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

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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

Hazard Assessment and Characterization

Dose Response Assessment and Characterization

Exposure Assessment and Characterization

Risk Characterization

Risk = Hazard \times Exposure

NAS four-step paradigm
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

Chemical Property Profile

Toxicant

Molecular Initiating Event

Target?
(many postulated, few confirmed)

Cellular Responses

Neurodev.
proliferation
differentiation
migration
neurite growth
synaptogenesis

Organ Responses

Nervous System
Δ connectivity
(morphology,
neurochemistry,
physiology)

Individual Responses

behavior
cognition

Target-based Screening
(known MIEs)

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

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

Cell-based assay

Functional electrophysiology

Intact organism

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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)

Cognition
Behavior

Proliferation

Differentiation/Migration

Apoptosis

Growth/Synaptogenesis

Myelination

Functional Network

Cognition
Behavior

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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