OVERVIEW OF REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY STUDIES

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REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

• OBJECTIVES
  • Describe the importance of Reproductive and Developmental Toxicity Testing
  • Describe the guidance for testing of reproductive and developmental toxicants
  • Provide an overview of reproductive and developmental toxicity endpoints
  • Describe the outcome of this type of testing
• Potential Adverse Effects of:
  • A Test Material
    • Drug
    • Medical Device
    • Chemical
  • On
    • Male and Female Fertility
    • Ability to Produce Offspring
    • Growth and Maturation of Future Generations
WHY IS THIS FIELD IMPORTANT?

- Smaller Family Size
- Planned/Timed Families
- Ever greater need to be able to produce healthy offspring when we want them
Birth defects occur in about 6% of all births.

- Drugs, chemicals and radiation: <1%
- Environmental Causes: 10%
  - Maternal disease states, metabolic deficiency, mechanical problem(s)
- Genetic Diseases: 10-25%
- Unknown: 65-75%

Adapted from Brent and Holmes. 1988. Teratology 38:241-251
WHY IS THIS FIELD IMPORTANT?

Sedative-hypnotic that produced over 5000 children with birth defects
GUIDANCE FOR TESTING

Today’s guidelines can be divided into those for **intentional** and **unintentional** exposure

**INTENTIONAL**
- Drugs, including small and large molecules
- Vaccines
- Medical Devices

**UNINTENTIONAL**
- Chemicals
- Consumer Products
- Foods
- Food Additives
GUIDANCE FOR TESTING

The outgrowth of Thalidomide and other tragedies lead to a series of guidelines and subsequent harmonization of these guidelines to aid in defining the hazard from exposure to a test material, whether it be a drug (small or large molecule), medical device or chemical (pesticide, food additive or consumer product).

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

DETECTION OF TOXICITY TO REPRODUCTION FOR MEDICINAL PRODUCTS & TOXICITY TO MALE FERTILITY

S5(R2)

Current Step 4 version
Parent Guideline dated 24 June 1993
(Addendum dated 9 November 2000 incorporated in November 2005)

This Guideline has been developed by the appropriate ICH Expert Working Group and has been subject to consultation by the regulatory parties, in accordance with the ICH Process. At Step 4 of the Process the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan and USA.
GUIDANCE FOR TESTING

<table>
<thead>
<tr>
<th>CHEMICALS</th>
<th>FOODS AND FOOD ADDITIVES</th>
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<tbody>
<tr>
<td>OECD Specific</td>
<td>EPA Specific</td>
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UNDERLYING PRINCIPLES

1. Dose-response relationships are expected
   a. Therefore more than one dose is used
   b. In most cases, three doses and a control group are standard

2. Testing up to doses that produce paternal or maternal (or very high multiples of the human dose) is necessary for adequate assessment of toxicity

3. Exposure during development of gametes (sperm or egg) or development of the conceptus (embryo or fetus) may result in different outcomes later in life depending on the exposure

4. Extrapolation of data across species to humans is most relevant when the test material is handled (metabolized) as in man
UNDERLYING PRINCIPLES

Example

- The spermatogenic cycle (production of sperm) is about 60 days in the rat plus an additional 10 days for the sperm to mature in the epididymis.
- Therefore, the effects on sperm may not be observed for a considerable time post dosing.

UNDERLYING PRINCIPLES

Example

• The first meiotic division of the ova (egg) takes place within the fetus.
  • Therefore, an effect on ova development may not be observed until that fetus is born, matures and produces the next generation.
  • Some antivirals are known to affect the ova with the effects observed only in the next generation.
UNDERLYING PRINCIPLES

Example

- Death of the conceptus or neonate
- Malformation (birth defect)
- Growth retardation
- Functional deficit
Susceptibility of Fetal Organs to Alterations as a Function of Time

Adapted from Wilson, 1965, p.256
UNDERLYING PRINCIPLES

Death & Failure to Thrive

Easy to measure in animal studies

a: dead, macerated
b: malformed
c: dead, compressed
d: live, externally normal
e: dead
UNDERLYING PRINCIPLES

Malformations

- Harder to detect over background when the spontaneous incidence of a malformation is not frequent
- Historical Control Databases very important
UNDERLYING PRINCIPLES

Variations

- Common findings observed in almost all studies across species
- Frequency of response could indicate relationship to treatment
UNDERLYING PRINCIPLES

Growth Retardation

Mouse fetuses – GD 18

May be evident at birth or only show up later in life
UNDERLYING PRINCIPLES

• Neurobehavioral changes are most commonly evaluated

• However, changes in the immune, cardiovascular, renal or other systems that can only be demonstrated if the specific system is challenged also need to be considered
GUIDANCE FOR INTENTIONAL EXPOSURE (DRUGS)

Since it is recognized that most drugs will have side effects the ICH guidance divides testing into phases or segments.

4.1.1. Fertility and early embryonic development

4.1.2. Pre- and postnatal development, including maternal function

4.1.3. Embryo-fetal development
GUIDANCE FOR INTENTIONAL EXPOSURE (DRUGS)

In addition, a segmented study design is recommended because human use of a drug product is usually limited to intervals, rather than occurring over the entire lifetime.

Christian, 2001
1. Rat Fertility & Early Embryonic Developmental Toxicity Study

Premating  Mate  GD 6  Euthanized GD 13/21

*Measures effects on fertility and early establishment of pregnancy*

2. Rat Fertility & Embryo-Fetal Developmental Toxicity Study

Premating  Mate  GD 17  Euthanized GD 21

*Measures effect on fertility, early establishment of pregnancy and organogenesis*
3. Rat & Rabbit Embryo-Fetal Developmental Toxicity Study

- Also known as teratology or organogenesis studies
- Assess effects of test material on development of offspring from implantation through closure of the hard palate, i.e., the period of organogenesis
4. Rat Pre- & Postnatal Developmental Toxicity Study

- Examines the effect of the test material on late fetal development through parturition and to weaning
- Typically extended to assessing sexual maturation, learning and memory and mating performance of the F1 generation (the offspring born to the dams that were dosed with the compound)
GUIDANCE FOR INTENTIONAL EXPOSURE (VACCINES)

• “Vaccination” is the deliberate exposure to an antigen under conditions that should not produce disease

• Vaccination still one of best means for preventing, rather than treating, infectious disease
F0 Generation

- 1st Dose
- Premating: 4 weeks
- Mating: 1 week
- Gestation: 4 weeks
- Lactation: 4 weeks

Day 1 of Study

F1 Generation

- ½ GD 29 Caesarean-Sectioning
- ½ Natural Delivery
- Postpartum: 4 weeks

- F1 Generation Euthanized
UNINTENTIONAL EXPOSURE

• When testing a chemical, it is important to establish the hazard for both developmental toxicity and male and female fertility

• The recommended studies are:
  1. Developmental toxicity studies in rodents and rabbits
  2. Multigenerational study in rodents
Rat & Rabbit Embryo-Fetal Developmental Toxicity Study

Pre-mating

Mate

Start of Dosing
GD 7 rabbits
GD 6 rats

End of Dosing
GD 28 rabbits
GD 20 rats

Euthanized
GD 29 rabbits
GD 21 rats

Health Effects Test Guidelines: Prenatal Developmental Toxicity Study, OPPTS 870.3700

OECD Guidelines For The Testing Of Chemicals. Prenatal Developmental Toxicity Study, No. 414 Section 4 Health Effects (Pink Pages)
GUIDANCE FOR UNINTENTIONAL EXPOSURE

OECD Guidelines For The Testing Of Chemicals. One Generation Reproduction Toxicity Study, No. 415 Section 4 Health Effects

Christian, 2001
GUIDANCE FOR UNINTENTIONAL EXPOSURE (MULTIGENERATIONS)

Two generational multigeneration study in rats. Adapted from Collins (1978).
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**F1 GENERATION**
(Daily clinical observations and sexual maturation, weekly body weights, feed consumption and blind observations)

**SUBSET 1**
100 Male and 100 Female Pups (one Male/Female pup/litter/dosage group)

- DG 6: Dosage Begins
- Acclimation 14 days
- Cohabitation 4 days
- DG 6 to DG 25 or DL 12
- DG 21: SCHEDULED SACRIFICE AND BRAIN WEIGHTS
- NEUROHISTOLOGICAL EXAMINATION
- 6 M & 6 F pups/dosage level
  (total of 30 male and 30 female pups)

- DG 21: First Possible Delivery
- DG 25: Last Possible Delivery
- DL 12: Scheduled Sacrifice

**SUBSET 2**
100 Male & 100 Female Pups (one Male/Female pup/litter/dosage group)

- DPs 23-30: PASSIVE AVOIDANCE TESTING
- DPs 59-67: WATERMAZE TESTING

- DP 5: Litters culled to 8 pups

**SUBSET 3**
100 Male & 100 Female Pups (one Male/Female pup/litter/dosage group)

- DPs 14, 18, 22 & 59: MOTOR ACTIVITY
- DPs 23 & 60: AUDITORY STARTLE HABITUATION
- DPs 67-69: SCHEDULED SACRIFICE

**SUBSET 4**
100 Male & 100 Female Pups (one Male/Female pup/litter/dosage group)

- DPs 80-85: SCHEDULED SACRIFICE
- NEUROHISTOLOGICAL EXAMINATION
- REGIONAL BRAIN WEIGHTS
- 6 M & 6 F/dosage group
  (30 M & 30 F total)
- DL 22: Scheduled Sacrifice (weaning)

**PARTURITION**
- DP 5: Litters culled to 8 pups

**Abbreviations:**
- DG=Day of (presumed) gestation
- DP=Day Postpartum
- DL=Day of Lactation
- M=Male
- F=Female

**Neurobehavioral Toxicity Study**

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PATERNAL/MATERNAL ENDPOINTS

In-Life Assessments
- Mortality
- Body Weight
- Body Weight Gain
- Food Consumption
- Water Consumption

Biomarkers/Exposure
- Clinical Pathology
- Bioanalytical Sampling

Terminal Procedures
- Necropsy
  - Gross Lesions
  - Organ Weights
  - Specifically Target Organs
- Histopathology
  - Could Include Tumor Evaluations
PATERNAL/MATERNAL REPRODUCTIVE ENDPOINTS

Paternal
- Mating and Fertility
- Reproductive Organ Weights
- Sperm Parameters

Maternal
- Mating and Fertility
- Estrous Cycling
- Ovarian Parameters
- Parturition, Lactation and Maternal Behavior
- Reproductive Organ Weights
DEVELOPMENTAL ENDPOINTS

- Number and Sex of Conceptuses
- Death
- Malformation/Variation (External, Soft Tissue, Skeletal)
- Retarded Growth
DEVELOPMENTAL ENDPOINTS

Functional Testing

- Reflex and Developmental Landmarks
- Functional Observational Battery (FOB)
- Passive Avoidance
- Simple Spatial Discrimination (M-Shaped Watermaze)
- Morris / Cincinnati Maze
- Delayed Spatial Alternation
- Classical Conditioning (Rabbit Eyeblink)
- Auditory Startle Habituation
- Motor Activity
OUTCOMES

• Hazard Assessment
  • Establish the ratio of the paternal/maternal toxicity levels to the reproductive and/or developmental toxicity levels.

• Ratios that are $\leq 1$ are common and expected.

• Ratios that are $> 1$ identify drugs or chemicals that might be toxic to processes of reproduction or development at dose levels that are therapeutic or appear “none” toxic in adults.

• NOAELs for Adults, Male and Female Fertility, Offspring
OUTCOMES

• Comparisons across species to man are most appropriate when the metabolism is similar across species and blood levels can be compared.

• The information on hazard derived from these studies will either be represented in the label for that product, the hazard rating for the chemical or the acceptable daily in-take.
CONCLUSIONS

• Importance of Reproductive and Developmental Toxicity Testing

• Guidance for testing of reproductive and developmental toxicants
  • ICH: http://www.ich.org
  • OECD: http://www.oecd-ilibrary.org/
  • EPA: http://www.epa.gov/ocspp/pubs/frs/home/testmeth.htm

• Reproductive and developmental toxicity endpoints
  • Paternal/Maternal
  • Reproductive
  • Developmental

• Outcomes
  • Establish No-observable-adverse-effect-level (NOAEL) for paternal/maternal toxicity, reproductive toxicity and developmental toxicity
  • Hazard Assessment