In vitro assays to identify developmental neurotoxicity hazard:
Promises and challenges

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Disclosure Statement

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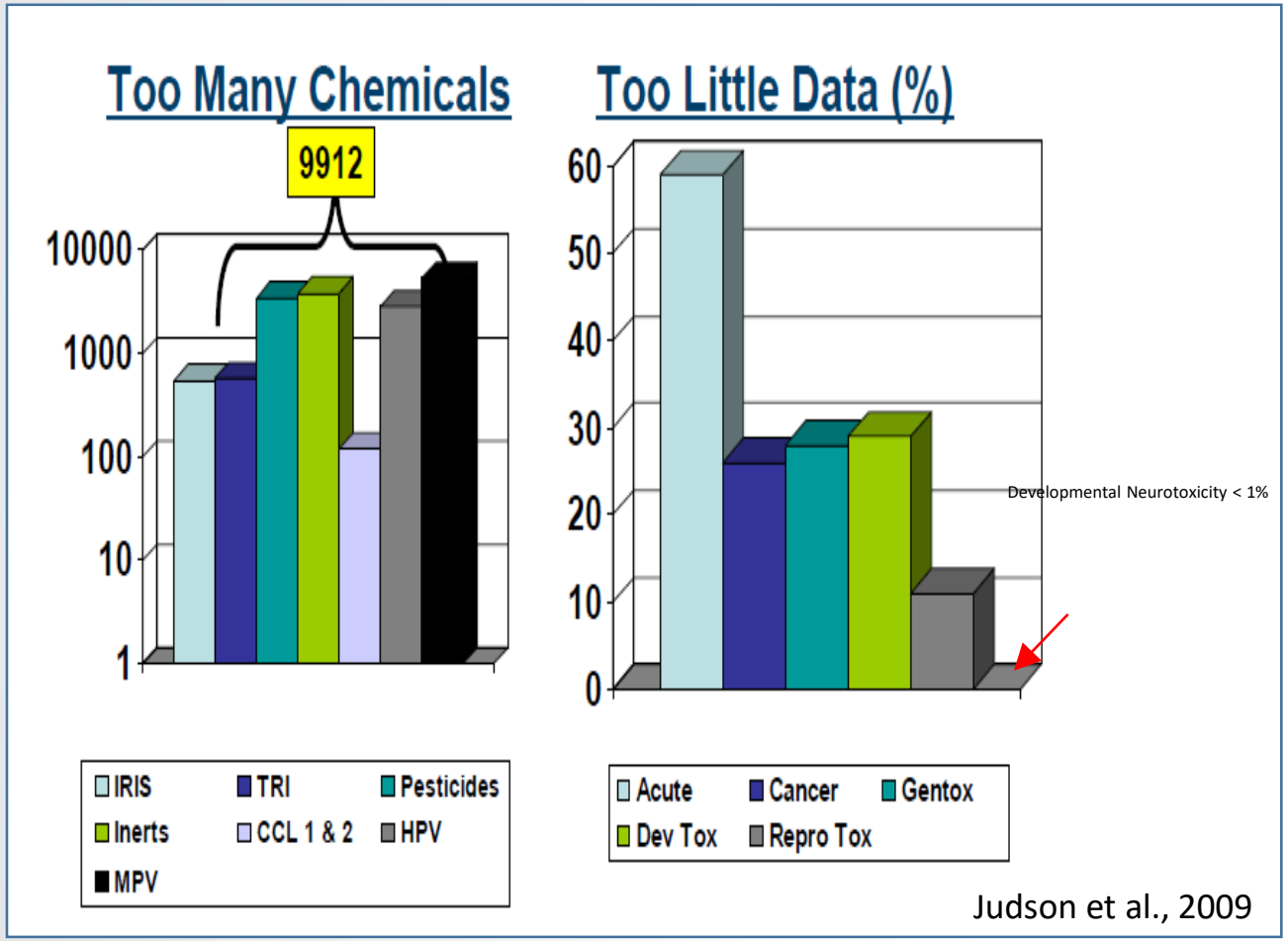
Many Chemicals Lack Developmental Neurotoxicity (DNT) Data



Judson et al., 2009

Current testing too slow

- Not Required under FIFRA
- Animal "Guideline" DNT; 1 chemical, $1M cost; 2 yr
- At current pace, ~150 chemicals in 20+ yrs
- Not often used (~25%) for point of departure values for risk assessment*

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

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

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

Solution: Faster, inexpensive and predictive methods are needed to detect and characterize compounds with developmental neurotoxicity hazard

- Develop high throughput, *in vitro* assays,
- Characterize chemicals for developmental neurotoxicity hazard
- Data from these assays can provide information for decision-making

International Efforts to Develop Alternatives for DNT Guideline Studies

- European Food Safety Organization
  - Funding research to develop and evaluate a battery of in vitro DNT assays
- Danish EPA
  - Supporting evaluation of DNT alternatives
  - Combination of structural and functional endpoints
  - Qualification of primary hits by secondary testing (same assay; and hit confirmation testing using an alternative assay)
  - Integration of dosimetry to improve hit prediction from screening results
- US EPA
  - Internal research on development of alternatives to DNT Guideline
    - Focus on Screening and Prioritization
- National Toxicology Program (NTP, National Institutes of Environmental Health Sciences (NIEHS))
  - Evaluating alternatives as a decision tool to best utilize limited resources for in vivo testing of nominated chemicals
  - Provided compounds for testing to a number of laboratories;
  - Built an interactive database (DNT DIVER) to house data and facilitate utilization of data for decision-making
- Organization for Economic Cooperation and Development (OECD)
  - DNT Expert Group

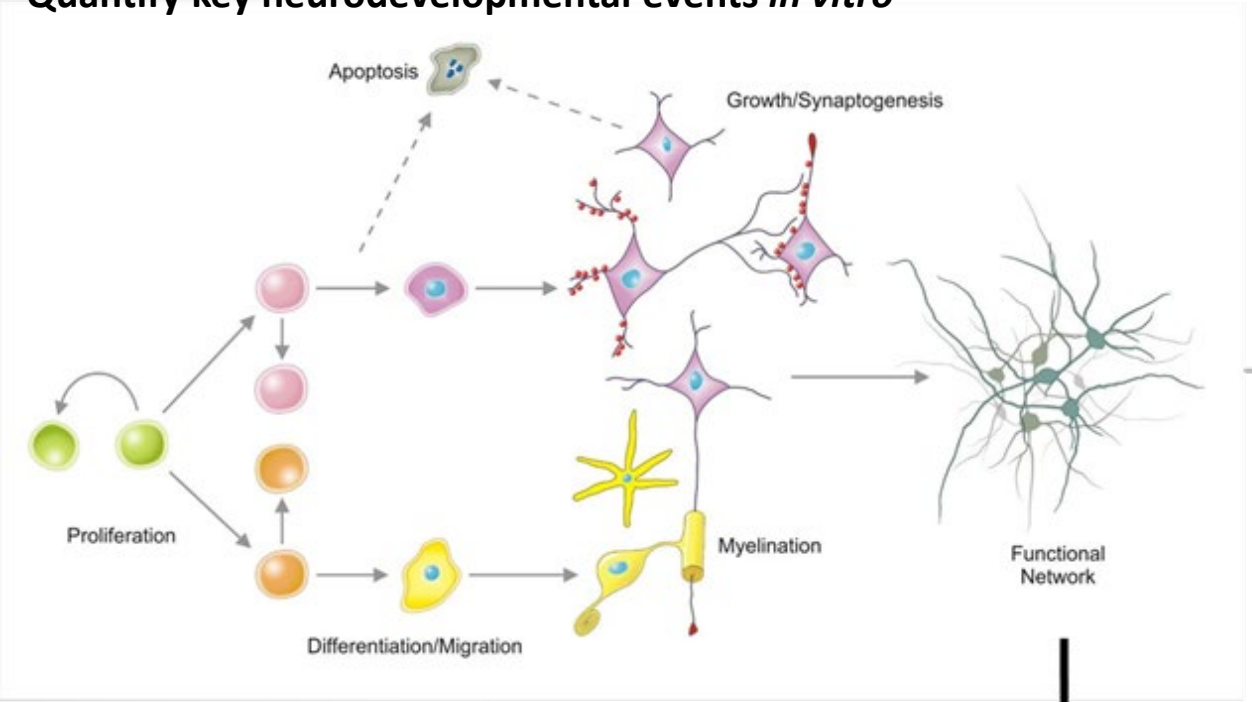
Challenges to Development of DNT Screens

- Central nervous system development is complex
  - Multiple potential targets
  - Time-dependent processes
  - Spatially dependent processes
- Which target? Where? When?

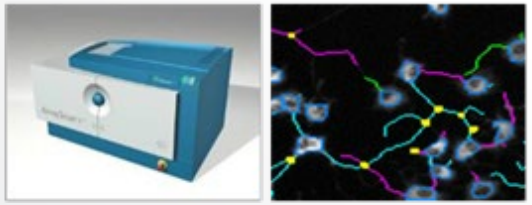
Research focus on *key neurodevelopmental processes*

Phenotypic Screening for DNT Hazard

Quantify key neurodevelopmental events *in vitro*



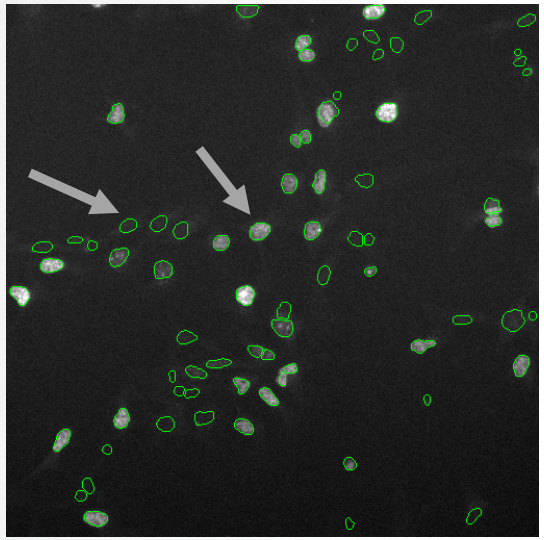
Key Neurodevelopmental Events (High Content Imaging)



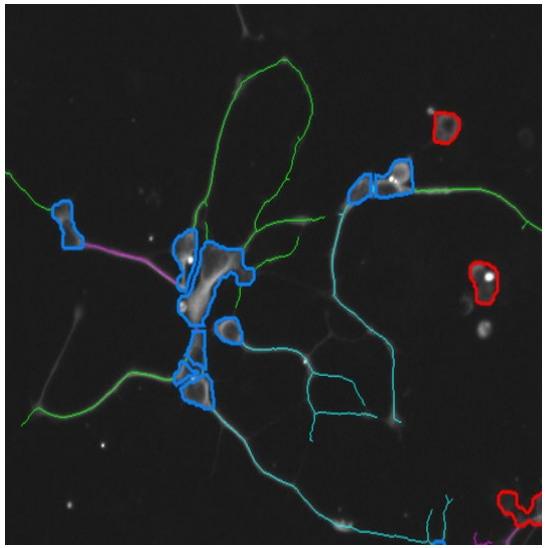
Network Formation (Multi-Electrode Array)

EPA Assays

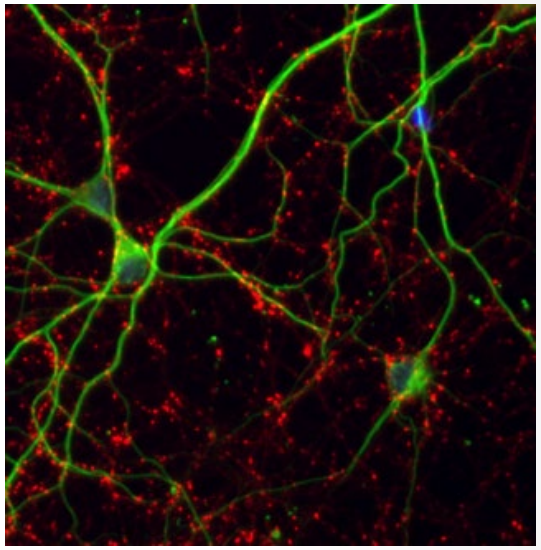
Proliferation



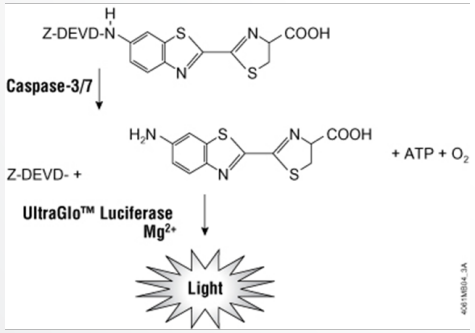
Neurite Outgrowth



Synaptogenesis

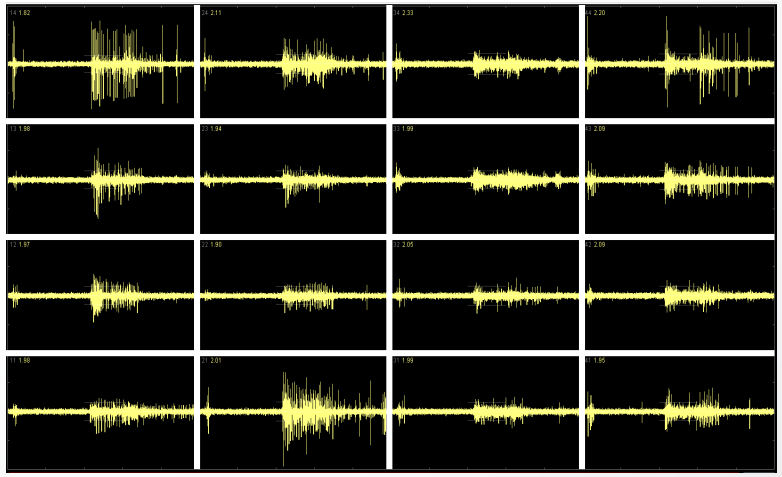


Apoptosis



luminescence

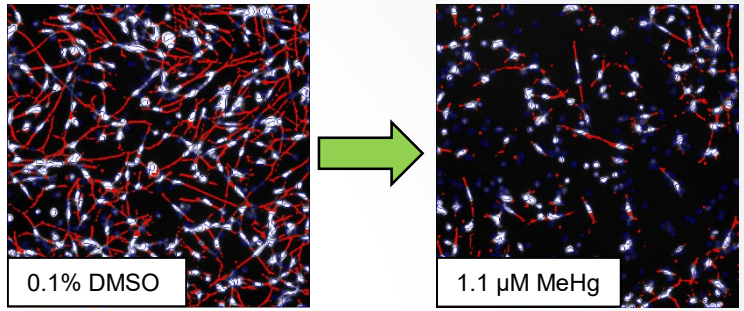
Network Function and Formation



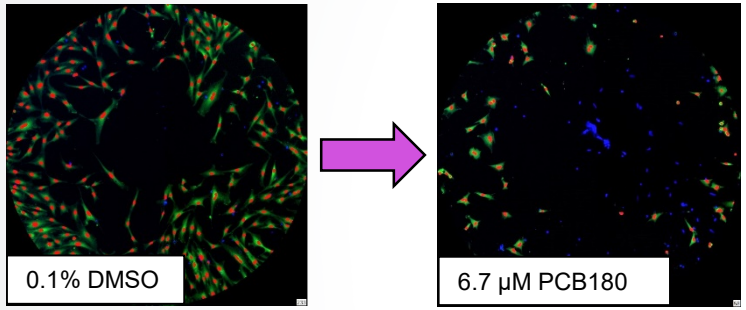
University of Konstanz Assays

DNT compounds

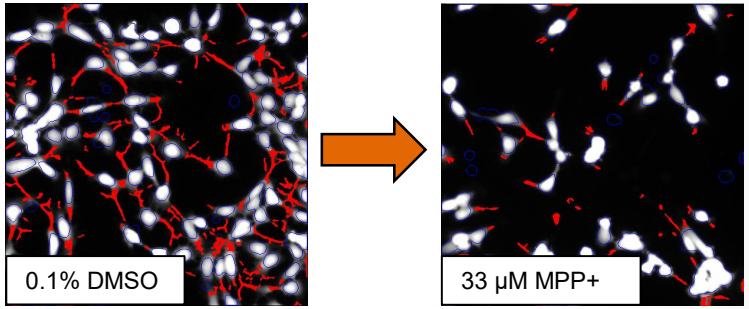
Effect on neurite outgrowth of PNS neurons

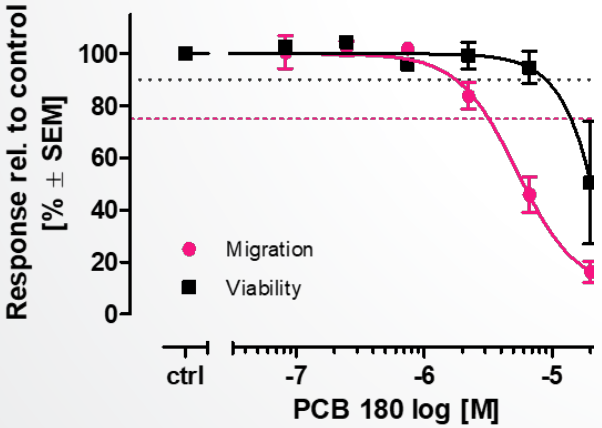


Effect on migration of neural crest cells



Effect on neurite outgrowth of CNS neurons

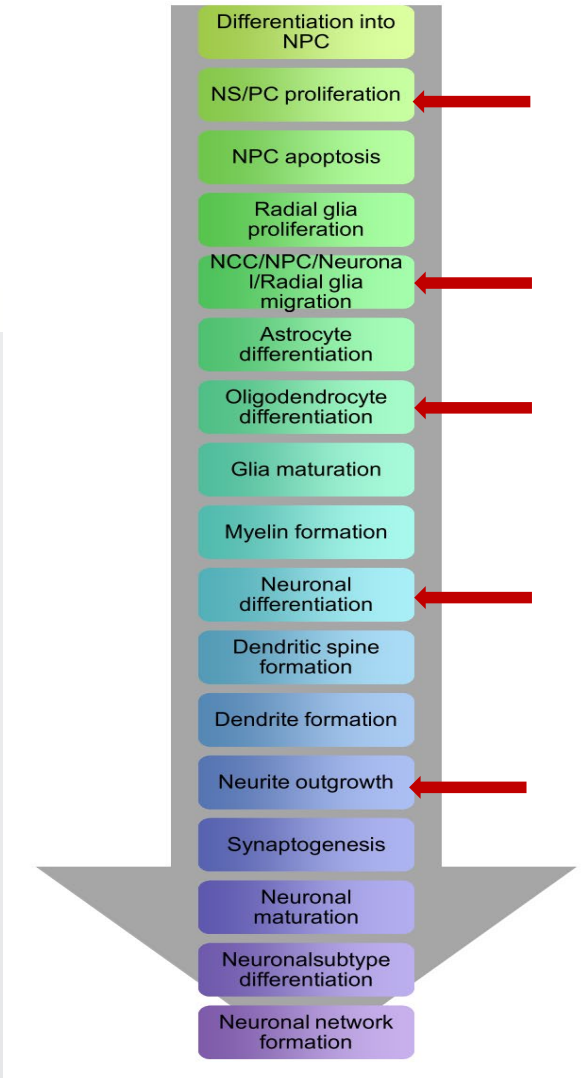


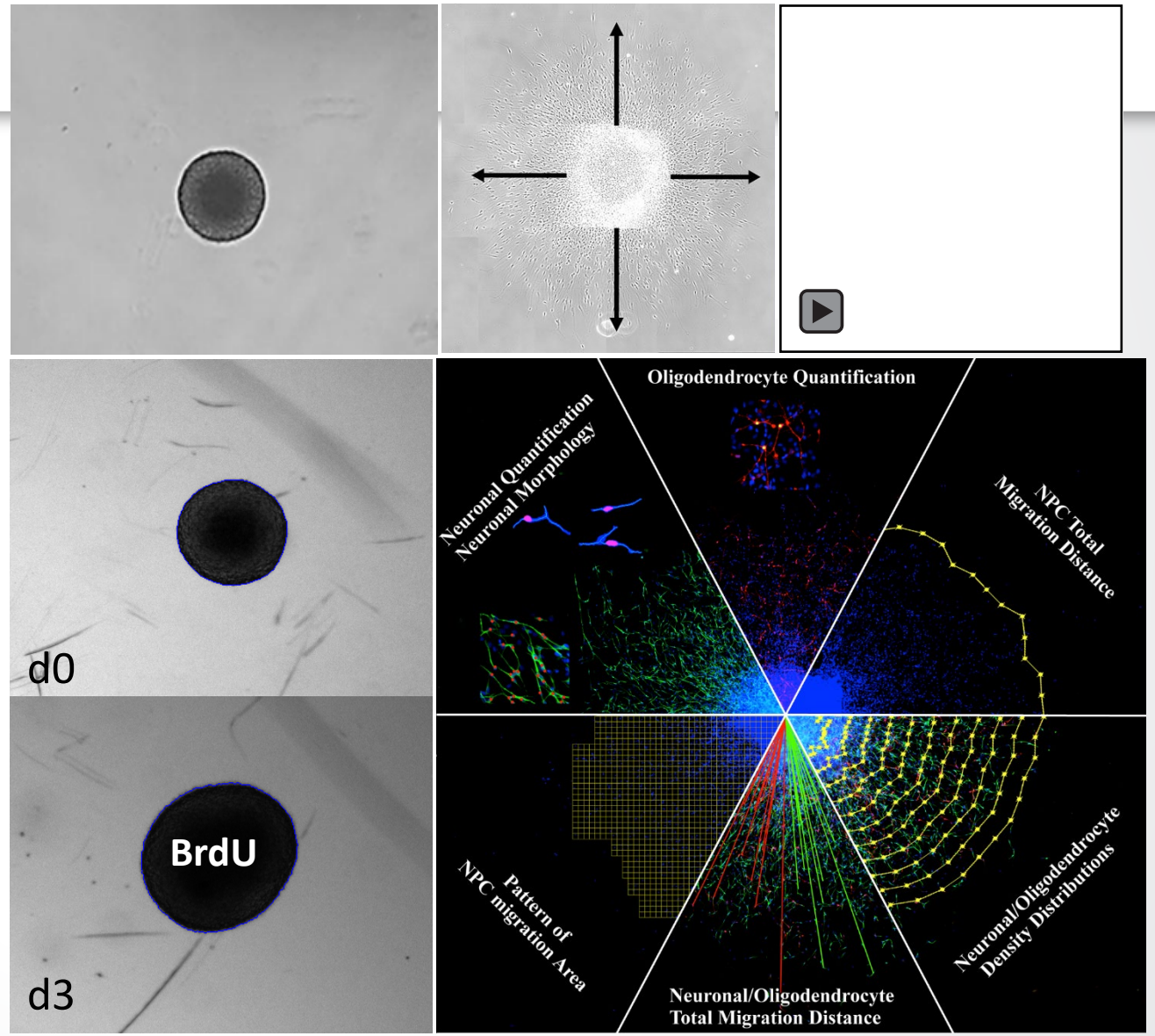


Prediction model & hit definition

Slide courtesy of M. Leist

The 'Neurosphere Assay' (Düsseldorf)



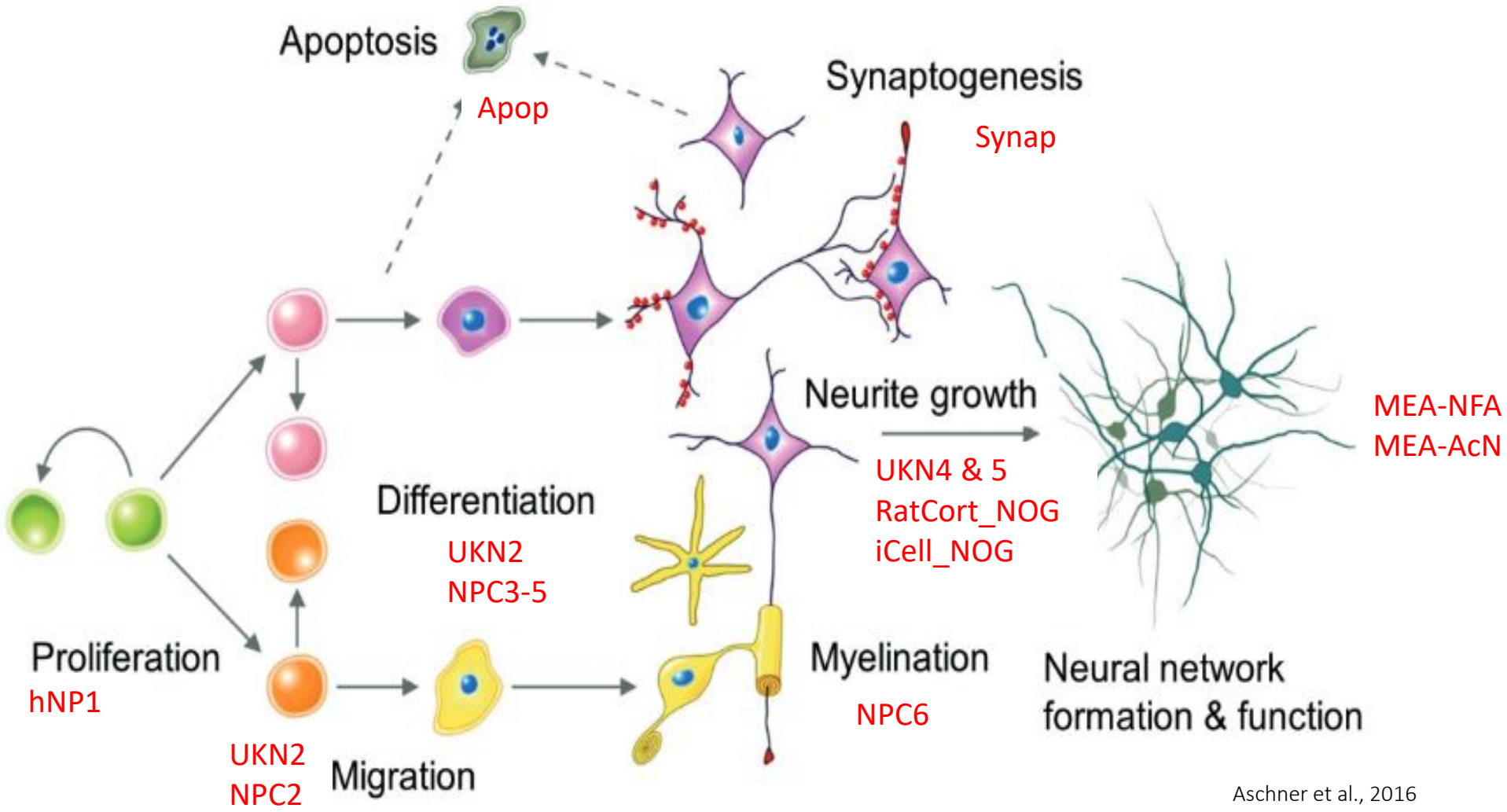


Baumann *et al.*, 2015 Methods in Pharmacology and Toxicology
Schmuck *et al.*, 2016 Archives of Toxicology
Masjosthusmann et al 2018 Toxicology and Applied Pharmacology

Slide courtesy of E. Fritsche

BrdU= bromdesoxyuridine

This Combination of Assays Provides Good Coverage of Neurodevelopmental Processes



Aschner et al., 2016

Needs to encourage Regulatory Use of Alternative Methods and for Guidance Document

- Data from alternative assays
  - Particularly for compounds that will be used for IATA case studies
- Understanding of how the assays work and what they measure
- Evaluation of individual assays and the battery of assays
- Understanding of what can be done with the data
- Accessibility to the data

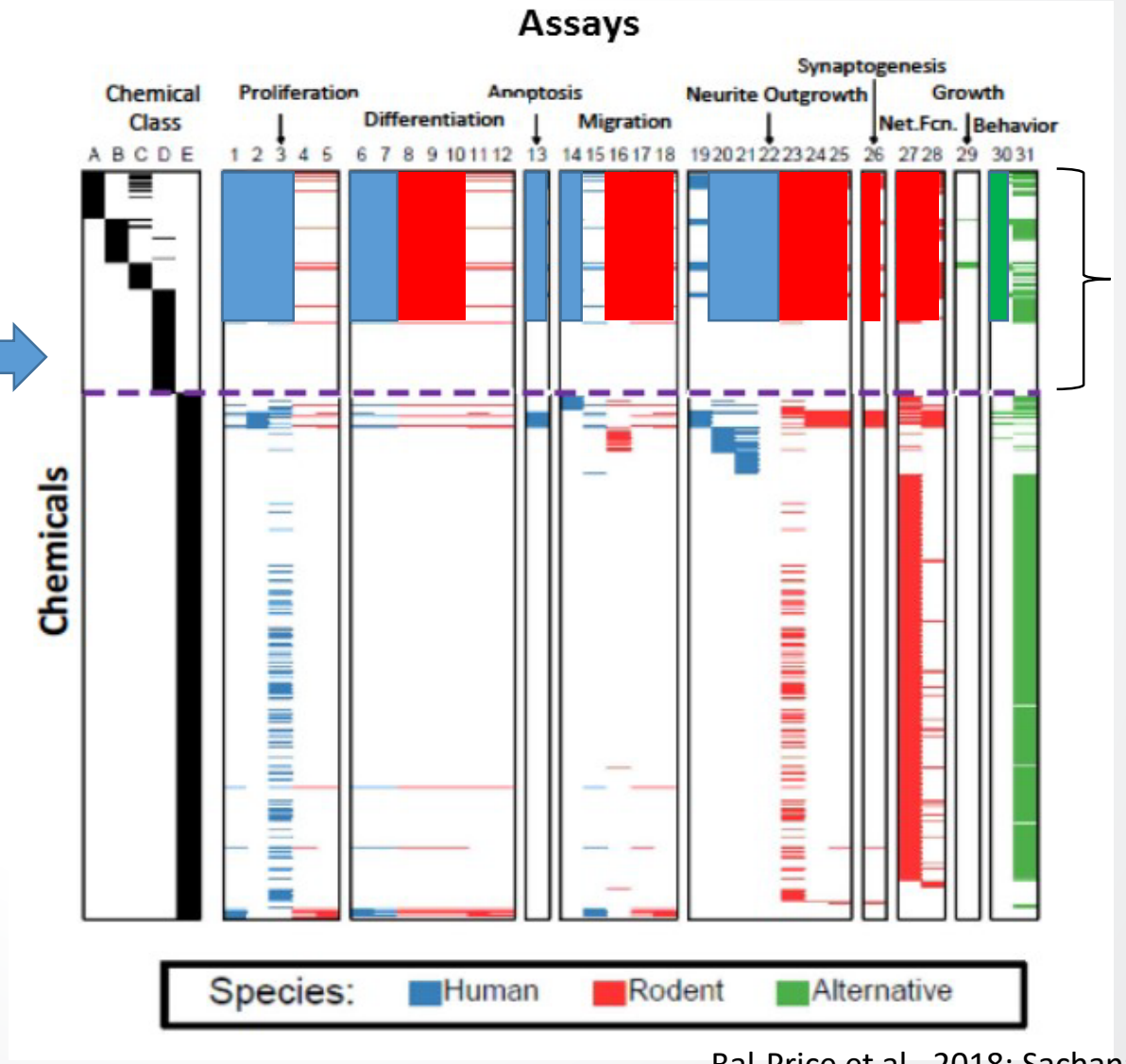
Regulatory decision-makers must have confidence in the assays and data in order to incorporate them into the decision-making process

The Need for More Data

Priority on compounds with in vivo DNT information

Assay-specific Compound Lists; Focused on in vivo DNT

Assay 1
Assay 2
Assay3…



Assay Evaluation

Bal-Price et al., 2018; Sachana et al., 2019

Development of a Chemical Library

- Identified ~136 compounds:
  - Compounds for which DNT Guideline studies are available
  - Compounds of interest for Integrated Approaches to Testing and Assessment (IATAs)
  - Compounds where the Danish EPA has in vivo data
  - Negative compounds
  - Modulators of developmental pathways
- These compounds will be tested in the 12 different DNT assays
- ToxCast has supplied most of these compounds

- Compounds will be tested by EPA, University of Konstanz and University of Dusseldorf in a variety of in vitro assays

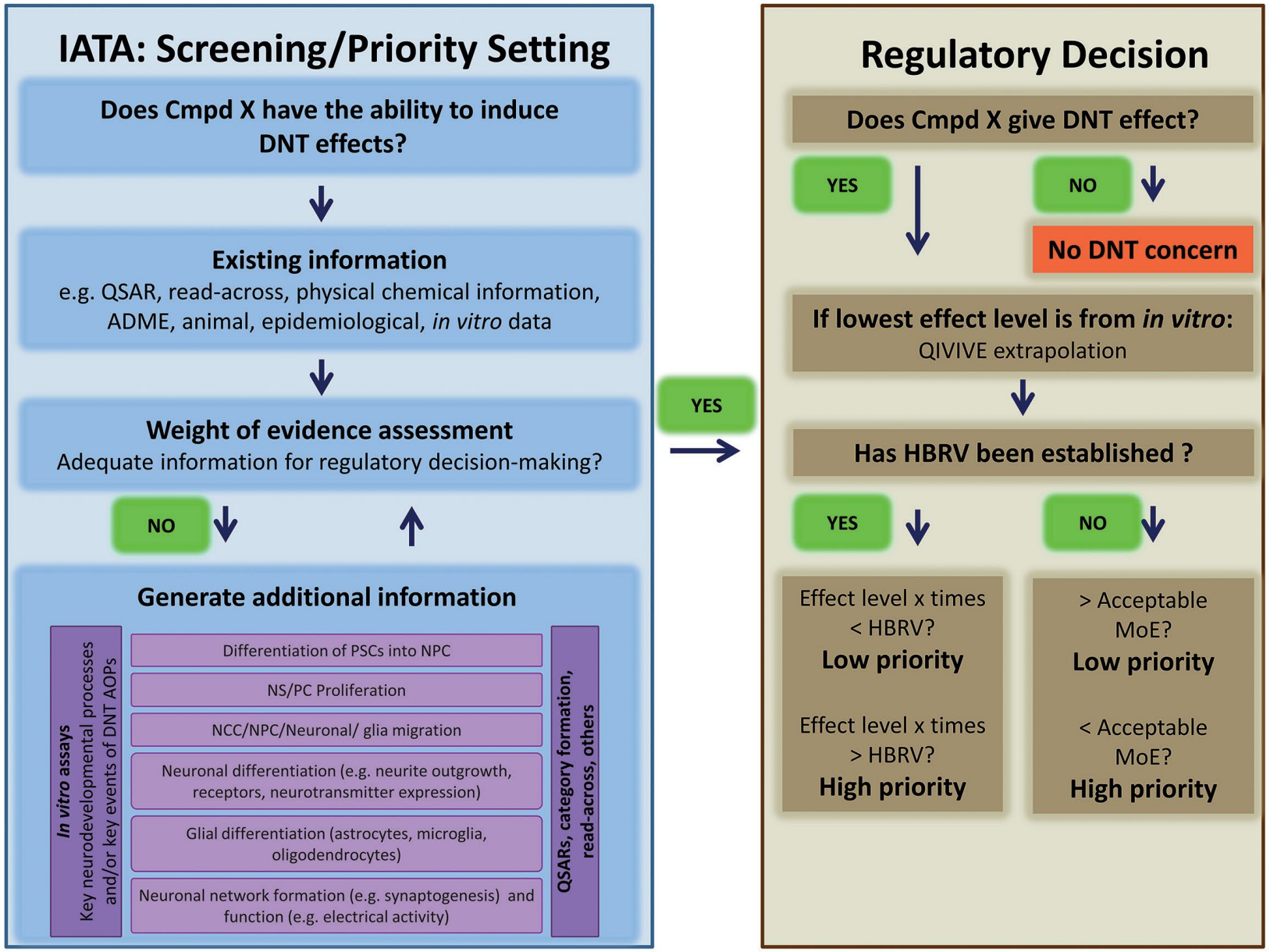
Status and Timelines

- Partners have received ToxCast compounds.
  - Testing is Completed at Konstanz and Duesseldorf
    - Report is expected to be released to public in October 2020.
  - EPA testing is nearing completion
    - Data expected in late 2020
  - Zebrafish behavioral testing
    - Focus on ~30 IATA compounds
    - Data collection has started and will be completed later in 2020.

Current Focus of the OECD DNT Expert Group

Development of a Guidance Document for the use of DNT alternative assays in Integrated Approaches for Testing and Assessment (IATAs)

- Introduction and Rational
- Issues with the Current Guideline testing approaches
- Guidance for incorporation of in vitro assays into IATAs
- **Case Studies**



HBRV = health-based reference value

Adverse Outcome Pathway Development

Well established AOPs involving Network Formation may reduce the uncertainty in using data from the MEAs

While several of the few DNT-relevant AOPs in the AOPWiki include alterations in network function as a key event, overall there are few established AOPs linked to Acute Neurotoxicity or DNT

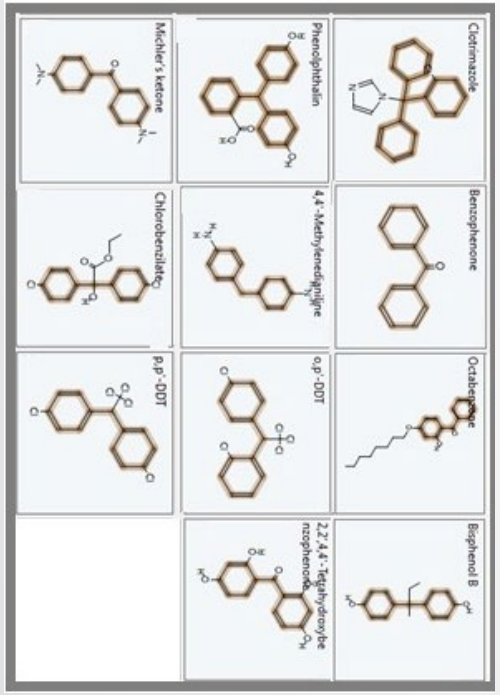
https://aopwiki.org/aops/15

High-throughput assays for DNT provide information for Adverse Outcome Pathway Development

Toxicant

Chemical Property Profile

→ "Chemotyping"



Molecular Initiating Event

? (many postulated, few confirmed)

→ **Target-based Screening** (known MIEs)

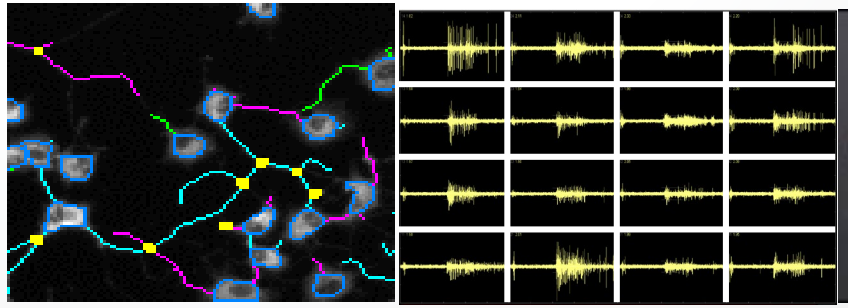
HTP Molecular assays (ToxCast)
ion channels
AChE
neurotransmitter receptors
growth factor receptors
transcription factors
kinases
…

Cellular Responses

Key Events
proliferation
differentiation
migration
neurite growth
synaptogenesis

→ **Phenotypic Screening** in physiologically-relevant models (MIE not assumed)

In Vitro Approaches



Organ Responses

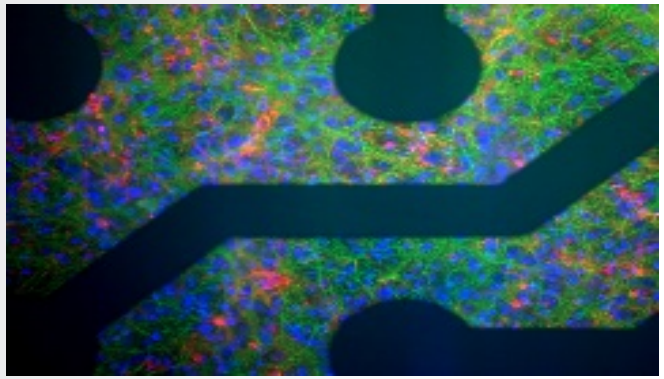
Nervous System
Δ connectivity (morphology, neurochemistry, physiology)

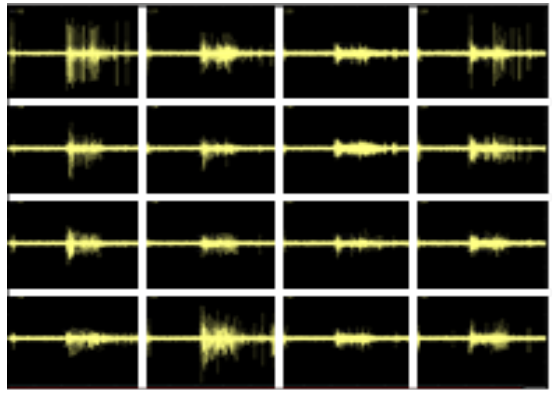
→ **Individual Responses**

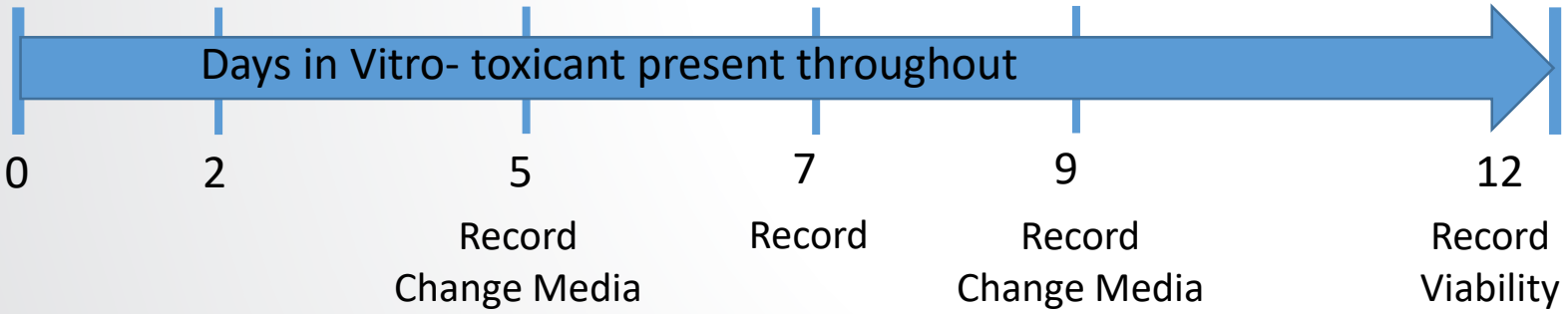
behavior
cognition

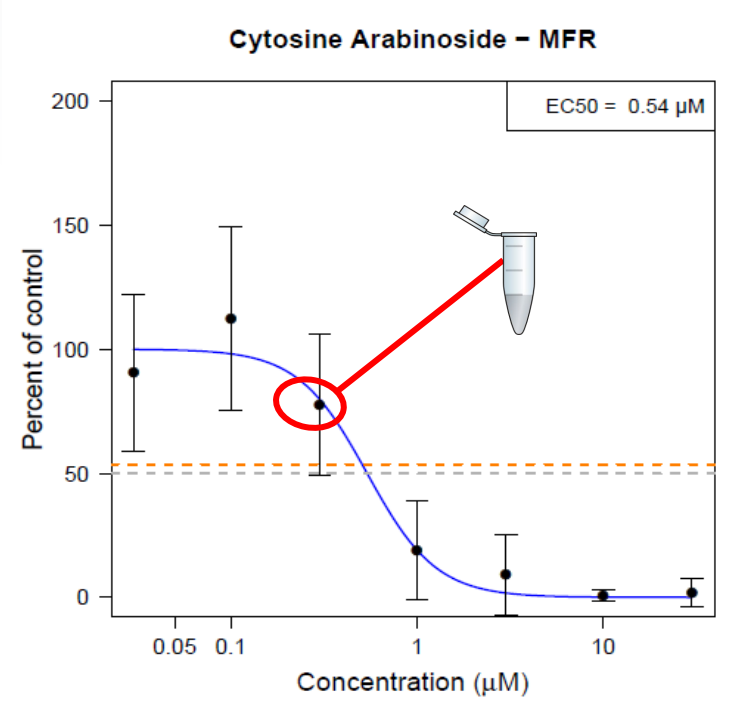
Behavior

Application of Transcriptomics and Metabolomics to in vitro DNT assays for AOP development





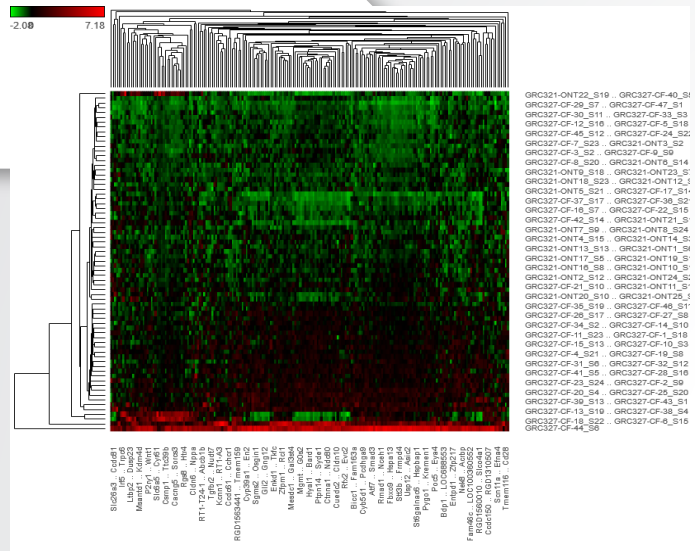




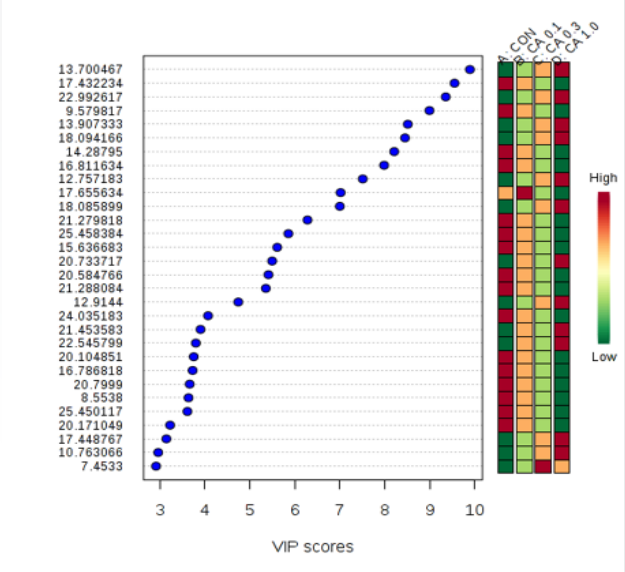
Critical concentration ("tipping point") determined

Six Chemical Proof of Concept

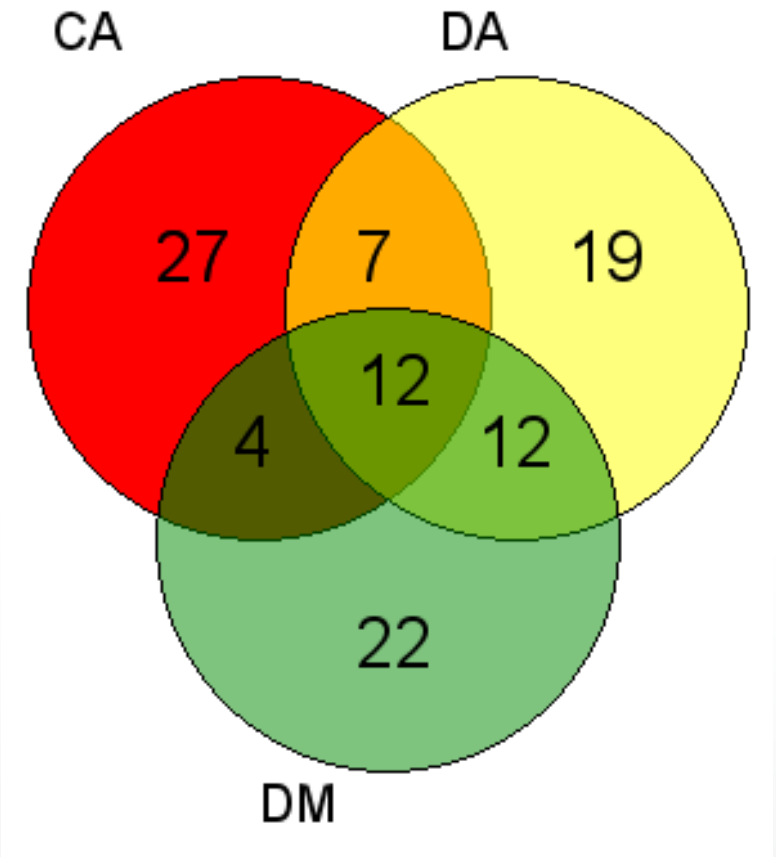
Domoic acid
Cypermethrin
Cytosine Arabinoside
Haloperidol
Deltamethrin
5-Fluorouracil



Canonical Pathway: Axonal Guidance



Transcriptomics



Metabolomics

Found in all three gene lists

ACTR3	EFNA5
ADAM15	EPHA7
ADAMTS5	FZD2
BMP7	FZD5
BRCC3	FZD7
EFNA4	GLI2

In vitro assays to identify developmental neurotoxicity hazard:

Promises and challenges

Promises:

- Data on DNT hazard for many more chemicals
- Characterization of DNT hazard on biologically-relevant processes
- Data from human models
- Substantially lower cost and faster results than *in vivo* studies

Challenges:

- Demonstration that the *in vitro* assays provide results that are equivalent to or better than animal models for DNT
- Development of additional case-studies using *in vitro* DNT assays
- Development of additional AOPs related to DNT that will increase confidence in using these assays
- Development of assays that cover areas of neurodevelopmental processes not well covered in the current battery

Thank you!
Questions?

OECD Expert Group on DNT

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