U.S. Regulatory Perspective on Developmental Neurotoxicity Studies: A Focus on Pesticides
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Introduction

• USEPA’s Office of Pesticide Programs is a licensing program regulating pesticide products in the U.S.
  • Review effects of pesticides on human and ecological health
• OPP is data rich
  • Acute, subchronic, developmental, reproductive, chronic/cancer, dermal, inhalation
  • Flexibility to waive or require more data
  • DNT is a conditionally required study
Introduction

- 870.6300 Developmental Neurotoxicity; footnotes 27-29
- Footnote 27. 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.
Introduction

• Footnote 28. Study required using a weight-of-evidence approach considering:

• (i) The pesticide causes treatment-related neurological effects in adult animal studies (i.e., clinical signs of neurotoxicity, neuropathology, functional or behavioral effects).

• (ii) The pesticide causes treatment-related neurological effects in developing animals, following pre- and postnatal exposure (i.e., nervous system malformations or neuropathy, brain weight changes in offspring, functional or behavioral changes in the offspring).
Introduction

• Footnote 28. Study required using a weight-of-evidence approach considering:
  
  (iii) The pesticide elicits a causative association between exposures and adverse neurological effects in human epidemiological studies.
  
  (iv) The pesticide evokes a mechanism that is associated with adverse effects on the development of the nervous system (e.g., SAR relationship to known neurotoxicants, altered neuroreceptor or neurotransmitter responses).

• Footnote 29. The use of a combined study that utilizes the 2-generation reproduction study in rodents as a basic protocol for the addition of other endpoints or functional assessments in the immature animal is encouraged.
Introduction

- The Food Quality Protection Act (1996) instructs EPA, in making its “reasonable certainty of no harm” finding, that in “the case of threshold effects, an additional tenfold margin of safety for the pesticide chemical residue and other sources of exposure shall be applied for infants and children to take into account potential pre- and postnatal toxicity and completeness of data with respect to exposure and toxicity to infants and children.”

- Section 408 (b)(2)(C) further states that “the Administrator may use a different margin of safety for the pesticide chemical residue only if, on the basis of reliable data, such margin will be safe for infants and children.”
The test substance is administered daily, generally orally, to mated females (rats are preferred) from the time of implantation (GD 6) throughout lactation (PND 21).

At least three dose levels and a concurrent control should be used and a total of 20 litters are recommended at each dose level.

Gross neurologic and behavioral abnormalities, and the evaluation of brain weights and neuropathology during postnatal development and adulthood.

~1100 animals, often more.

Requires many months/year to conduct & analyze all aspects of the study.
OPP DNT Challenges

- Challenges on interpretation across the studies
  - Motor activity:
    - multiple types of measurements (i.e. beam breaks v. distance traveled) and quantifications make data hard to compare across compounds
    - High variability (50% or higher)
  - Morphometric data:
    - orientation of slices differs between studies and contract laboratories.
    - parameters measured (i.e. length, width, height) also differ which also makes comparison difficult
    - Learning and memory methods differ across studies
- Labs providing inadequate statistical analyses on behavioral data
- Lack of comparable methodology across studies
OPP DNT Determination

- Weight of Evidence Approach for Requiring a DNT
  - Quantitative susceptibility observed in database
  - Neurotoxicity observed or plausible
  - AOP knowledge
  - Current risk assessment endpoints may not fulfill FQPA lifestage safety finding, 10x retained if uncertainty remains
Status of DNT Studies at OPP

• Currently 101 DNT studies reviewed by OPP
• 24 DNTs currently used as points of departure
  • All 24 DNTs are based on offspring effects without corresponding maternal effects
    • Pup mortality (5)
    • Brain morphology (9)
    • Pup weight (4)
    • Behavioral changes (5)
    • Developmental delays (1)
Status of DNT Studies at OPP

• Endpoints of the 24 DNTs used as points of departure:
  • offspring brain morphology (9) and behavioral changes (5) unique to the DNT
  • pup mortality (5): effects identified in the reproduction studies
  • pup weight (4), and developmental delay (1) were also observed in the reproduction studies
Status of DNT studies at OPP

• Registration Review- a 15 year re-evaluation
  • 2 DNTs required during Registration Review
    • 1 DNT required due to unknown AOP
    • 1 DNT required but option for alternative study design; on-going
  • 13 DNT studies waived from RED requirements
    • 1 was an OP
    • 2 the liver was the target organ
    • 10 No susceptibility and no neurotoxicity or only at high doses
• DNTs also waived for pyrethroids
• DNTs waived for other OPs
DNT Study Alternatives: OPs

• In 1999 a data-call-in issued for DNTs for the organophosphates (OPs)
• 18 DNTs submitted for the OPs
  • None of the DNTs used in risk assessment
• Comparative Cholinesterase Study (CCA):
  • -10% Cholinesterase (ChE) inhibition
    • Acute (juvenile and adult)
    • Repeat (juvenile and adult)
    • Gestational (fetal)
DNT Study Alternatives: OPs

• USEPA typically reviews the CCA protocol before conducted:
  • RBC and brain ChE sampled
  • Time course data to determine the time to peak effect
  • Dose selection
    • Typically 3 doses & control
    • However, sometimes 4-5 doses but limit the sample size or only 1 sex to limit animal use.
  • High quality studies
  • Nearly all registered OPs in the US have part or all of the CCA
  • Benchmark dose analysis on the studies
DNT Study Alternatives: NMCs

- **N-Methyl Carbamates (NMCs)**
  - DNTs available for 3 NMCs
  - CCA studies available for all 8 NMCs
  - Specifically designed based on AOP
    - RBC and brain ChE
    - Time to peak (15-45 minutes) in adults and juvenile
    - Time to recovery (minutes to hours)
    - Adult and juvenile (PND11-17)
  - High quality studies
  - Benchmark dose analysis on the studies
  - ChE data relied upon for risk assessment
Conclusions

• AOP knowledge has worked well for OPs and NMCs
• CCA studies for OPs and NMCs
• CTA studies now being called in for developmental thyroid AOPs
• Lifestage data derived differences
• In vitro/alternative assays to the in-life DNT could inform:
  • a screen for future pesticides;
  • prioritize testing of pesticides;
  • potential for lifestage susceptibility and the FQPA factor