Session III: Practical methods to integrate the biological variable “sex” into research projects

Toxicology Testing Guidelines Used in Preclinical Research

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Methods and Techniques for Integrating the Biological Variable “Sex” in Preclinical Research

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The views expressed in this presentation are those of the speaker and do not necessarily represent the views or policies of the USEPA.
Testing Guidelines

Standardized testing protocols (study designs) that are developed through a rigorous and transparent process, including peer review and public comment. Guidelines are published and publicly available.

Organized into categories by topic area.

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EPA: http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm
Generally Conducted in Accordance with Good Laboratory Practice (GLP) Regulations

- Regulations that are intended to assure the quality and integrity of data for regulatory decisions
- Include specifications on:
  - Organization and personnel; facilities; equipment; animal care; standard operating procedures (SOPs); test, control, and reference substances
  - Study Director roles and responsibilities
  - Quality Assurance Unit roles and responsibilities
  - Data documentation, recording, archiving, reporting
- GLPs increase confidence in study conduct and reporting but do not address appropriateness or adequacy of study design

USEPA (TSCA), 1989, 40 CFR Part 792.
What Studies Need to be Conducted?

- Specified by (for example):
  - Toxicology Testing Requirements (FIFRA 40 CFR Part 158)
  - Data Call-In
  - Test Rules
  - Published recommendations or guidance
  - Negotiated agreements between industry and regulatory agency

- Might include non-guideline studies
Example: EPA Toxicology Data Requirements for Food-Use Pesticides

- **Acute testing**
  - Acute oral, dermal, and inhalation
  - Primary eye and dermal irritation
  - Dermal sensitization
  - Acute neurotoxicity*
- **Subchronic testing**
  - 90-day oral, dermal, and/or inhalation
  - 21/28-day dermal
  - 90-day neurotoxicity*
- **Chronic testing**
  - Chronic oral
  - Carcinogenicity
- **Developmental toxicity and reproduction**
  - Prenatal developmental toxicity
  - Reproduction and fertility effects
  - Developmental neurotoxicity*
- **Mutagenicity testing**
  - Bacterial reverse mutation assay
  - In vitro mammalian cell assay
  - In vivo cytogenetics
- **Special testing**
  - Metabolism and pharmacokinetics
  - Dermal penetration
  - Immunotoxicity*

40 CFR Part 158 (Subpart F – Toxicology)
Toxicology Testing Strategies are Influenced by Multiple Factors

- **Environmental agents (EPA)**
  - Broad screening studies to identify hazard and dose-response for use in risk assessment
  - Often a lack of important information, e.g., mode of action, toxicokinetics → rigid adherence to guidelines
  - Human exposure assumptions may be based on proposed use patterns; human biomonitoring data are often not available; generally the goal is to avoid or limit human exposure

- **Pharmaceuticals (FDA/ICH)**
  - Studies designed to focus on specific questions regarding target organ toxicity and dose safety
  - Generally an extensive database of background information is available → flexibility in study design
  - Human exposure is intentional
Toxicity Test Guidelines are Designed to Evaluate a Range of Issues

- Life stages (pre-conception to death)
- Durations of exposure (acute, subchronic, chronic)
- Routes of exposure (oral, inhalation, dermal)
- Multiple treatment levels
- Species differences
- Gender differences
- Multiple target organs
- Structural and functional effects
- Mechanistic data
- Kinetics
Regulatory Testing Guidelines

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Note that some FDA (ICH) guidelines are “guidance”.

Detailed recommendations are provided for each study design.
Most Guidelines Specify Evaluation of Both Sexes

Repeated-Dose Mammalian Toxicity Studies

Note: FDA Redbook (2000) (for safety assessment of food ingredients) includes *General Guidelines for Toxicity Studies* that specify both males and females should be tested in guideline studies.

http://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/ingredientsadditivesgraspackaging/ucm078330.htm
Some Reasons that Only One Sex Might be Used

- Strategy to reduce the number of animals used
  - Replace animals with alternative
  - Reduce the number of animals used
  - Refine the testing

- One sex has been shown to be more responsive to treatment
  - Toxico(pharmaco)kinetics (ADME)
  - Physiological differences
  - Target organ specificity
  - Life stage issues (e.g., pregnancy)

- Intended test substance administration is to the fetus (via the maternal animal)
- Human exposure is only anticipated in one sex (pharmaceuticals)
Example of Study Dosing Only Females Subjects:

**Prenatal Developmental Toxicity Study**

OPPTS 870.3700; OECD 414

**Embryo-Fetal Developmental Toxicity Study**

ICH S5(R2)

- Exposure of dams during major period of fetal organogenesis or during entire duration of gestation
- Laparohysterectomy conducted immediately prior to expected day of parturition
- Maternal evaluation:
  - Clinical observations
  - Body weight, food consumption, and/or water consumption
  - Necropsy findings
    - Macroscopic pathology
    - Ovarian corpora lutea counts
    - Non-reproductive organ weights are optional
  - Evaluation of gravid uterus
    - Gravid uterine weight
    - Implantation status
      - Counts (live, dead, early and late resorptions, empty implantation sites)
      - Placement in uterine horns
    - Examination of placental and amniotic fluid
- Fetal evaluation:
  - Fetal weight and sex
  - External examination
  - Visceral (soft tissue) examination
  - Skeletal examination

Images from www.irdg.com and Google Images
Example: Impact of Sex on Reference Value Derivation for Environmental Chemicals in the IRIS Database

- IRIS = Integrated Risk Information System (www.epa.gov/iris)
  - Contains over 550 final chemical assessments
- IRIS provides hazard identification and dose-response information that can be used in risk assessments, including reference values (RfC and RfD) for lifetime exposures
- A search of critical effects for all reference values in the database identified a number of chemicals with toxicity in only one sex at the lowest NOAEL

| IRIS Reference Values Based on Toxicity in Single Sex |
|-----------------------------------------------|-----|
|                  | Male Only | Female Only |
| Oral RfD        | 13        | 13          |
| Inhalation RfC  | 3         | 2           |

**Reference Value Derivation:**

\[
RfV = \text{NOAEL (BMDL)} / \text{UF}
\]
Inclusion of Both Sexes in Toxicity Testing Can Provide Useful Information

- Identifying gender-related susceptibility
  - Establishing safe levels for pharmaceutical administration
  - Hazard identification and dose response for risk assessment of environmental chemicals
- Data from guideline studies can provide insight for:
  - Designing clinical or epidemiology protocols
  - Identifying additional research needs