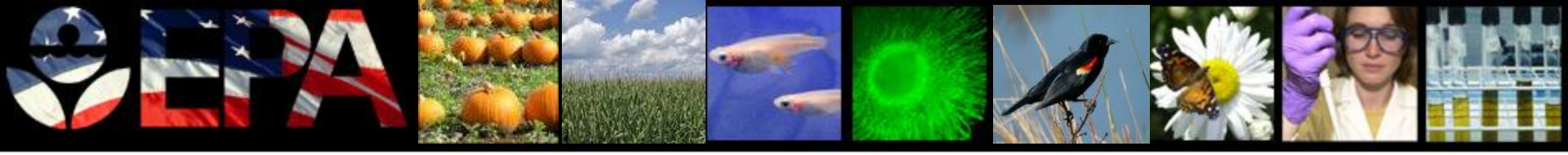
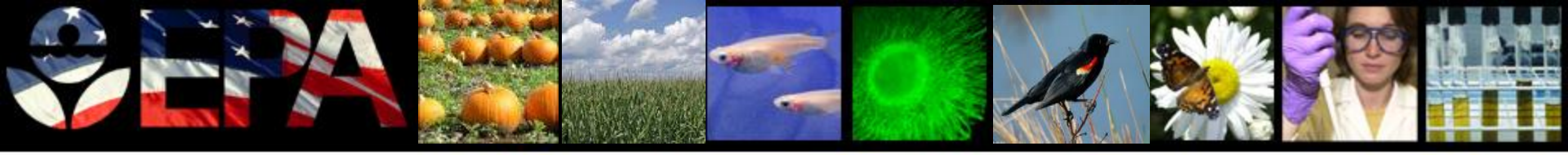


New Alternative Methodologies (NAMs) and DNT: A Regulator's Perspective

Elizabeth Méndez, Ph.D.
Senior Science Advisor
Health Effects Division
Office of Pesticide Programs
US EPA



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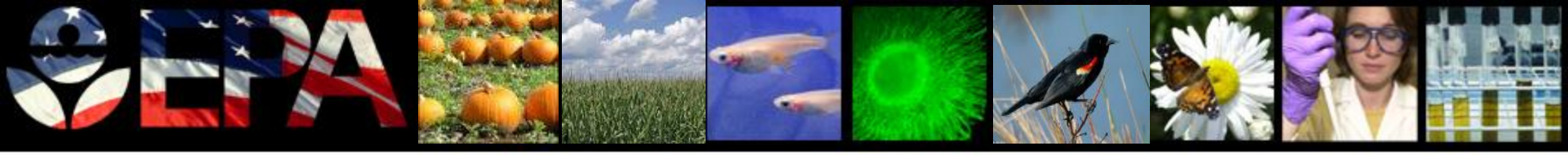
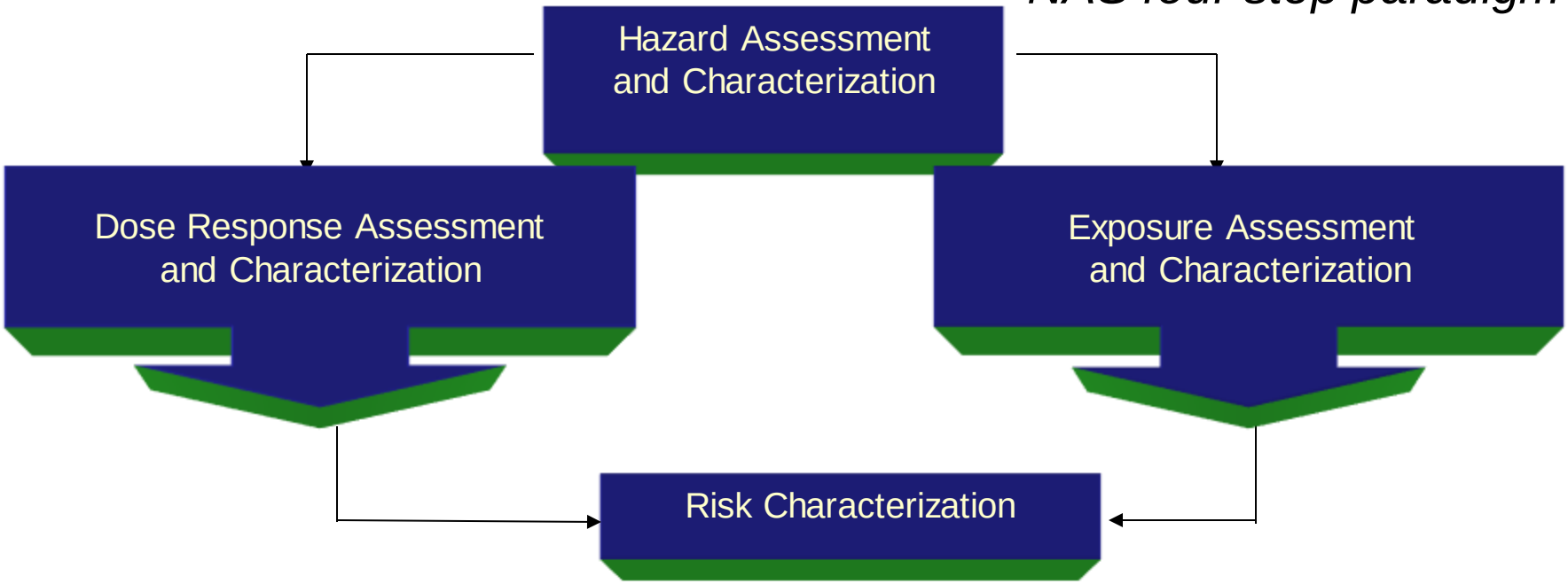


Introduction

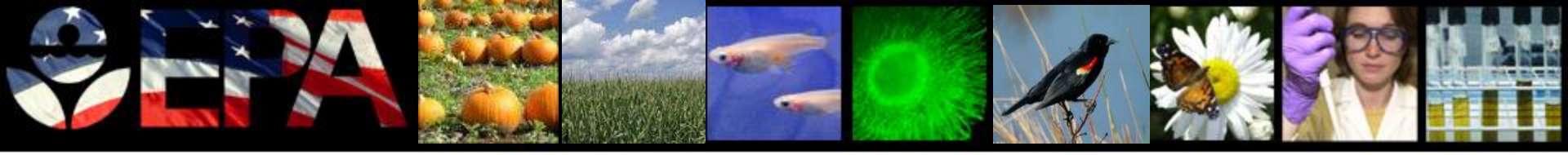
- US EPA's Office of Pesticide Programs is a licensing program regulating pesticide products in the US
  - The process of registering a pesticide is a scientific, legal, and administrative procedure.
  - Consider the ingredients of the pesticide; the particular site or crop where it is to be used; the amount, frequency, and timing of its use; and storage and disposal practices.
  - Develop risk assessments that evaluate the potential for
    - Harm to humans, wildlife, and plants, including endangered species and nontarget organisms.
    - Contamination of surface water or ground water from leaching, runoff, and spray drift.
  - The company that wants to produce the pesticide must provide data from studies that comply with our testing guidelines.

Risk-Based Approach

NAS four-step paradigm

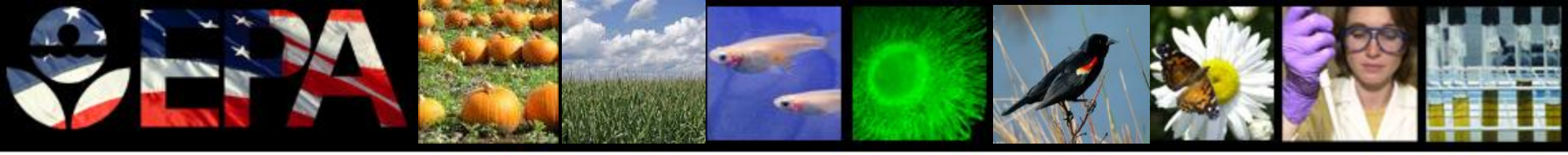


Risk = Hazard x Exposure



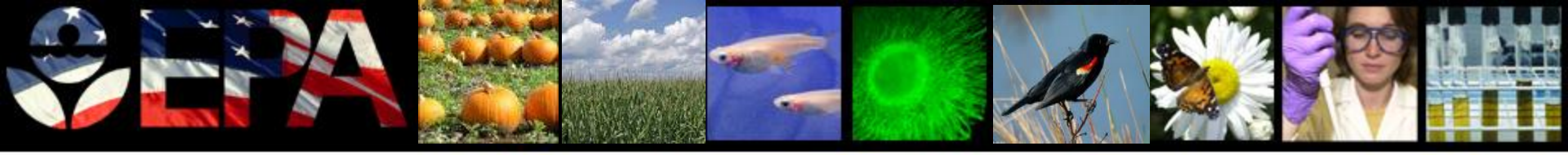
DNT study

- Conditionally required using WOE approach if:
  - The pesticide causes treatment-related neurological effects in adult animal studies
  - The pesticide causes treatment-related neurological effects in developing animals, following pre- and/or postnatal exposure
  - The pesticide elicits a causative association between exposures and adverse neurological effects in human epidemiological studies
  - The pesticide evokes a mechanism that is associated with adverse effects on the development of the nervous system



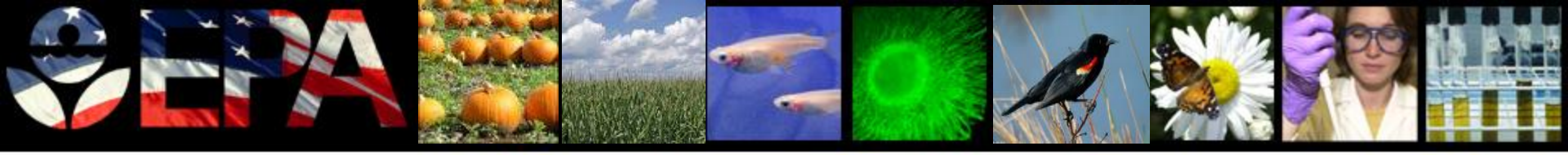
DNT Study (Cont'd)

- An information-based approach to testing is preferred, which utilizes the best available knowledge on the chemical (hazard, pharmacokinetic, or mechanistic data) to determine whether a standard guideline study, an enhanced guideline study, or an alternative study should be conducted to assess potential hazard to the developing animal, or in some cases to support a waiver for such testing. Registrants should submit any alternative proposed testing protocols and supporting scientific rationale to the Agency prior to study initiation.
- Considers risk picture



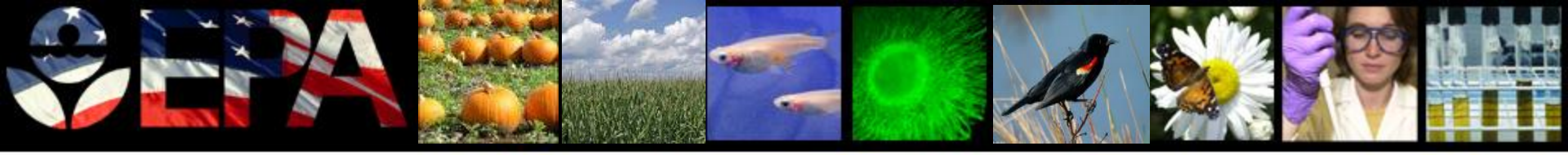
DNT Guideline Challenges & Limitations

- Reliable detection, measurement, and interpretation of treatment-related DNT effects depends on appropriate study design and conduct
- Infers DNT effects on the basis of apical endpoints with little or no information on the underlying biological processes
- Interpretation hampered by a number of limitations including high variability, low precision, and being resource intensive
- Difficult to interpret isolated findings where a change in one endpoint is not substantiated by other endpoints
- Challenges correlating behavioral and/or neuropathological effects in the animal model to complex neurological deficits in the human population



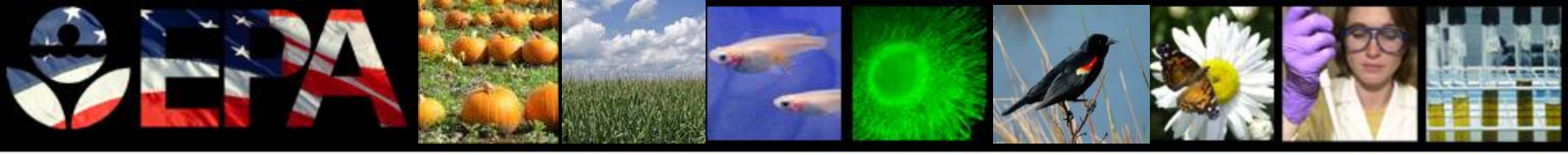
Status of DNT Studies at OPP

- Approximately 100 DNT studies reviewed by OPP
- Approximately two dozen DNTs used to set points of departure
  - All are based on offspring effects (pup mortality – 5, brain morphology – 9, pup weight – 4, behavioral changes – 5, developmental delays – 1) without corresponding maternal effects
- 32 DNT studies available for known neurotoxicant chemical classes (OPs, *N*-methyl carbamates (NMCs), and pyrethroids)
  - None provided the most sensitive endpoint for human health risk assessment
- Required alternative studies based on known mode of action



DNT Study Alternatives

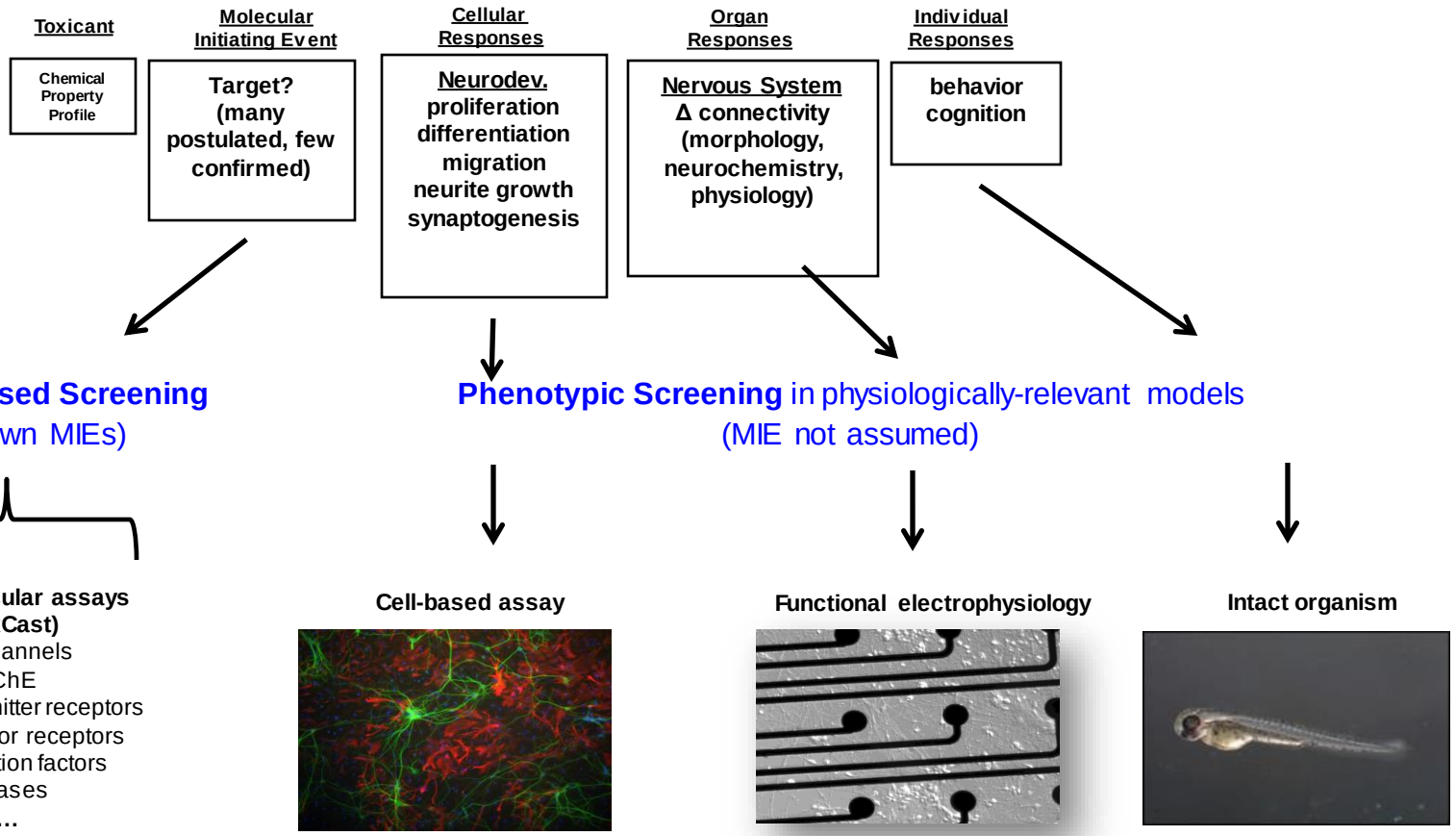
- Testing strategy shift from DNT guideline study → targeted testing based on accepted MOAs
  - OPs and NMCs: comparative cholinesterase assays (CCA)
    - AChE inhibition across lifestages
  - Pyrethroids: *in vitro* studies and physiologically based pharmacokinetic (PBPK) modeling
    - Interaction with voltage-gated sodium channels leading to neurotoxicity
  - Thyroid toxicants: comparative thyroid assay (CTA)



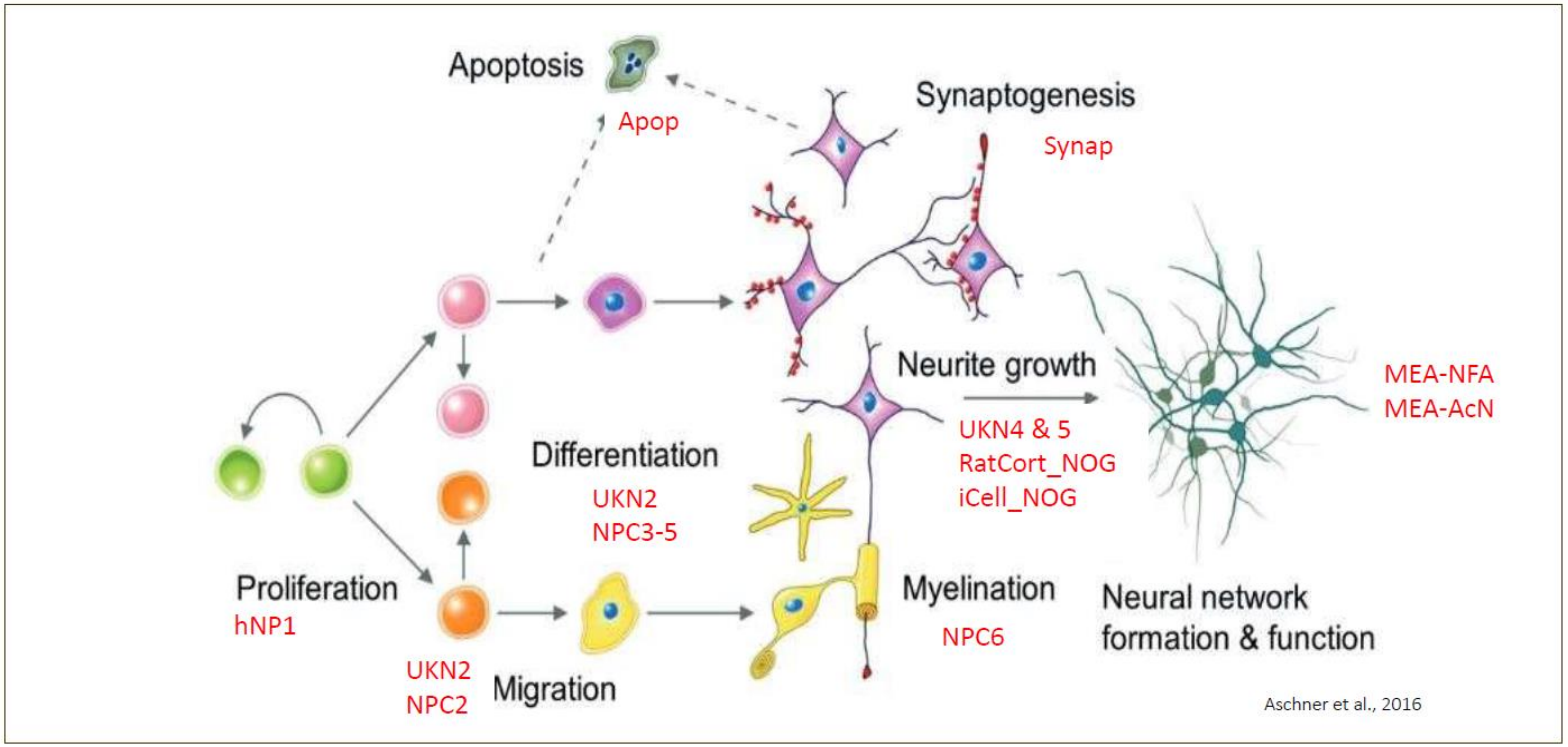
DNT NAMs Battery Development

- Next logical step in EPA's efforts to implement more human relevant and efficient approaches
  - Normal brain development depends upon coordinated expression of critical neurodevelopmental processes that are conserved across species and can be modeled in vitro and in small, non-mammalian organisms
- On-going work at ORD-NHEERL, Univ. of Konstanz, Univ. Düsseldorf, and several Zebrafish labs developing a screening battery for evaluating DNT
  - Approach:
    - Identify key events in neurodevelopment at increasing levels of biology
    - Use model systems that recapitulate key neurodevelopmental processes
    - Apply new technologies for high-throughput assessment of endpoints at cell, tissue, and intact organism level

Assays Based on Measuring Key Events in AOPs

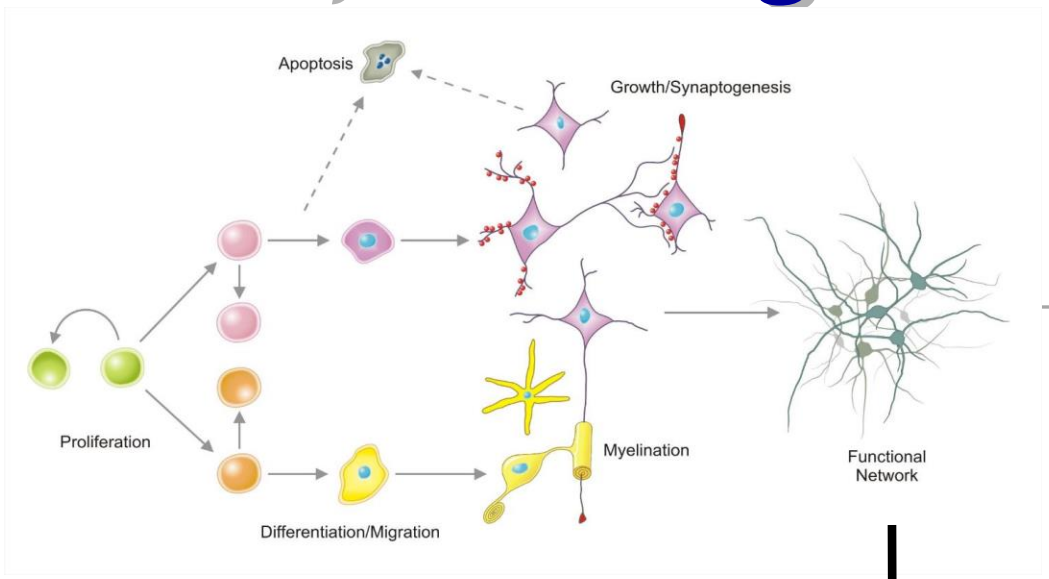


DNT NAM Coverage of Neurodevelopmental Processes

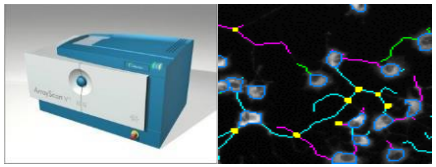


Hypothesis: *If a compound alters a neurodevelopmental process in vitro, then there is a concern it could be an in vivo DNT hazard*

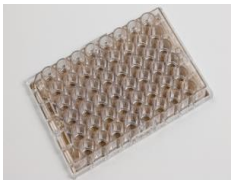
High-throughput Assays at Cell, Tissue, and Organism Level



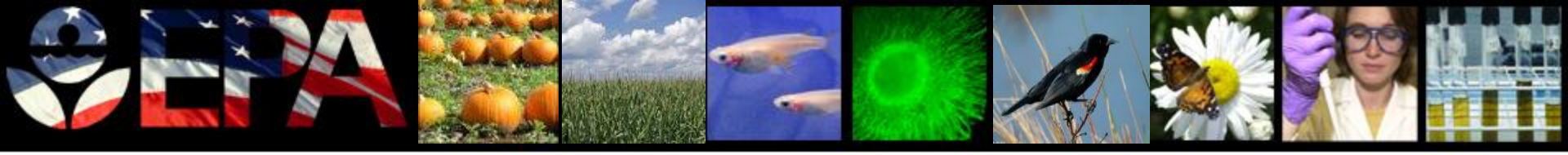
Key Cellular Events (High Content Imaging)



Network Formation (Multi-Electrode Array)

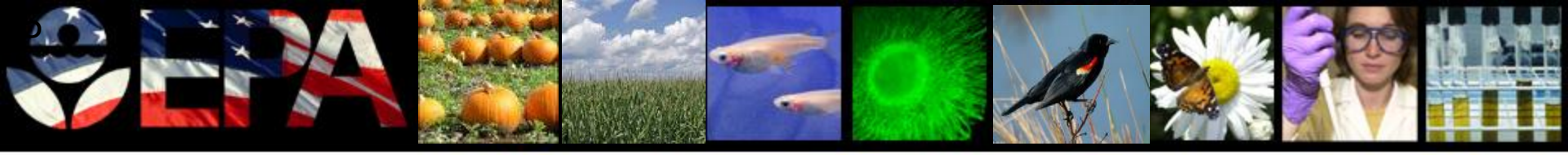


Zebrafish behavior (Noldus video tracking)



Integrating NAMs into Risk Assessment

- Integration process is a continuum
  - Screening and prioritization
  - Weight-of-evidence
    - Aid in interpretation of *in vivo* observations
    - Aid in deciding if additional studies are/aren't needed
  - Elucidate MOAs/AOPs
  - Tailor *in vivo* testing
    - Leverage understanding of underlying biological processes
  - Point of departure derivation – not yet!
- Different levels of uncertainty tolerated depending on context of use



Conclusions

- AOP knowledge has worked well for OPs, NMCs, pyrethroids and thyroid toxicants
- NAMs could inform:
  - Screen for new pesticides
  - Prioritize testing of pesticides
  - Potential for identifying lifestage susceptibility
  - MOA/AOP discovery
  - Identify data needs
  - Interpretation of *in vivo* observations
  - Tailor future *in vivo* studies
  - Eventually, point of departure derivation