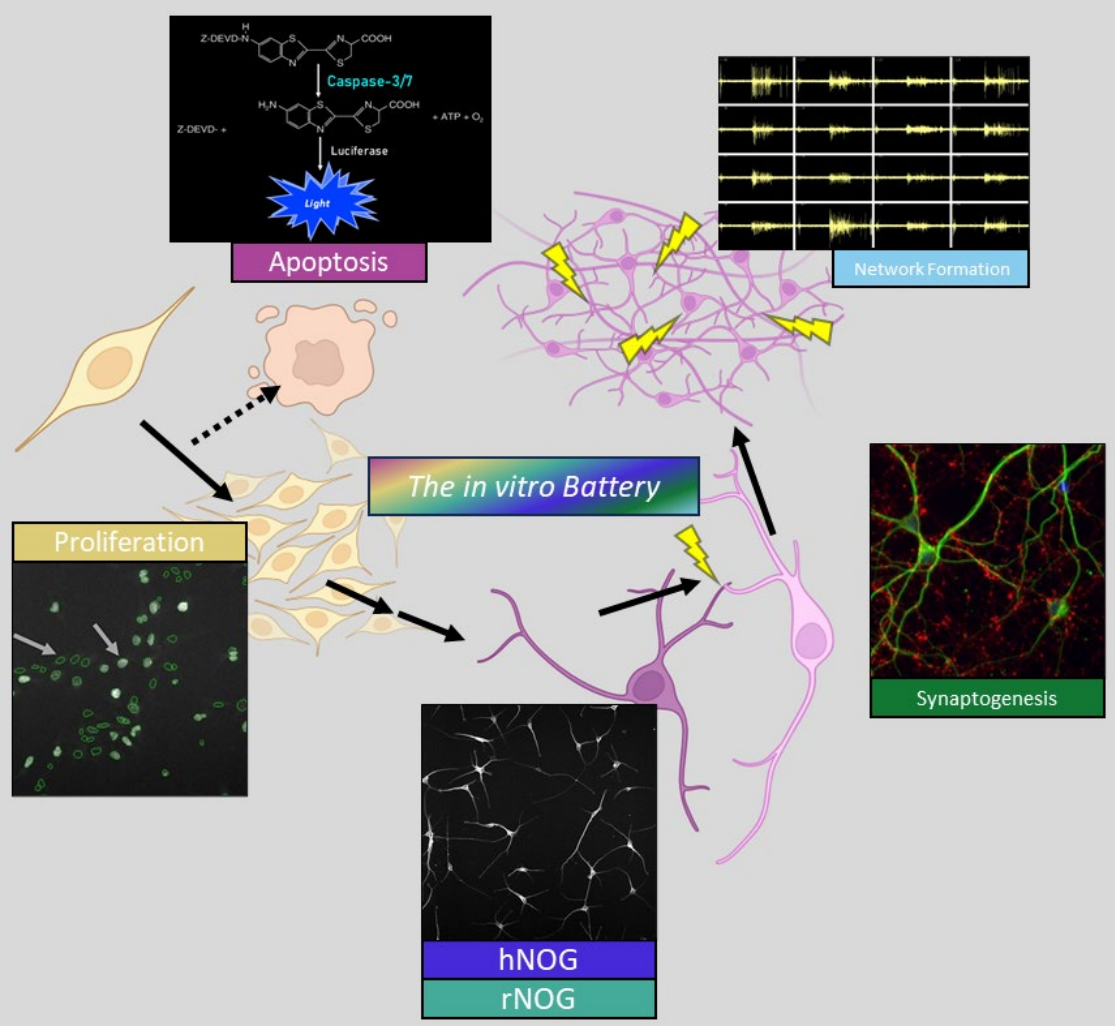
While picrotoxin increases activity





DNT Signaling Pathways

- Purpose: Determine whether 22 developmentally relevant signaling pathways are recapitulated in our battery
- Method: Utilize specific chemical pathway inhibitors/activators in our normal battery to characterize selectivity in our assays



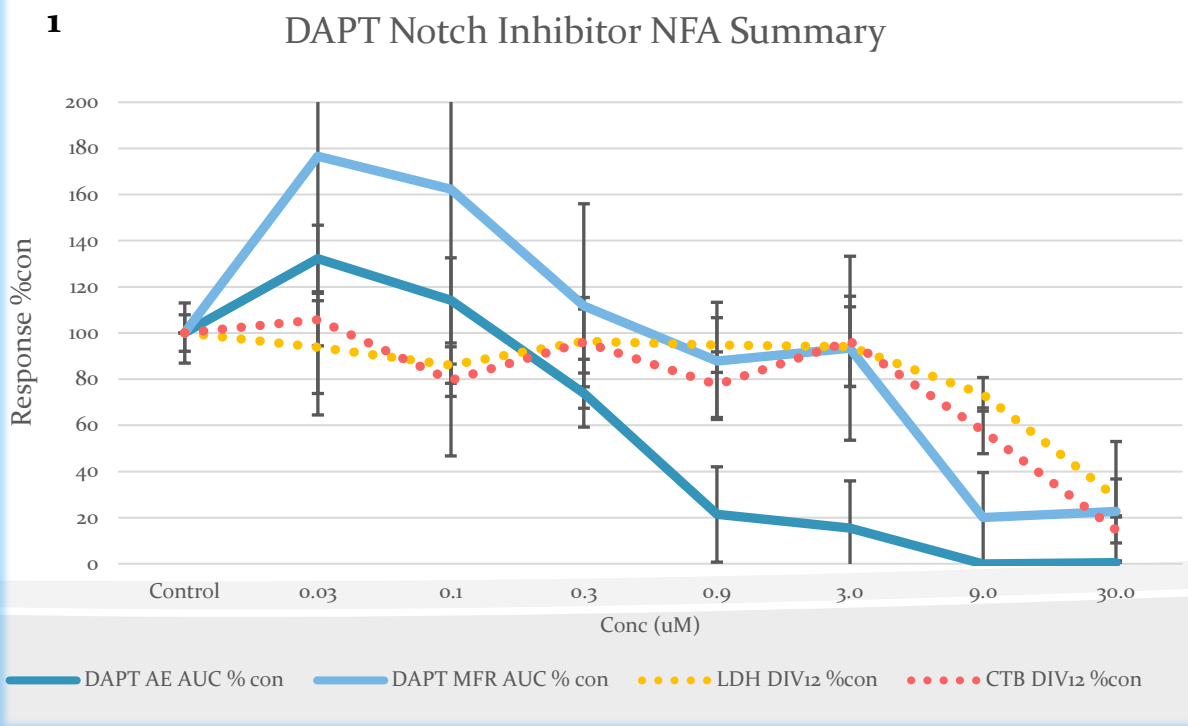
Pathway	Compound
BMPR	BMP2 BMP7
Notch	DAPT
EGFR	PD153035
WNT	CHIR99021 IWP2
PPAR-γ	Pioglitazone HCl
COX-2	Celecoxib
mTORC	MHY1485 Rapamycin
AKT	MK-2206
PDGFR	CP-673451
PKC	BIS-1/BIM-1
RHO	Narciclasine
EP1-4	PGE2
CREB	KG-501
AKT	SC79 (Activator)
ETC I	Rotenone
EGFR	AG1478
NO-cGMP	ODQ
ROCK	Y 27632
Protein Synthesis	Cycloheximide
HDAC	SAHA/Vorinostat
PARP1 (DNA repair)	Talazoparib/BMN-673
ERK/MAPK	SCH772984

Work in progress
Evaluated qualitatively while awaiting quantitative analysis in ToxCast.

	BMPR	Notch	EGFR	WNT	PPAR-γ	COX-2	mTORC	PDGFR	PKC	RHO	Microtubule	EP1-4	CREB	AKT	ETC I	EGFR	NO-cGMP	ROCK	Protein	HDAC	PARP1	ERK/MAPK	p38/MAPK
hNP1 Apoptosis		3																					
hNP1 Prolif.																							
hNOG								4					5										
rNOG				2	6								7										
Cortical Syn.																							
NFA		1																					

Active Selective
Active
Inactive/Cytotoxic
Awaiting Data

1



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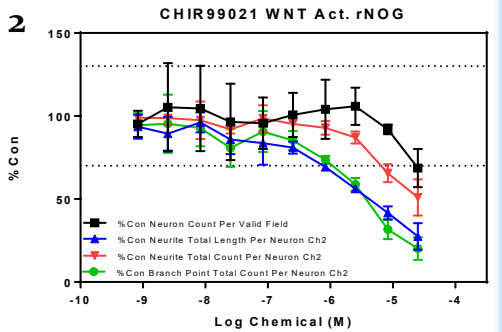

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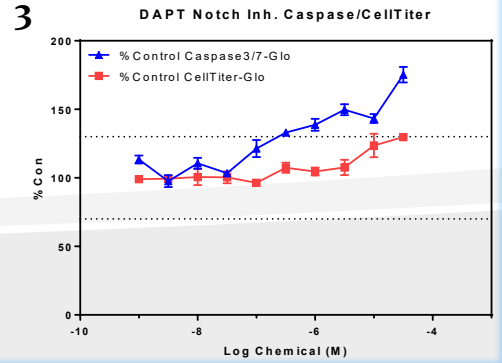

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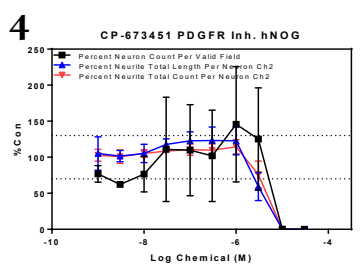

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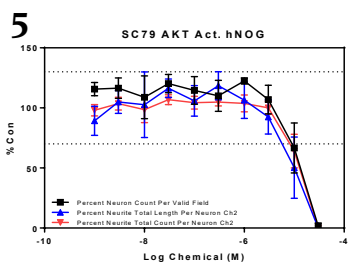
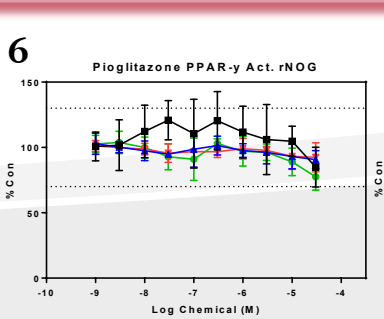


6



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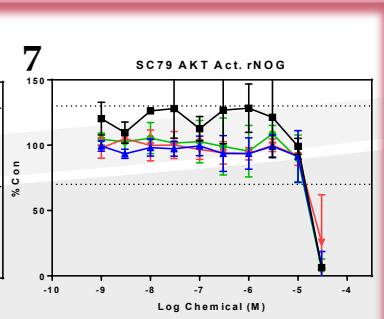
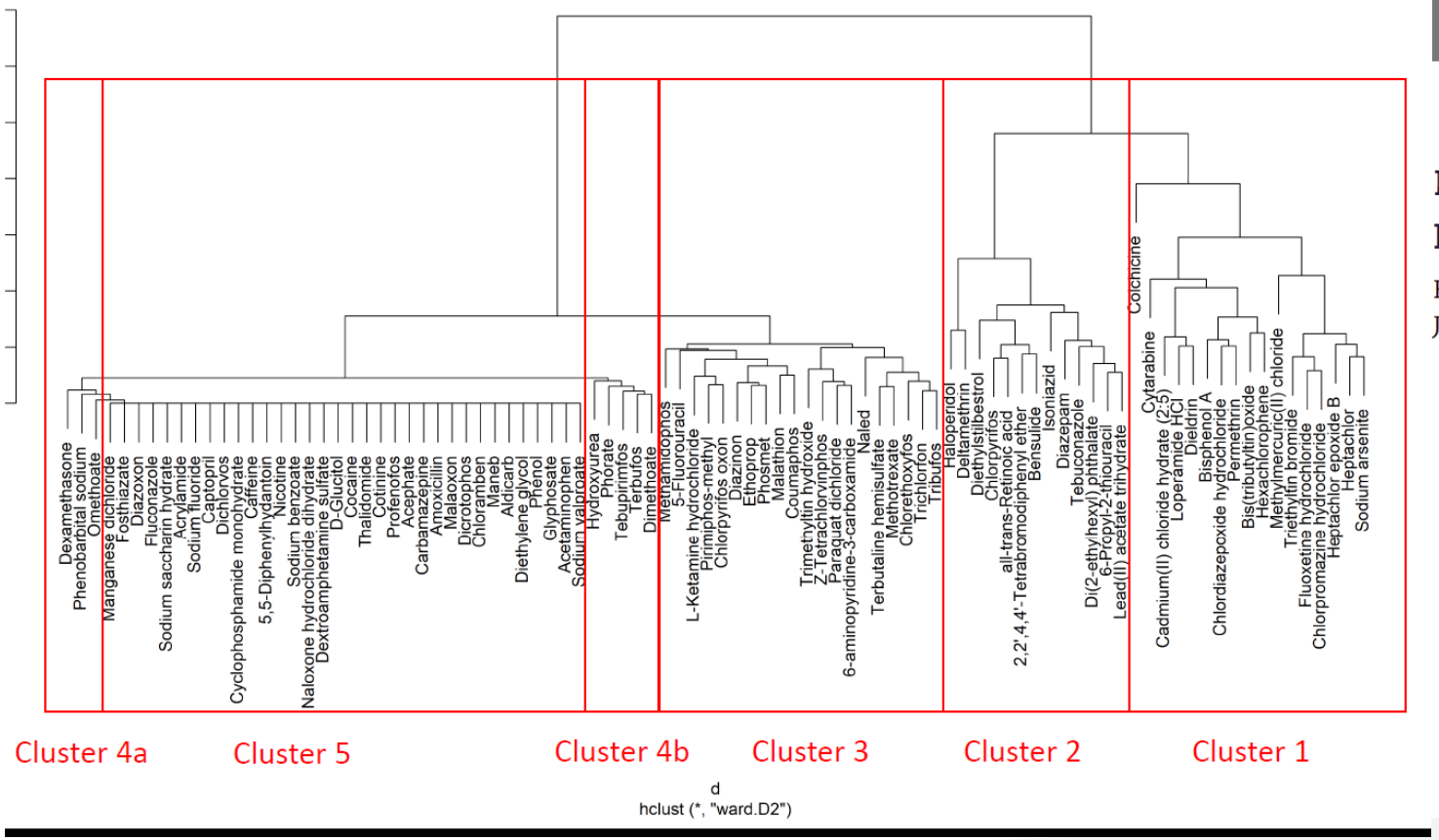


Performance of the assays- False Negatives



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Research article

Integrating Data From *In Vitro* New Approach Methodologies for Developmental Neurotoxicity

Kelly E. Carstens,*,† Amy F. Carpenter,*,† Melissa M. Martin,* Joshua A. Harrill,* Timothy J. Shafer,* and Katie Paul Friedman*,1

Sensitivity:

93% including cytotoxicity
74% using only selective hits

But…..14/53 putative positive compounds clustered in the “inactives” space (Cluster 5)

Reasons for a “false negative” response

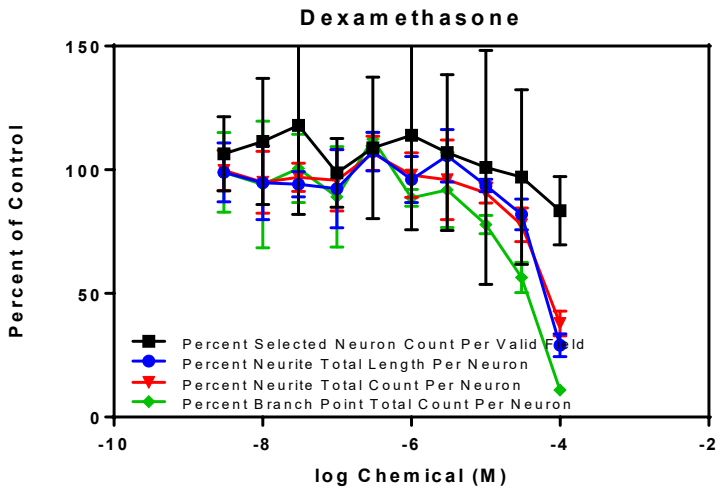
- Testing at too low of a concentration
- Chemical instability/insolubility/wrong solvent
- Need for metabolism
- Testing of less active or inactive enantiomers
- Lack of necessary biology (e.g. critical receptor not expressed in the assay)

Goal: Re-screen 19 false negative compounds in the EPA assays

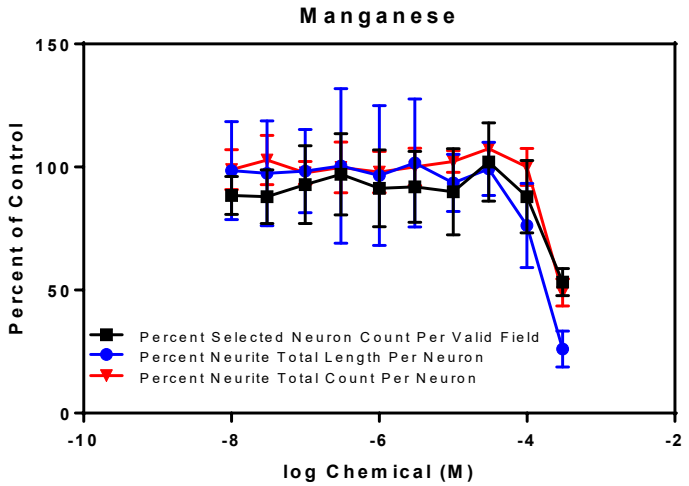
- Understand underlying reason for negative response.

Preliminary data

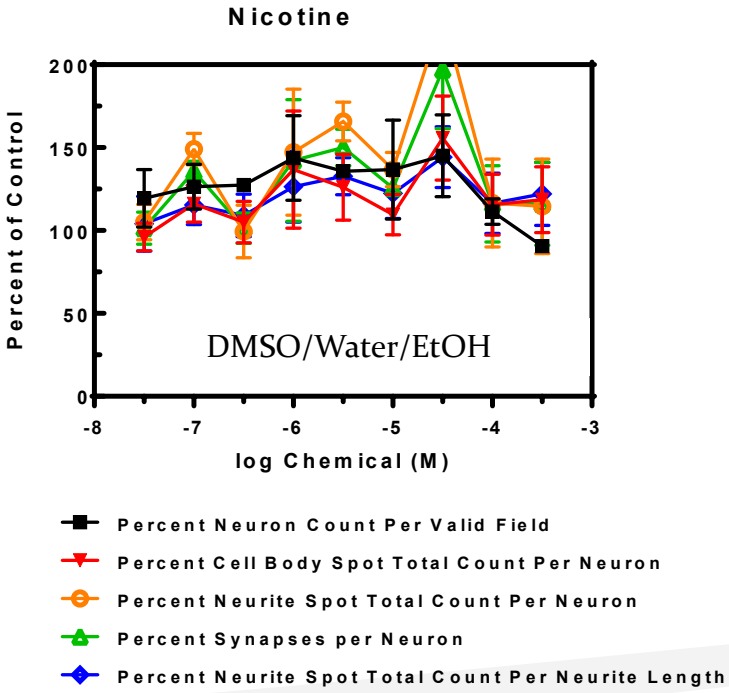
NOG Primary Cortical cells



NOG CDI Igluta cells



Synaptogenesis (rCort)



Overall Summary

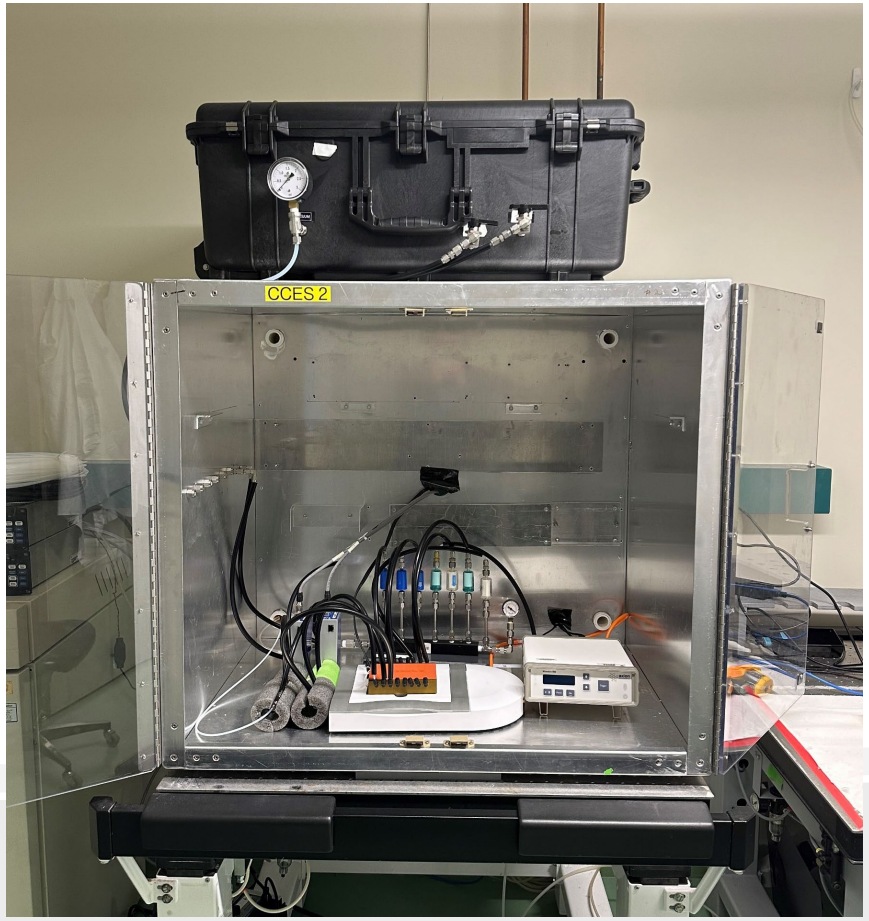
Solvent	Chemical Name	µM	NFA	Nog	Synap	NOG	Cyto	Apop		Overall
DMSO	5,5-Diphenylhydantoin	1000	Y	Y	N	N	?	?		Y
DMSO	Dexamethasone	100	Y	Y	N	N?	?	N	Old	Y
DMSO	Dexamethasone	100	Y	Y	N	Y	N	N	New	Y
DMSO	Nicotine	300	N	Y (cyto)	N	N	N	N		Y
DMSO	Maneb	10	Y	Y (cyto)	N	Y	N	N		Y
DMSO	Thalidomide	100	N	N	N	N	N	N		N
DMSO	Phenobarbital sodium	100	Y	N	N	N	N	N		Y
DMSO	Carbamazepine	100	N	N	N	N	N	N		N
Media	Sodium valproate	10000	Y	Y	Y	Y	N	N		Y
Water	Cocaine	100	N	Y	N	N	N	N		Y
Water	L-Ketamine	100	Y	N	N	N	N	N		Y
Water	Terbutaline hemisulfate	100	N?	N	N	N	N	N		N
Water	Caffiene	300	N	N	N	N	N	N		N
Water	Dextroamphetamine sulfate	100	Y	Y (cyto)	N	N	N	N		Y
Water	Acrylamide	300	Y	Y (cyto)	Y	N	N	N		Y
Water	Hydroxyurea	300	Y	Y (cyto)	Y	?	Y	Y		Y
Water	Sodium fluoride	300	N	Y (cyto)	Y	N	N	N		Y
Water	Manganese dichloride	300	Y	Y (cyto)	Y	Y	N	N		Y
Water	Naloxone hydrochloride dihydrate	100	N	Y (cyto)	N	N?	N	N		Y
Water	Cyclophosphamide monohydrate	100	N?	Y (cyto)	Y	N?	N	N		Y

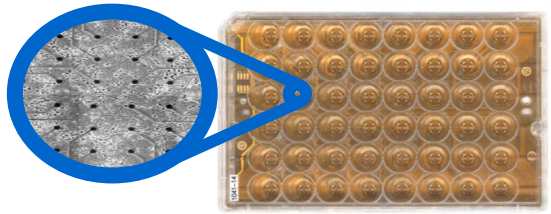
15/19 “False Negatives” appear to have some biological activity upon retesting with fresh solutions, different enantiomers and higher concentrations.

This should improve the sensitivity of the battery

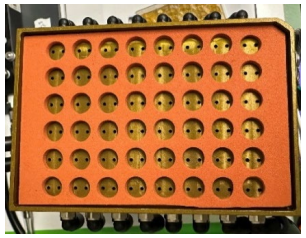
Screening volatile compounds for neuroactivity

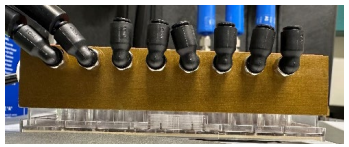
Volatile compounds are not amenable to *in vitro* screening because they have low solubility in aqueous solution and evaporate rapidly, especially at 37 °C



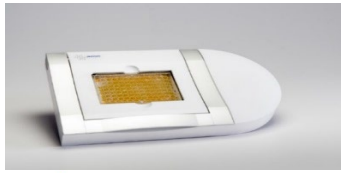


48-Well MEA plate with primary cortical neural networks growing on electrodes

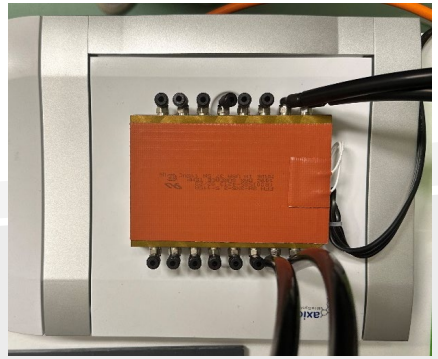




Manifold with input and output vents for gas exchange



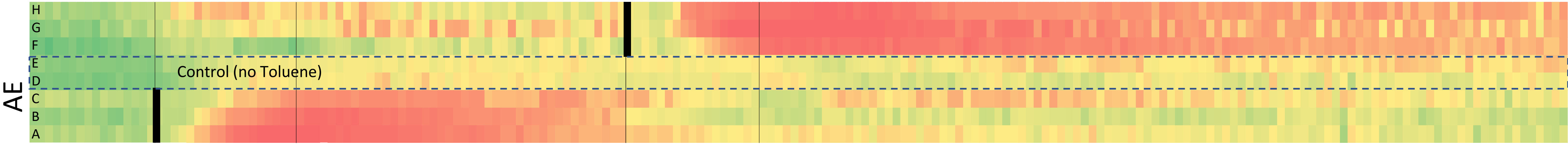
Decreasing Volumes should lead to quicker equilibrium between gas and media



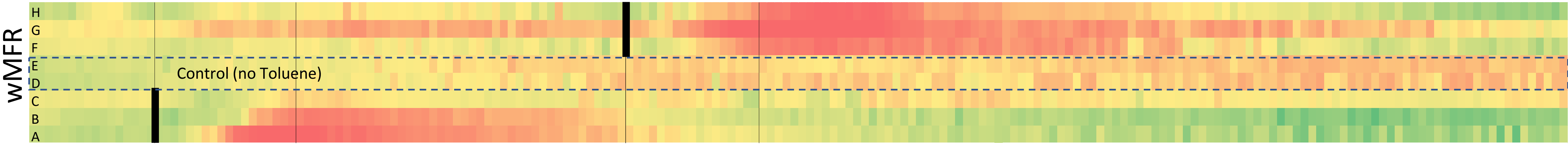
Manifold sits on top of MEA plate in the Maestro amplifier

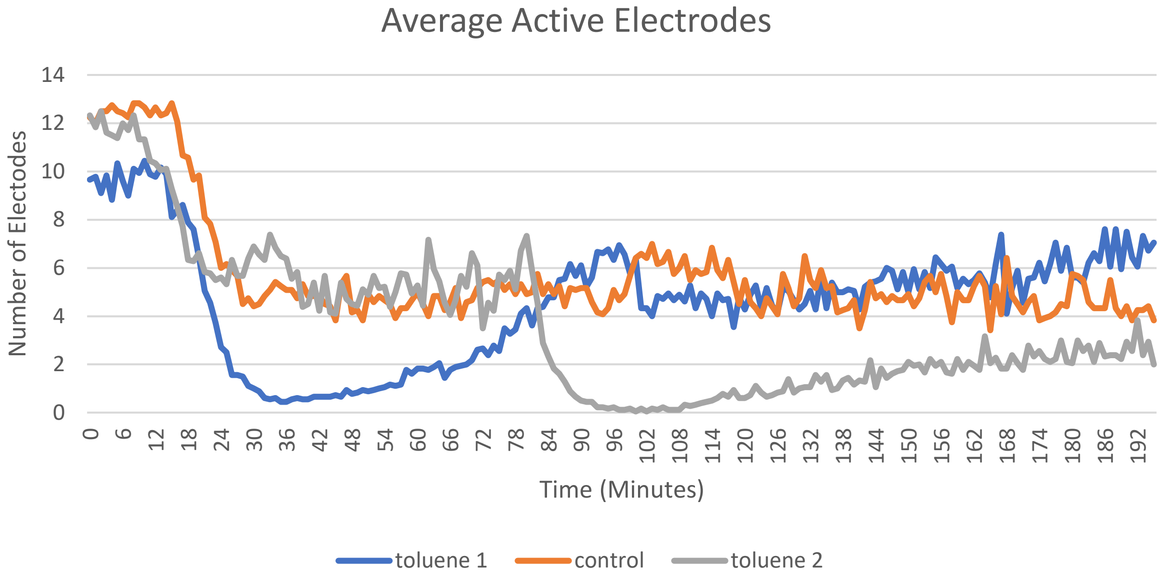
Proof-of-concept: Toluene

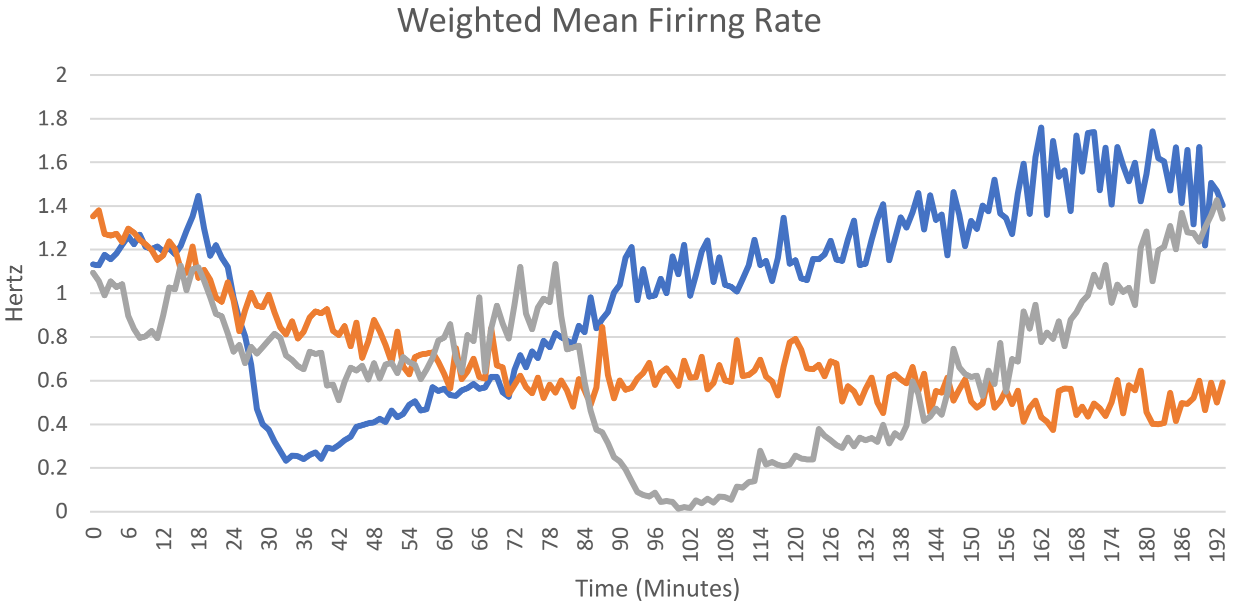
A: 100uL	B: 100uL	C: 100uL	500uL	500uL	F: 100uL	G: 100uL	H: 100uL
A: 100uL	B: 100uL	C: 100uL	500uL	500uL	F: 100uL	G: 100uL	H: 100uL
A: 100uL	B: 100uL	C: 100uL	500uL	500uL	F: 100uL	G: 100uL	H: 100uL
A: 100uL	B: 100uL	C: 100uL	500uL	500uL	F: 100uL	G: 100uL	H: 100uL
A: 100uL	B: 100uL	C: 100uL	500uL	500uL	F: 100uL	G: 100uL	H: 100uL
A: 100uL	B: 100uL	C: 100uL	500uL	500uL	F: 100uL	G: 100uL	H: 100uL



Baseline 0 - 16 min	Toluene Flowing 20000 ppm **A-C** 17 - 34 min	Air Flow A-C 35 - 76 min	20000 ppm Tol Flowing **F-H** 76 - 93 min	Air Flow A-C, F-H 93 – 196 min







Thank you!
Questions?

EPA ORD Colleagues:
- Kathleen Wallace
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Students:
- Carmen Marable (ORISE)
- Amy Carpenter (ORISE)
- Hunter Fitzpatrick (ORISE)
- Jessica Conley (ORISE)
- Olivia Rice (ORISE)
- Gabby Byrd (ORISE)

EPA Program Office Colleagues
- Anna Lowit
- Liz Mendez
- Monique Perron
- Sarah Dobreniecki
- Mike Metzger

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- Ellen Fritsche (IUF)
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