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

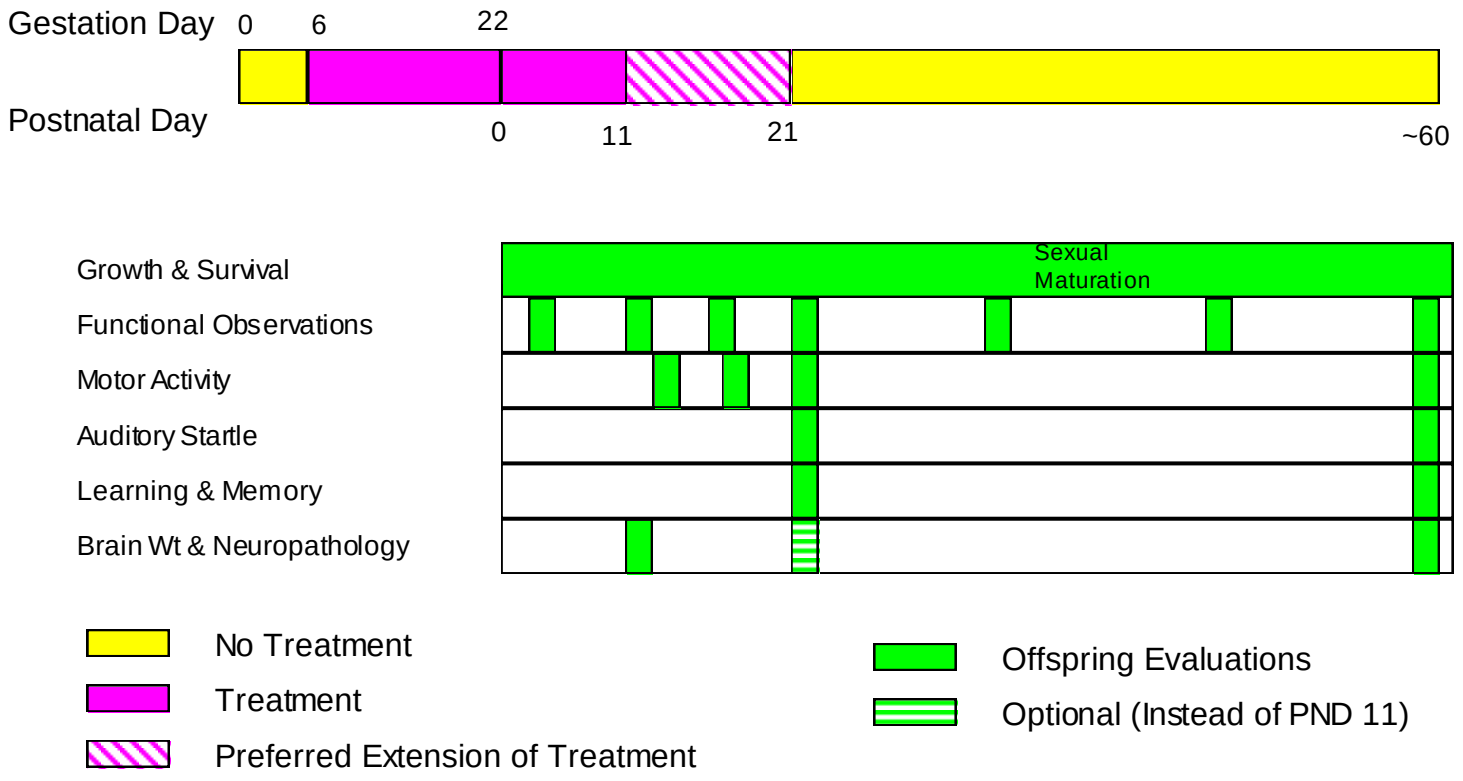
- Footnote 28. Study required using a weight-of-evidence approach considering:
- (iii) The pesticide elicits a causative association between exposures and adverse neurological effects in human epidemiological studies.
- (iv) The pesticide evokes a mechanism that is associated with adverse effects on 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."

Developmental Neurotoxicity Study

- 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