The Developmental Neurotoxicity (DNT) In Vitro Test Battery (DNT-IVB)

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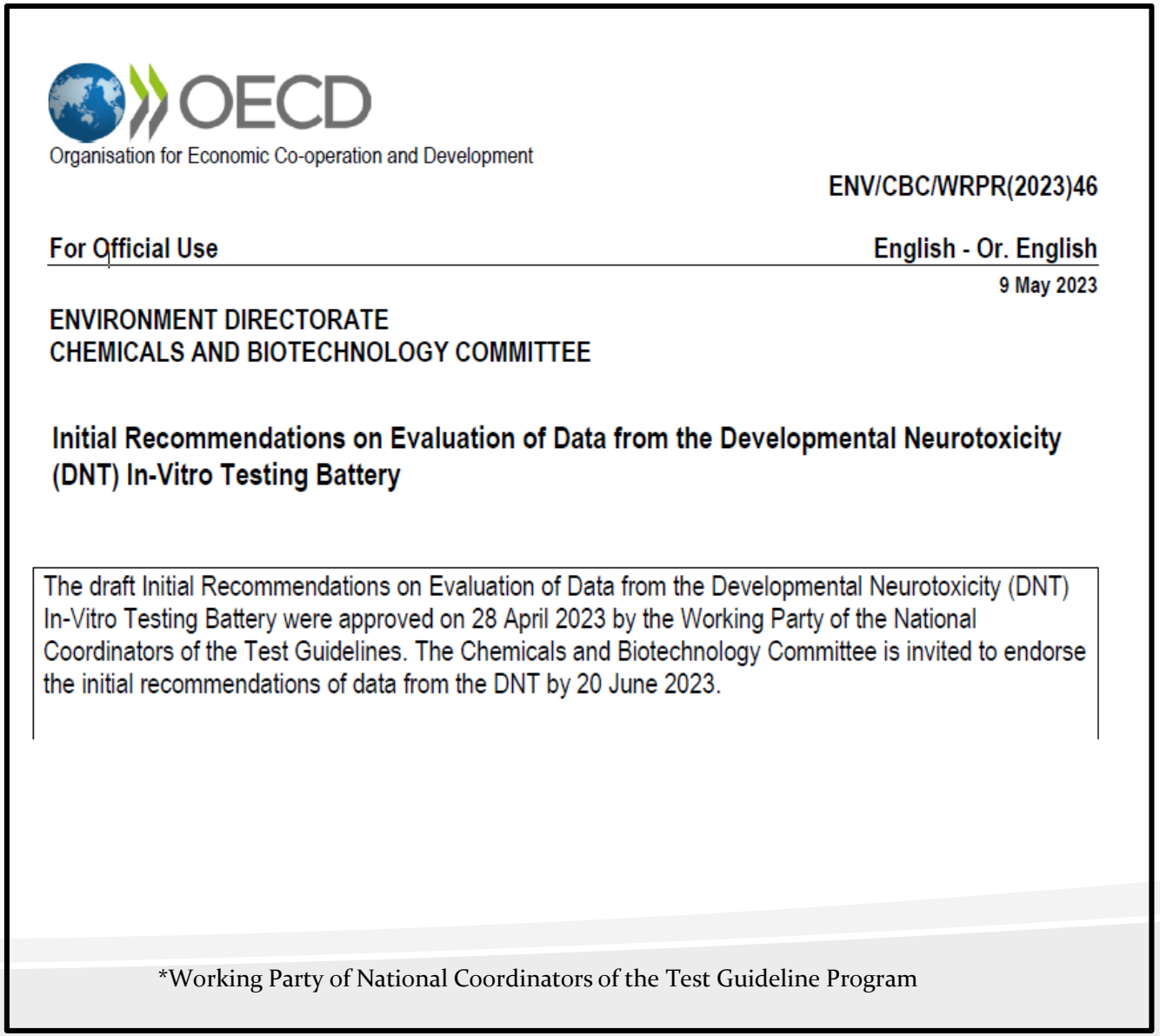

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ENV/CBC/WRPR(2023)46

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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

Towards regulatory DNT testing: Alternative methods

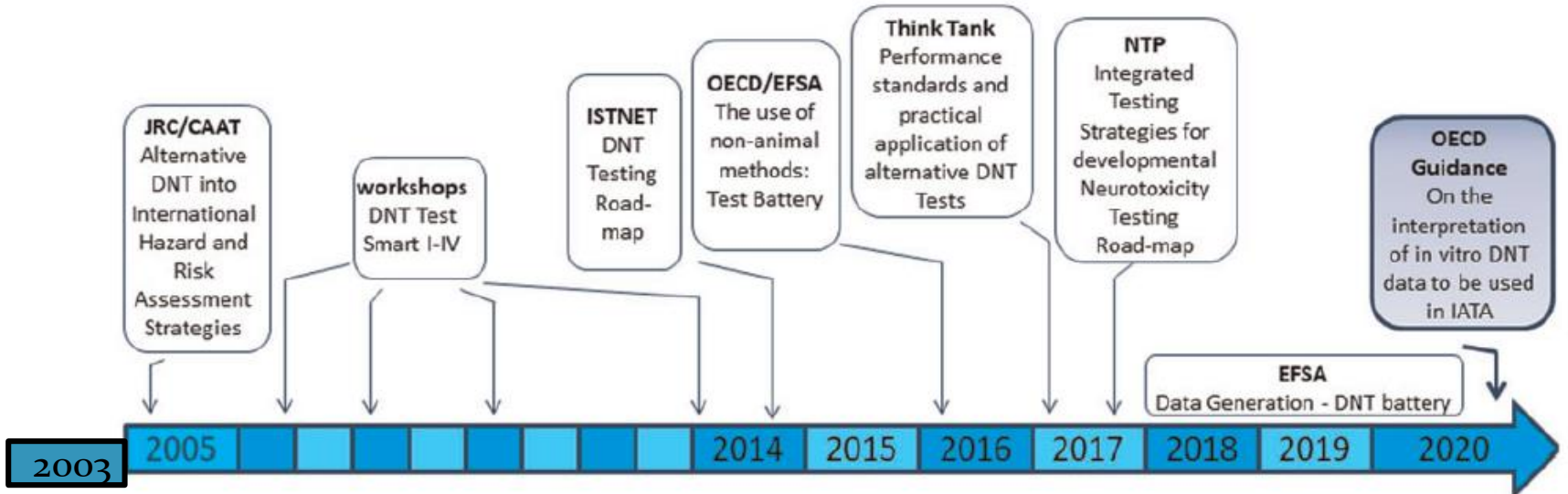

Figure 1. Timeline of efforts to develop and implement new alternative methods for developmental neurotoxicity.

Toxicology Forum

Research

Meeting Report: Alternatives for Developmental Neurotoxicity Testing

Pamela Lein,[1,2] Paul Locke,[1*] and Alan Goldberg[1]*

[1]Department of Environmental Health Sciences and Center for Alternatives to Animal Testing (CAAT), Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland, USA; [2]Center for Research on Occupational and Environmental Toxicology, Oregon Health & Science University, Portland, Oregon, USA

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In vitro and other alternative app
neurotoxicity test

Pamela Lein[a,b,*], Ellen Silbergeld[a], Pa

Research

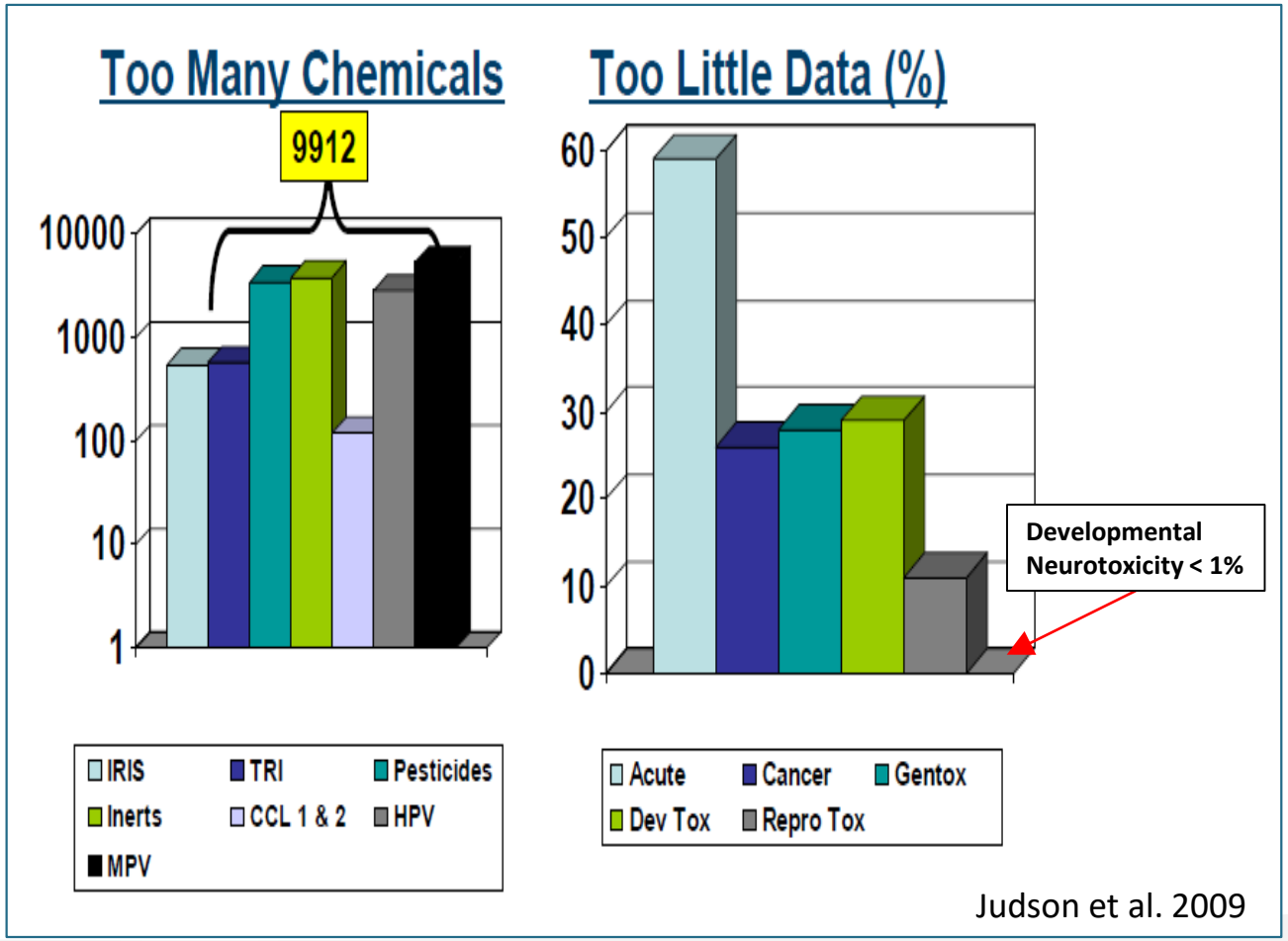
Workgroup Report: Incorporating *In Vitro* Alternative Methods for Developmental Neurotoxicity into International Hazard and Risk Assessment Strategies

Sandra Coecke,[1] Alan M Goldberg,[2] Sandra Allen,[3] Leonora Buzanska,[1,4] Gemma Calamandrei,[5] Kevin Crofton,[6] Lars Hareng,[1] Thomas Hartung,[1] Holger Knaut,[7] Paul Honegger,[8] Miriam Jacobs,[1] Pamela Lein,[9] Abby Li,[10] William Mundy,[6] David Owen,[11] Steffen Schneider,[12] Ellen Silbergeld,[2] Torsten Reum,[13] Tomas Trnovec,[14] Florianne Monnet-Tschudi,[8] and Anna Bal-Price[1]

Overview

I. Introduction to the problem
II. The DNT *in vitro* battery (DNT-IVB)
III. Establishing Confidence in the DNT-IVB
IV. Using the Battery: Case-studies
V. Future Directions

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

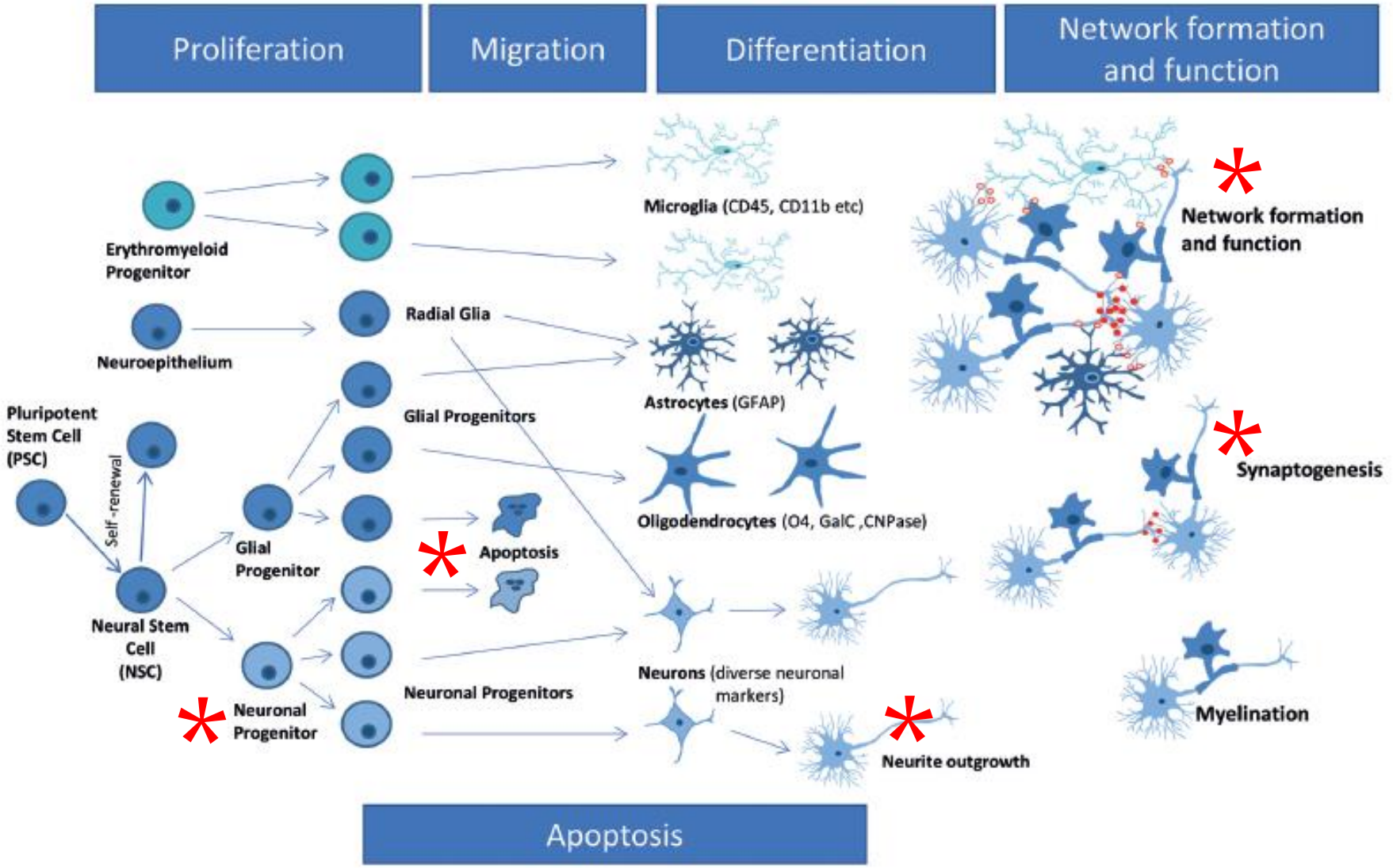
Current testing is too slow; "Guideline" DNT:

- 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.

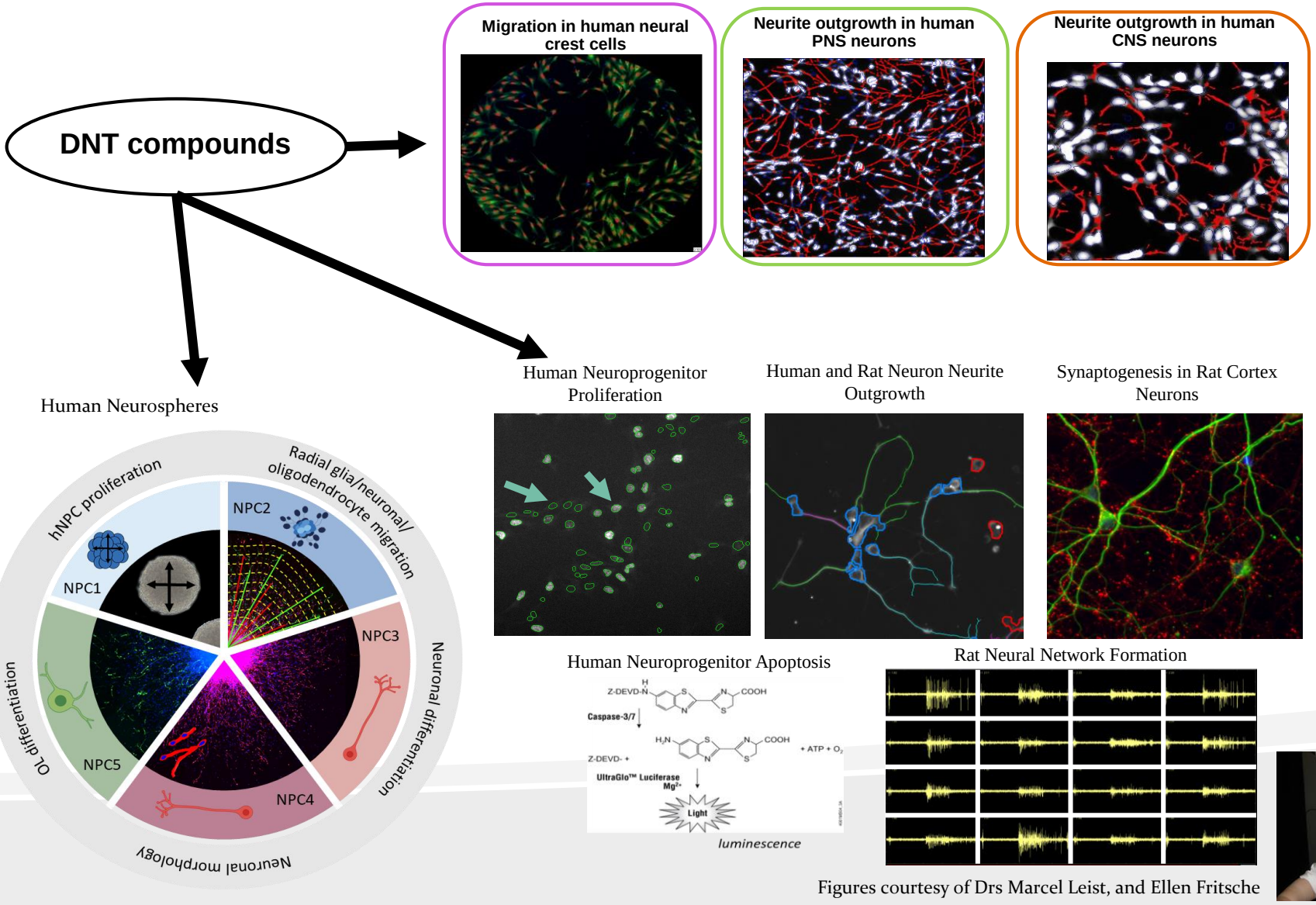
*Raffaele et al. The use of **developmental neurotoxicity** data in pesticide risk assessments. Neurotoxicol Teratol. 2010 Sep-Oct;32(5):563-72.

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



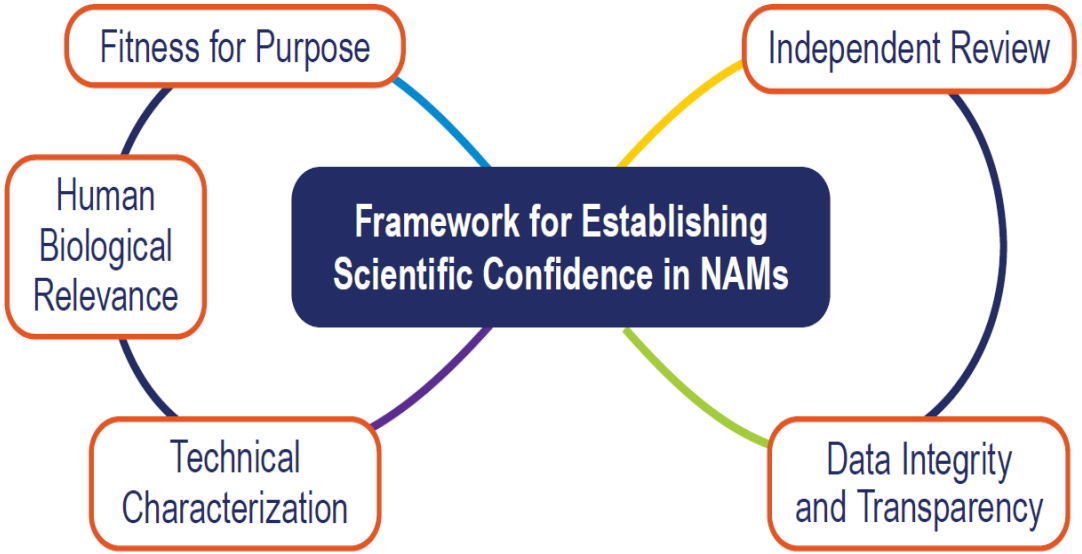
From Bal-Price et al. 2018 ALTEX. 2018;35(3):306-352. doi: 10.14573/altex.1712081

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



Figures courtesy of Drs Marcel Leist, and Ellen Fritsche

Establishing Confidence in the Assays



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

Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies

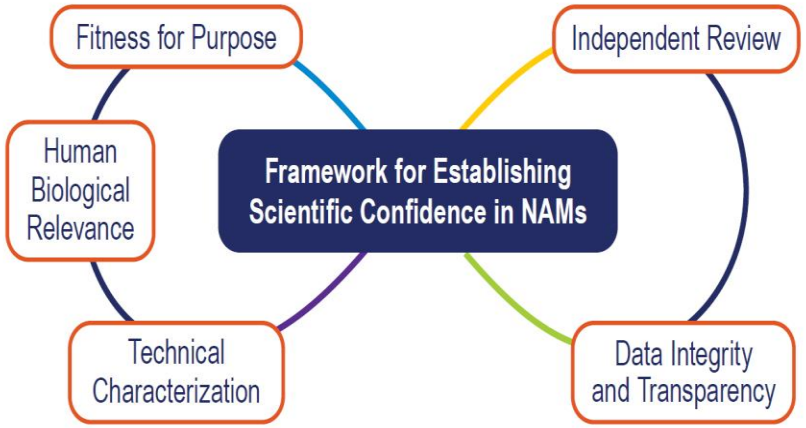
A Report of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Validation Workgroup

2023

Assay Inclusion in the Battery:

- Deemed ready for use in screening and prioritization (Fritsche et al. 2017; Bal-Price et al. 2018)
- 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)

Establishing Confidence in the Assays: Independent Review



All assays in the battery have been described in the peer-reviewed literature.

The Developmental Neurotoxicity Battery- DNT-IVB

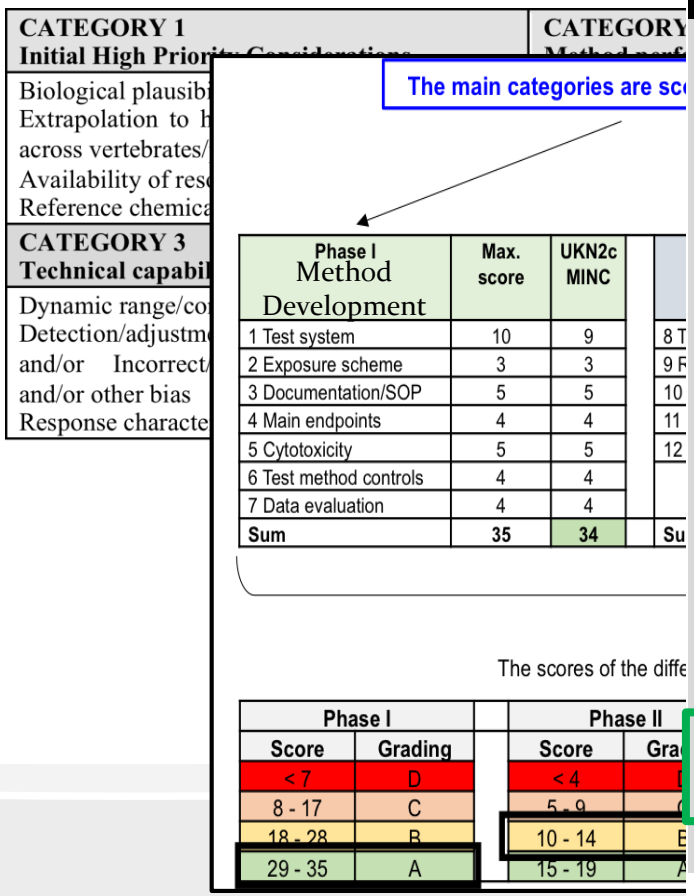
Table 2. Proposed Assays for Evaluation As an *In Vitro* DNT Battery

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)

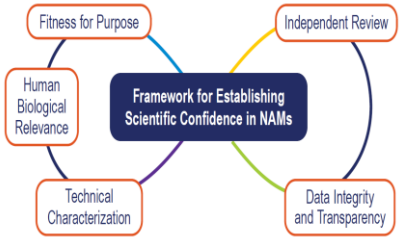
From Sachana et al., Toxicol Sci. 2019 Jan 1;167(1):45-57. doi: 10.1093/toxsci/kfy211

Establishing Confidence in the Assays: Independent Review

Ranking Parameters



Readiness/ Test method	Phase I	Phase II	Phase III	Overall readiness
UKN1	A	B	B	B+
NPC1	A	A	A	A
NPC2	A	A	A	A
NPC3	A	A	B	A-
NPC4	A	B	C	B
NPC5	A	A	B	A-
NPC6	A	B	B	B+
UKN2 (cMINC)	A	B	A	A-
MESn	C	D	D	D+
UKN4 (NeuriTox)	A	A	A	A
NSR	C	D	D	D+
SYN	B	B	B	B
Nnff	B	A	B	B+
3Dh	B	C	C	C+



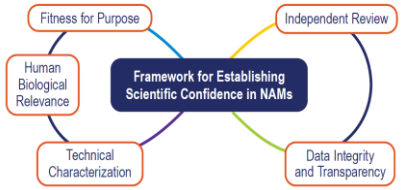
Phase I: Method Development

Phase II: Performance & Replicability

Phase III: Screening

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

Review of DNT *in vitro* battery



OECD WNT* accepts the inclusion of the DNT Project in its workplan

1st Draft of Guidance Document July 2021

Sept- EPA SAP† review of DNT-IVB

Expert Group comments

2nd Draft

WNT Comments& Revisions 3 rounds

April 2023-WNT approval

2017 | 2018 | 2019 | 2020 | 2021 | 2022 | 2023

Release of DNT-IVB data in ToxCast

OECD
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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,*]

*Working Party of National Coordinators of the Test Guideline Program

†SAP=Scientific Advisory Panel (an external review board)

Review of DNT *in vitro* battery

Consensus

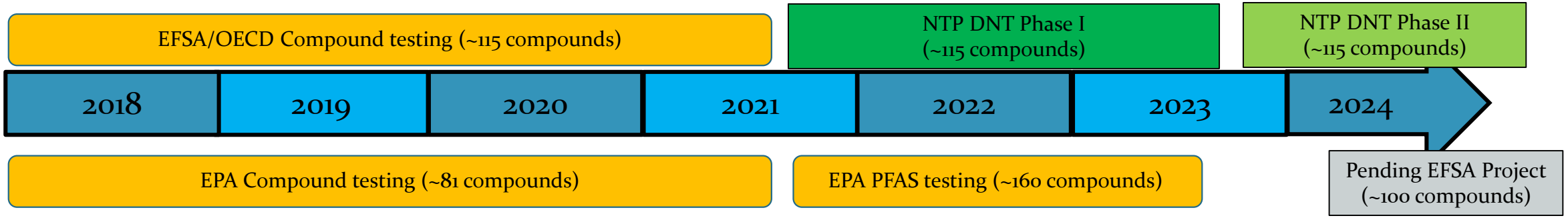
All three reviews of the DNT IVB agreed that it could be used for:

- Screening and Prioritization
- Weight of Evidence Decision-Making

and

- The battery should be a “living process” that should evolve

DNT *in vitro* battery: Compound Testing

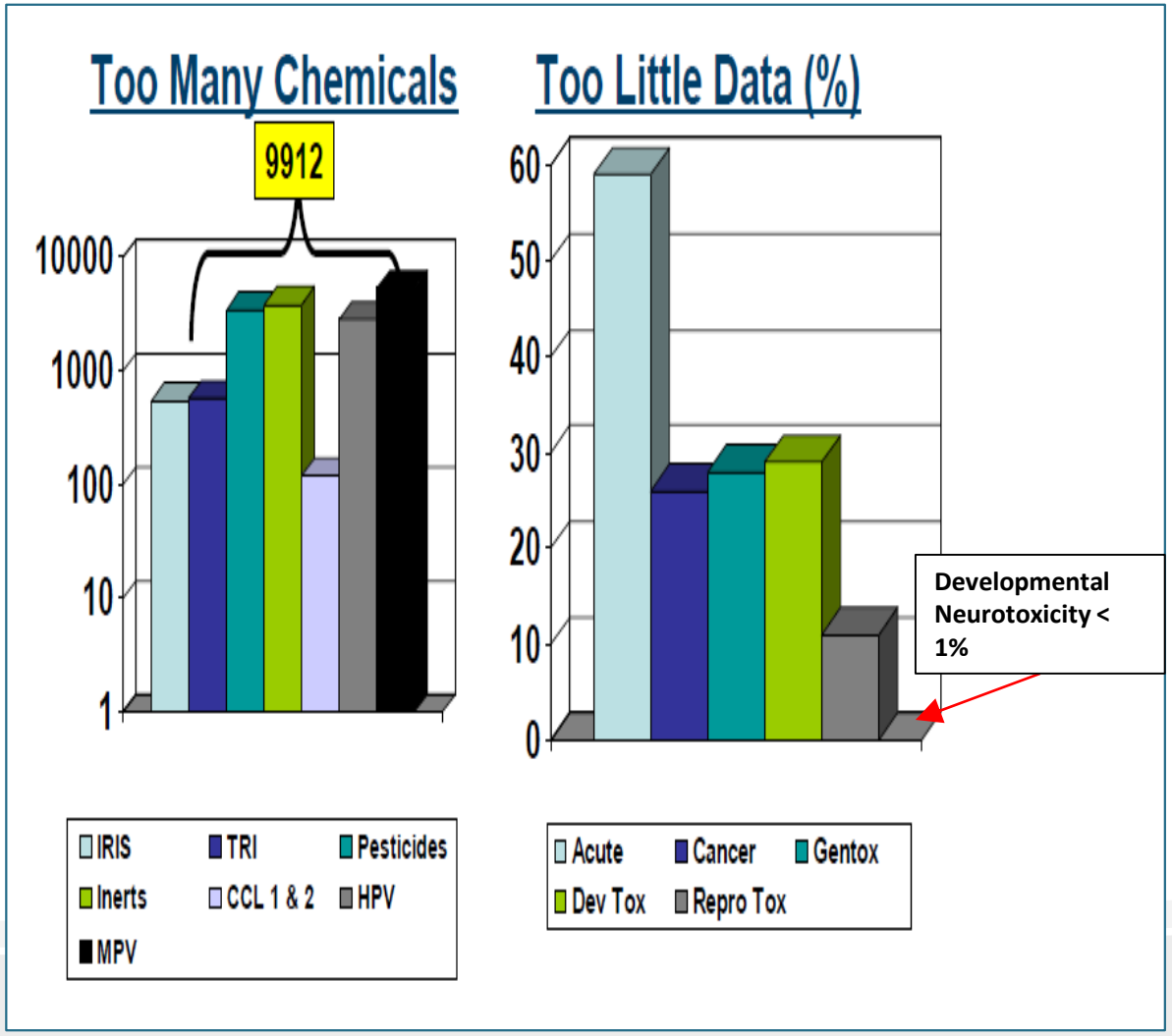


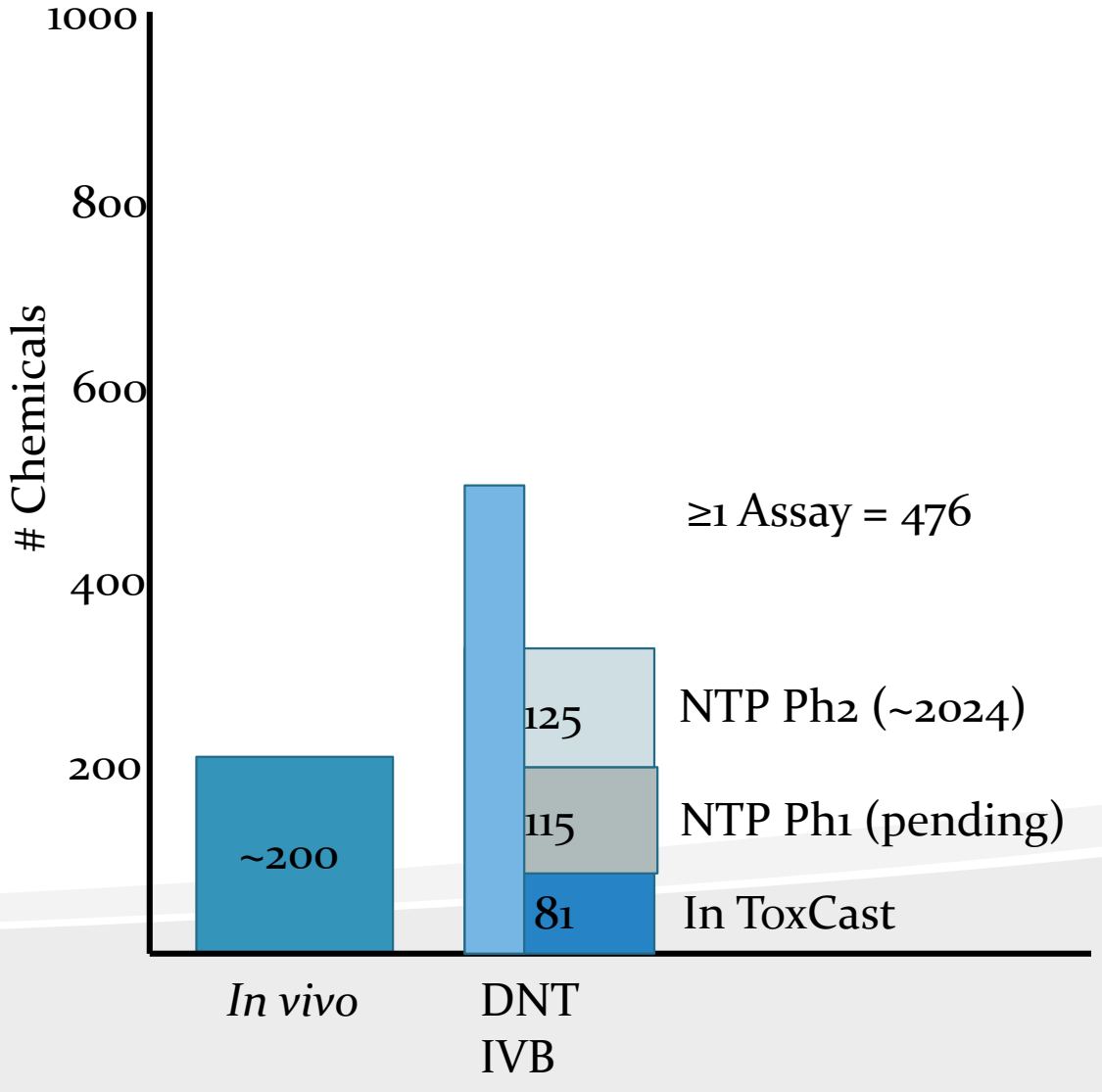
Testing has focused on:

- "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 invitrodb
- Tested, data pending in invitrodb
- Testing initiated
- Testing in planning process

Have we made any progress?





DNT NAMs are providing data

Is that data being used?

Screening Level Information for 160 PFAS Compounds

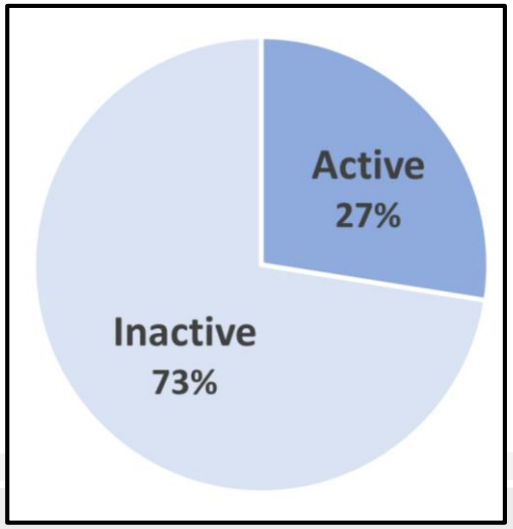
- Structurally diverse group of chemicals
- Little *in vivo* toxicological information on DNT
- DNT evidence is conflicting
  - epidemiological studies are equivocal
  - neurodevelopmental effects associated with exposure to PFAS in rodent and other animal studies

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



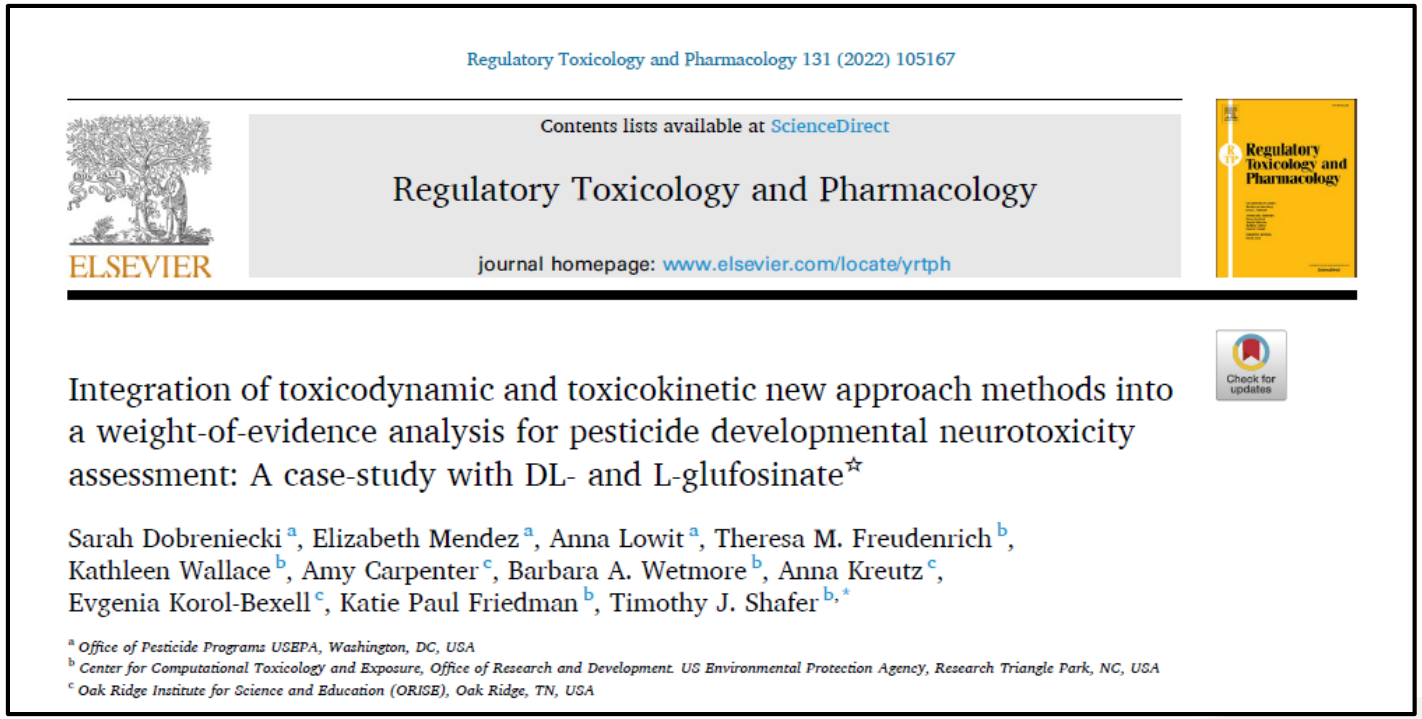
- Out of a set of 160 PFAS, 118 were inactive, leaving 42 active PFAS that decreased measures of neural network formation, neurite outgrowth, proliferation, or apoptosis
- 24 PFAS demonstrate moderate or low selective activity

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

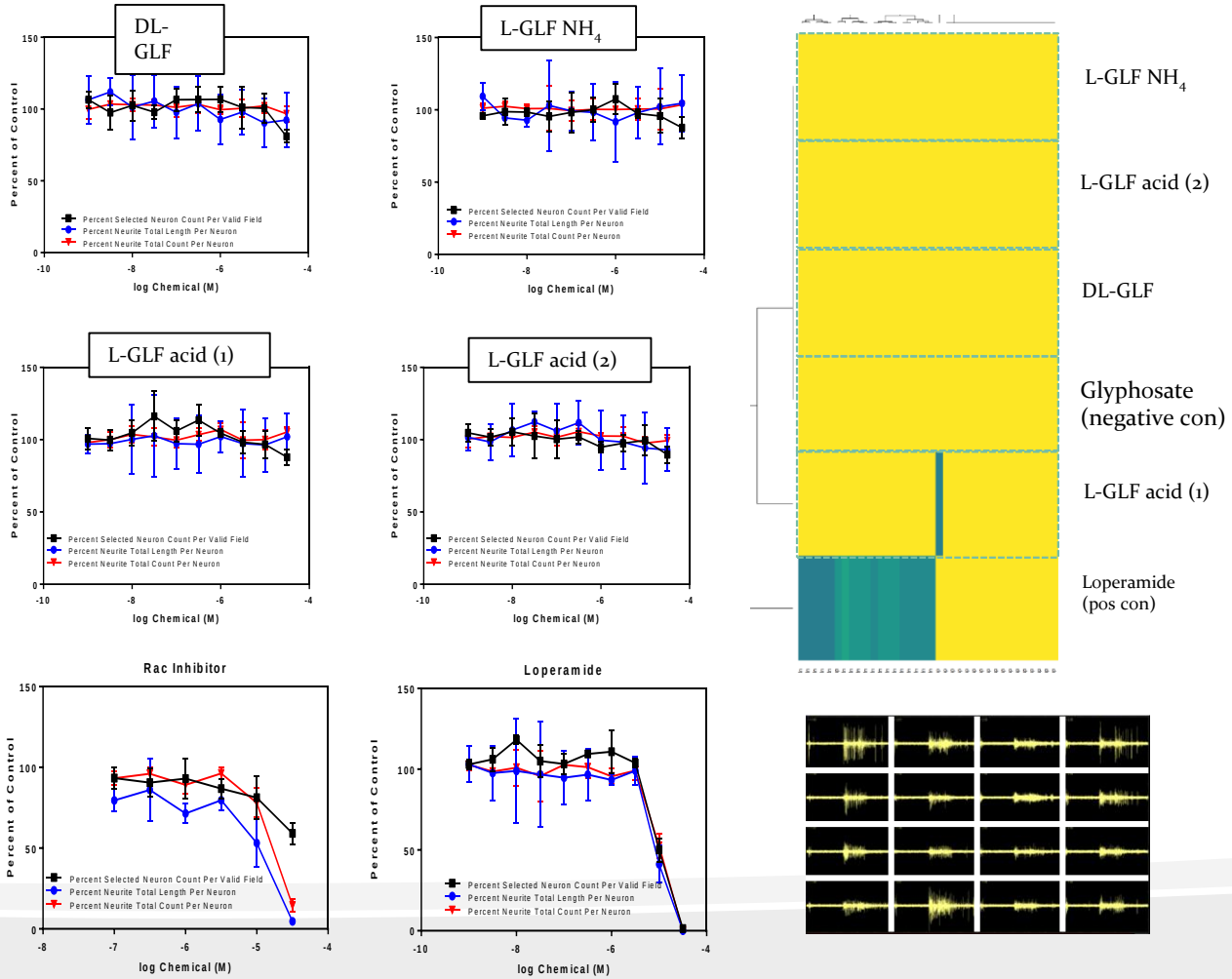
journal homepage: www.elsevier.com/locate/yrtph

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)

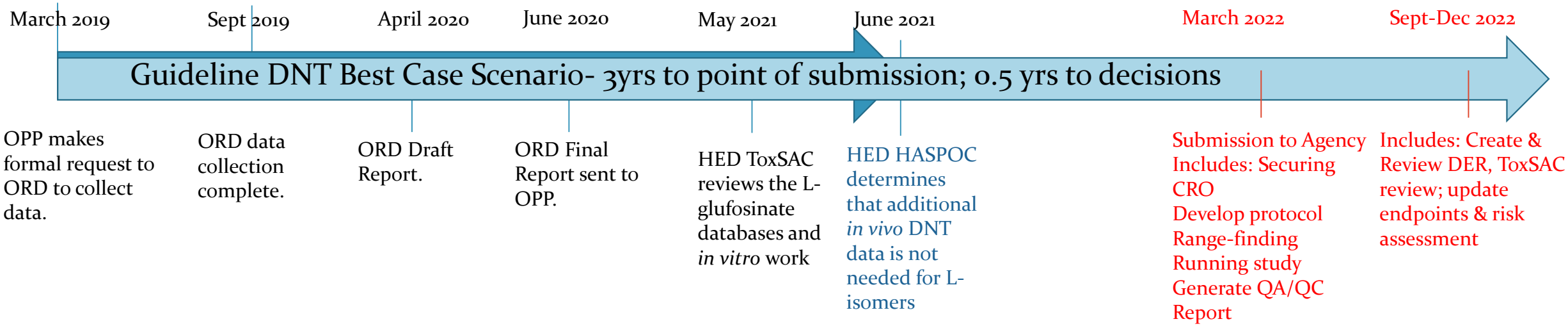
- Tested concentrations *in vitro* > PODs selected for L-glufosinate risk assessment

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)

Other Case Studies

Year	No.	Title	Key words provided by the authors	AOPs (When a case study includes a AOP that has a AOP-Wiki No., the AOP-Wiki No. is listed.)	Other AOP wiki number	Uncertainty reporting	NAMs
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	• In vitro developmental neurotoxicity testing battery (DNT-IVB) • Pyrethroids • In vivo developmental neurotoxicity study	X		X	X
2021	2	Case study for the integration of in vitro data in the developmental neurotoxicity hazard identification and characterisation using flufenacet	• In vitro developmental neurotoxicity testing battery (DNT-IVB) • Flufenacet • In vivo developmental neurotoxicity study	X		X	X
2021	3	Case study on the use of Integrated Approaches for Testing and Assessment for DNT to prioritize a class of Organophosphorus flame retardants	• DNT – developmental neurotoxicity • Prioritisation • Flame retardants • Zebrafish	X		X	X
2021	4	Case Study on the use of Integrated Approaches for Testing and Assessment for developmental neurotoxicity hazard characterisation of acetamiprid		X		X	X
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		X		X	X

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

Future Directions

Identified Gaps in the DNT-IVB

Consensus:

- Important neurodevelopmental processes not well covered
  - Glia, microglia, neurovascular unit, plasticity
- Interlaboratory transferability not demonstrated
- Tiered testing strategy lacking

Summary and Conclusions

- Over the past 15 years a battery of *in vitro* DNT NAMs has been developed

- There is consensus that this DNT-IVB is ready for use in decisions regarding:
  - Screening and prioritization
  - Weight of Evidence

- Several Case-Studies using DNT-NAMs now exist

- These case-studies demonstrate that data from the DNT-NAMs can:
  - Speed decision making
  - Reduce costs
  - Contribute to health protective decisions.

- There is consensus that the science behind DNT NAMs will continue to evolve and improve.

Thank you!
Questions?

EPA ORD Colleagues:
- Kathleen Wallace
- Theresa Freudenrich
- Bill Mundy (retired)
- Kevin Crofton (retired)
- Josh Harrill
- Jasmine Brown
- Katie Paul Friedman
- Melissa Martin
- Kelly Carstens
- Megan Culbreth
- Gabby Byrd
- Amy Carpenter (ORISE)
- Seline Choo (ORISE)
- Richard Judson
- Grace Patlewicz

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

International Collaborators
- Ellen Fritsche (IUF)
- Marcel Leist (U. Konstantz)
- Andrea Terron (EFSA)
- Iris Mangas (EFSA)

OECD
- Magda Sachana