

A fluorescence microscopy image of neurons. The cell bodies (soma) are stained blue, while the cytoplasm and processes (dendrites and axons) are stained green and red. The background is black.

US EPA Case studies: Prioritization, Weight of Evidence and Waiving *in vivo* DNT test studies

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.

This is a scientific presentation only. Some or all of the data presented in this presentation may be preliminary and subject to change.

This presentation does not represent EPA policy and mention of products or tradenames does not constitute a recommendation for use or endorsement. **Do not cite or quote this presentation.**

Photograph by Thresa Freudenrich, CCTE



Case studies using DNT NAMs



- Screening and Prioritization
 - 160 PFAS compounds (Carstens et al., 2023)
 - Organophosphate flame retardants (DTT; OECD case-study)
- Weight of Evidence
 - Glufosinate DNT Guideline Waiver (Dobreniecki et al., 2022)
 - Deltamethrin and flufenacet (OECD Case-studies)
 - DCNA (Dichloran)
 - Required the DNT guideline study; based on WoE and positive effects in acute MEA study
 - Organophosphates
 - Evaluate DNT potential and relative sensitivity to AChE inhibition to inform FQPA determinations
 - Individual OP WoE assessments
 - Acephate, methamidophos, others pending



EPA Case Studies with assays from the DNT-IVB



1) Present 2 Case Studies from EPA

- PFAS Compounds (Prioritization)
- L-glufosinate isomers (Weight of Evidence)

2) Discuss lessons learned, uncertainties, benefits, challenges & solutions

Note: While these case studies used IATA principles, they have not been submitted as formal IATAs.

Case study 1: Screening 160 PFAS Compounds



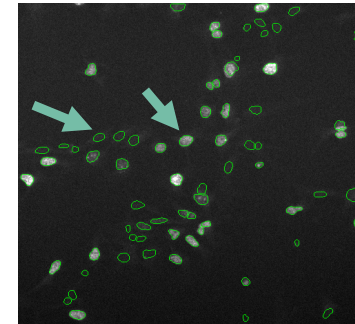
Problem Formulation

- Widespread human exposure to a structurally diverse group of chemicals
- Little *in vivo* toxicological information on DNT
- DNT evidence in peer-reviewed literature was conflicting
 - epidemiological studies are equivocal
 - neurodevelopmental effects associated with exposure to limited numbers of PFAS in rodent and other animal studies

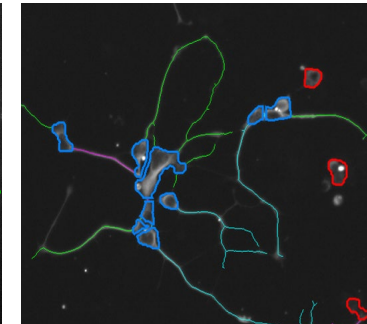
Approach

- EPA Developed a test set of ~160 PFAS compounds that represented a diverse structural cross section of PFAS
- These were tested in 4 EPA DNT NAMs assays
- These PFAS were also tested in other ToxCast assays
 - ACEA, Attagene, Bioseek, HTr & HTTP, Thyroid, Zebrafish development
- Analytical chemistry was also conducted

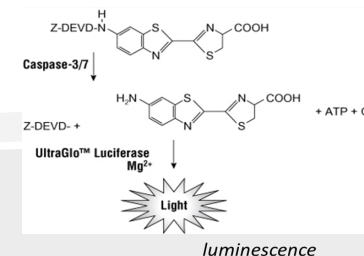
Human Neuroprogenitor Proliferation



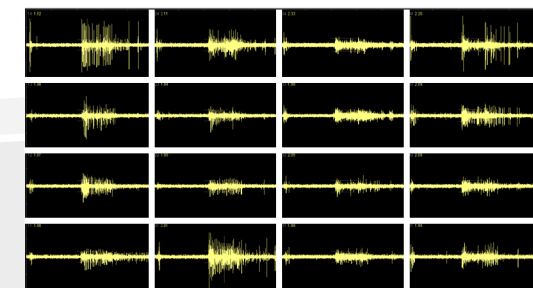
Human Neuron Neurite Outgrowth



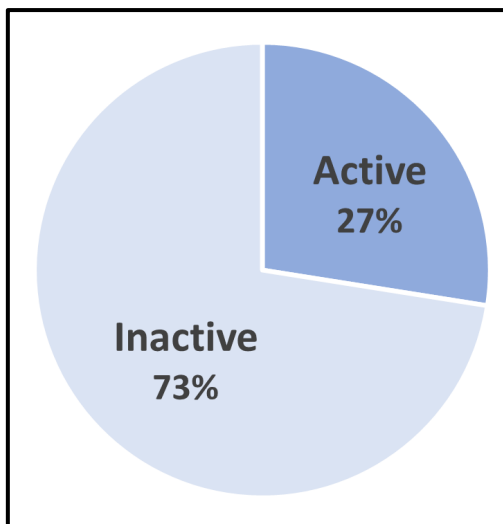
Human Neuroprogenitor Apoptosis



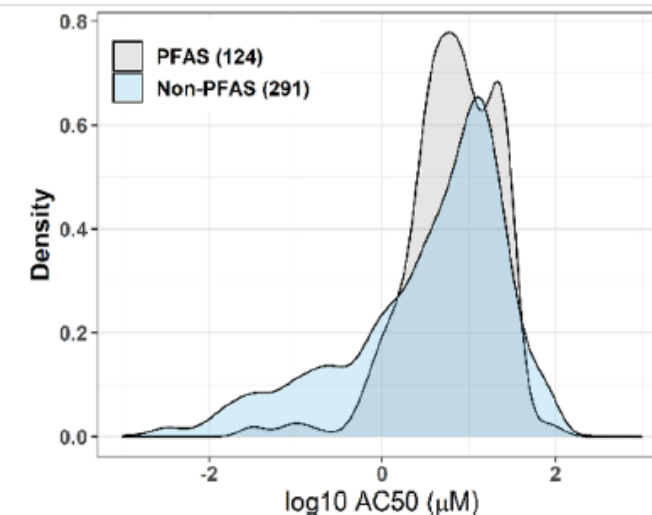
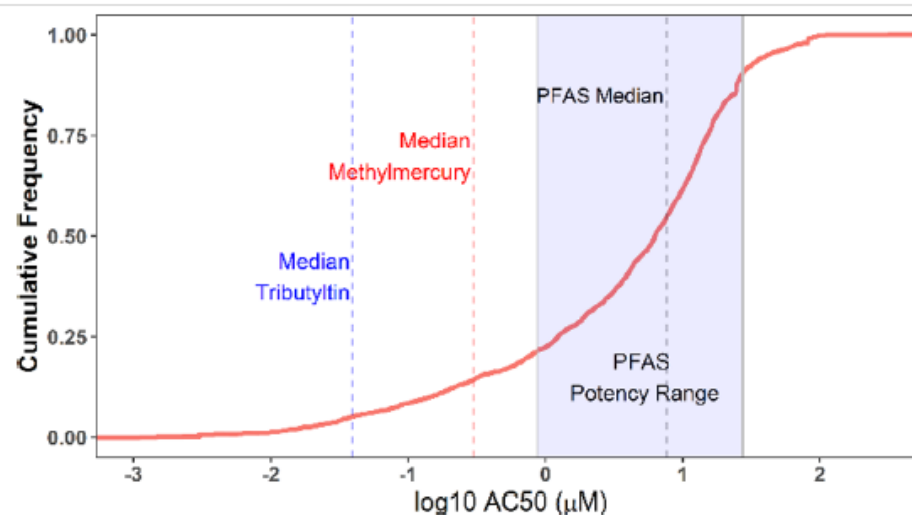
Rat Network Formation Assay



Screening 160 PFAS Compounds- Results



- 118/160 PFAS inactive,
- 42/160 active PFAS
 - 24 PFAS moderate or low selective activity

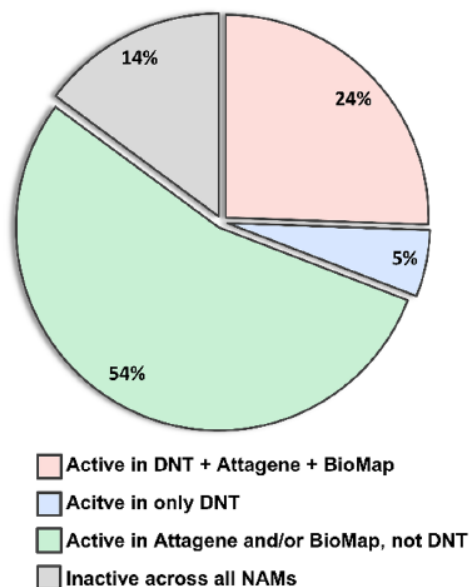


- Potency of Active PFAS were near the median potency of 415 non-PFAS compounds tested in the EPA DNT NAMs
- PFAS were less potent than two other well established DNT compounds

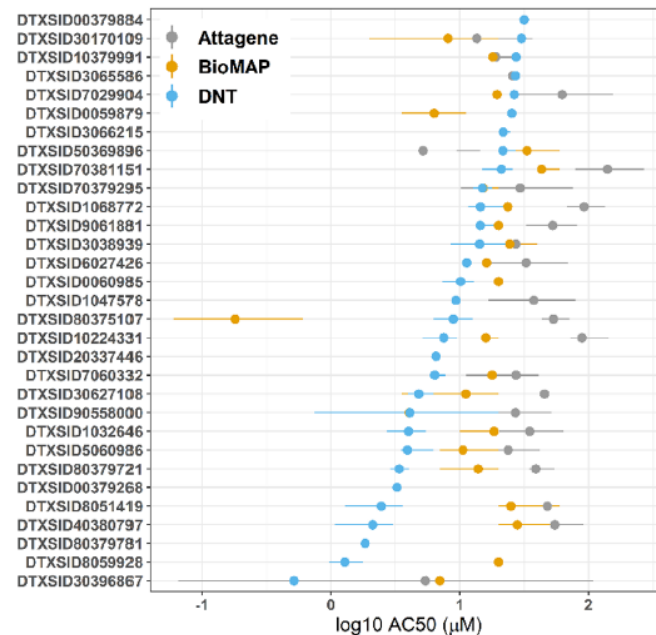
Screening 160 PFAS Compounds- Results



A. PFAS bioactivity in DNT NAMs versus BioMAP and Attagene assays



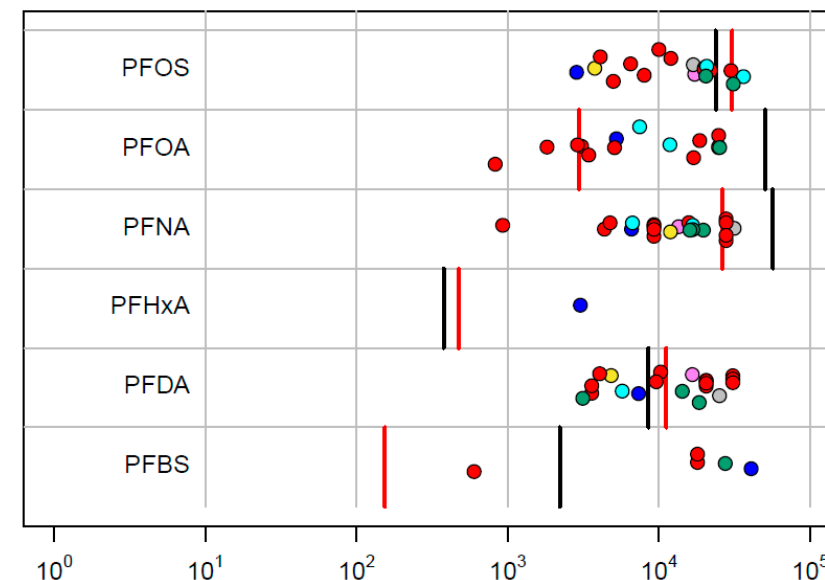
B. PFAS potency in DNT NAMs versus BioMAP and Attagene assays



From Carstens et al, 2023, Chem Res Toxicol. 2023 Mar 20;36(3):402-419.

- A small number of PFAS compounds were active only in DNT assays
- In general, DNT NAMs were less sensitive than other NAMs assays

Points of Departure from NAMS vs internal concentrations from animal study Lowest Effective Concentrations (vertical lines)



From Judson et al, 2024, Toxics 2024, 12, 271.
<https://doi.org/10.3390/toxics12040271>.



Screening 160 PFAS Compounds- Results



Challenges:

- COVID 19
- Compound stability
- Compound cost and availability

Uncertainties:

- Lack of data from other DNT NAMs assays
- Compound stability and in vitro kinetics
- Unknown ability to cross the BBB
 - S. Ramaiahgari @ EPA working on this
- Many untested PFAS compounds

Benefits:

- Tested 160 compounds in 4 assays in <2 yrs during a global pandemic
 - Less time than 1 guideline DNT study
- Data would be unattainable using animal studies

Lessons Learned:

- Analytical chemistry support was invaluable for these compounds.
- Structure was important
 - Chain length >7; high C:F ratio; carboxylic acid moiety more likely to be active



Case Study 2: DNT Waiver Evaluation for L-Glufosinate



Problem Formulation

- 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)

Available Data

- 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
 - Morphometry changes were not robust and difficult to interpret
- DL-glufosinate also has acute neurotoxicity effects
- Literature report of altered network activity *in vitro*, following acute exposure (Lantz et al., 2014)

Question:

- *Is the Guideline DNT for DL-glufosinate sufficient to inform decisions for L-glufosinate isomers?*

Need:

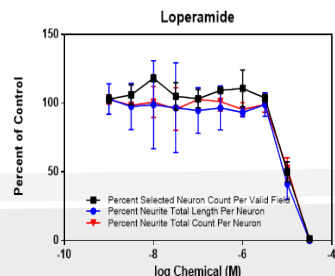
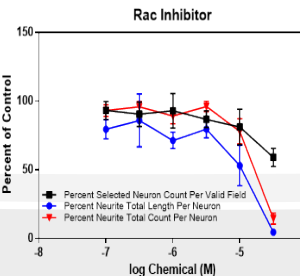
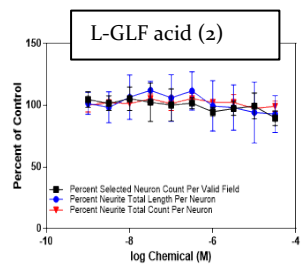
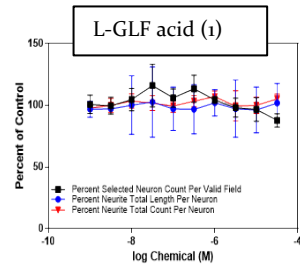
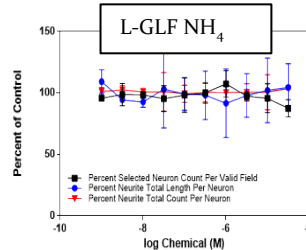
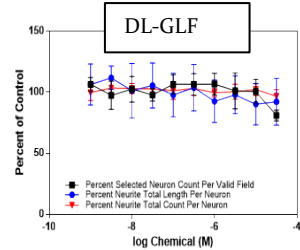
- Comparative bioactivity data for DL- vs L-Glufosinate isomers

DNT Waiver Evaluation for L-Glufosinate isomers

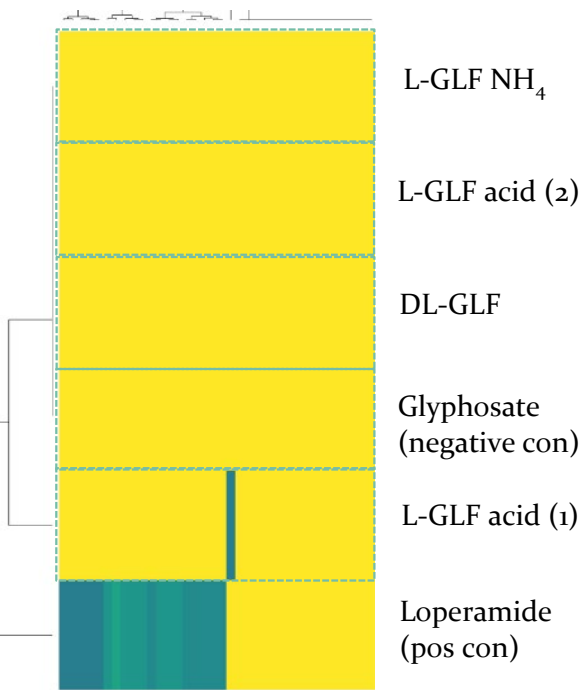


Approach

Neurite Outgrowth in human iPS Neurons



Network Formation in rat primary cortical cultures



Weight of Evidence Evaluation

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-isomers 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.*



DNT Waiver Evaluation for L-Glufosinate isomers



Challenges:

- Data Pipelining & Analysis (now solved)

Benefits:

- See next slide

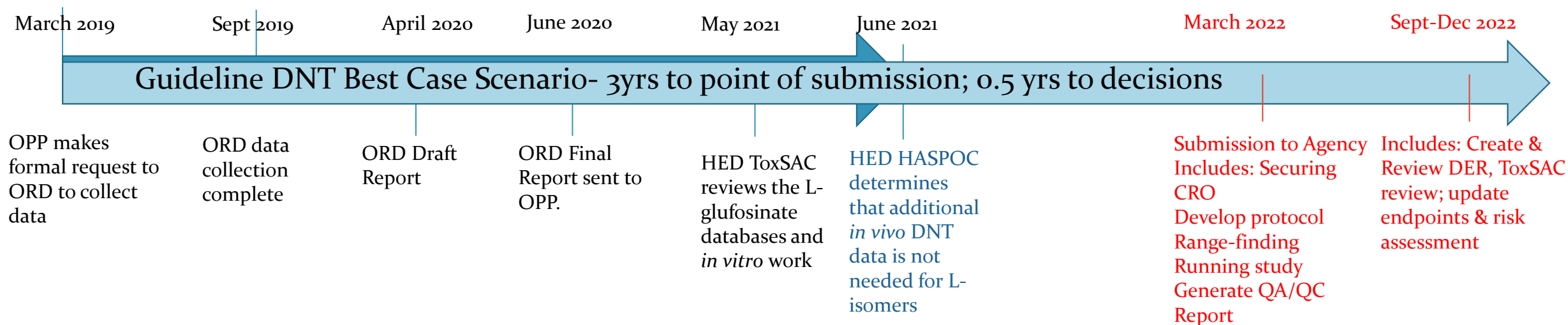
Uncertainties:

- Lack of data from other DNT NAMs assays
 - DL-glufosinate is negative in UKN and IUF assays
- Negative Data; Addressed by:
 - Inclusion of assay positive controls
 - Replication of previous results with acute exposures on MEA

Lessons Learned:

- Communication between Agency regulators and scientists, as well as registrants was key to success
- Negative data in the DNT NAMs evaluation can still provide useful information for WOE decisions

Glufosinate case study demonstrates the impact of DNT NAMs



Animals Used:

- *In vitro* study- 3 Pregnant Dams (~12-15 pups)
- 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)

Additional Lessons from Case Studies

There are potentially a wide variety of use-scenarios for the DNT-IVB

- Screening and Prioritization
- Weight of Evidence
- Interpretation of in vivo data
- Inform decisions about more complex and/or in vivo studies

Submission/publication of more examples of these as IATA case studies would be helpful to the community

Some challenges exist that need to be addressed:

- Transferability
 - Performance criteria for transfer and “me too” assays need to be articulated (I believe that they exist)
 - Involvement of experts (ICCVAM, ECVAM, OECD) in assay transfer is needed
- Clarification of data analysis and interpretation approaches
 - Network Formation Assay
- Defined Approaches need to be developed



Summary and Conclusions



- EPA has used data from the DNT and other NAMs for:
 - Screening and Prioritization
 - Weight of Evidence decisions
- This is consistent with recommendations from the 2020 SAP
- While formal IATAs were not developed for the examples here, the decision process incorporated concepts from the DNT IATA framework.
 - Our experience indicates that the DNT IATA framework is flexible enough to accommodate a variety of different decision-making scenarios related to DNT.
- All of the DNT NAMs data in these examples were analyzed using *tcpl*, *httk* and IVIVE
 - This suggests that Data Interpretation Protocols could be developed
- Defined Approaches could also be developed for several different scenarios related to DNT.
 - This may be more challenging due to a variety of different scenarios that might be encountered

Thank you! Questions?



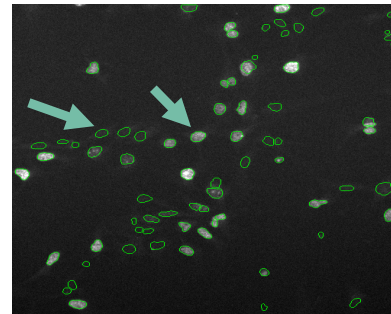
EPA ORD Colleagues:

- Kathleen Wallace
- Theresa Freudenrich
- Bill Mundy (retired)
- Kevin Crofton (retired)
- Josh Harrill
- Jasmine Brown
- Katie Paul Friedman
- Kelly Carstens
- Megan Culbreth
- Richard Judson
- Grace Patlewicz

EPA Program Office Colleagues

- Anna Lowit
- Liz Mendez
- Monique Perron
- Sarah Dobreniecki
- Mike Metzger (retired)

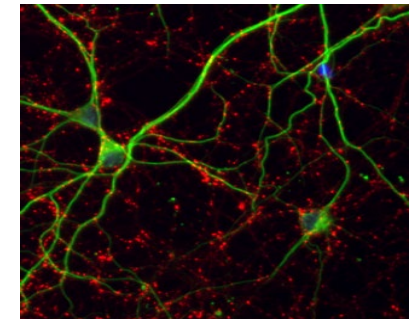
Human Neuroprogenitor
Proliferation



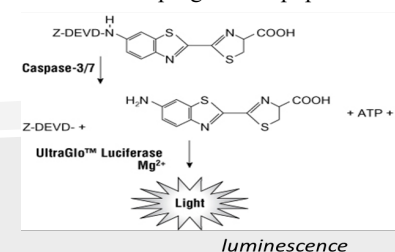
Human and Rat Neuron
Neurite Outgrowth



Synaptogenesis in Rat
Cortex Neurons



Human Neuroprogenitor Apoptosis



Rat Network Formation Assay

