Toxicology for Industrial and Regulatory Scientists

Reproductive & Developmental Toxicology Studies

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Reproductive Toxicity
Testing for Pharmaceuticals

Goals
- Overview of reprotox study design & evaluation endpoints
- Allow better communication with reprotox study director
- Understand and interpret reprotox data
- Allow better communication of reprotox data to Project Team, Clinicians, Regulators, etc.

Importance of nonclinical reprotox studies
- Difficult/unethical to perform these studies in humans (lack of human data to assess risk to reproduction)
- Data will be used for risk assessment in product label
Reproductive Toxicity
Testing for Pharmaceuticals

Outline

- Biology/hormonal regulation of mammalian male and female reproductive systems
- Mammalian reproductive cycle and development
- Fertility and early embryonic development study
- Embryo-fetal development studies
- Pre- and postnatal development study
- Juvenile animal study
Male Reproductive System

Rodent (Frontal View)  Human (Frontal View)
Male Reproductive System: Testis

Section of seminiferous tubule

- Spermatid (n)
- Secondary spermatocyte (n)
- Primary spermatocyte (2n)
- Tight junction*
- Spermatogonium (2n)

* Forms the blood-testis barrier

- Spermatozoa (n)
- Leydig cell
- Sertoli cell

* Forms the blood-testis barrier
Cycle of Seminiferous Epithelium

Seminiferous tubule

Rats ≈ 60 days
Men ≈ 74 days

Transit of sperm from testis to cauda epididymis

Rats ≈ 4 days
Men ≈ 2 days

Cycle Figure from Russell et al., Histological and Histopathological Evaluation of the Testis, Cache River Press, Clearwater, FL, 1990, with permission.
Rat Testis: Cross-Section

Roman numeral in lumen indicates stage of spermatogenesis
Hormonal Control of Testicular Functions

- Hypothalamus
- GnRH
- Inhibin
- Testosterone
- Pituitary
- FSH
- LH
- Leydig cells
- Seminiferous tubule
- Testosterone
- DHT
- ABP

Male sexual development, protein synthesis, cell growth, etc.

ABP: androgen-binding protein
DHT: dihydro-testosterone
Male Reproductive System

- Hypothalamus: receives input from the CNS and rest of the body; secretes GnRH to stimulate the pituitary.
- Pituitary: responds to GnRH by releasing LH and FSH.
- Testis: responds to LH/FSH, produces sperm, secretes
  - Testosterone from Leydig cells
  - Androgen-binding protein and inhibin by Sertoli cells
- Epididymis*: a single tube that modifies sperm to confer motility and fertilizing ability.
- Accessory Sex Organs (Prostate & Seminal Vesicles)*: contribute fluid that carry sperm, provide beneficial environment for sperm motility and transport.

* Functions regulated by testosterone and dihydro-testosterone
Female Reproductive System

Rodent (Frontal View)       Human (Frontal View)
Ovulation, Fertilization, & Implantation

Theca cells (stimulated by LH, participate in estradiol synthesis)
Female Reproductive System: Hormonal Control

- GnRH from the hypothalamus stimulates the pituitary gland to release FSH and LH.
- FSH stimulates the growth of ovarian follicles and the differentiation of granulosa and theca cells.
- LH stimulates ovulation and the growth of the corpus luteum.
- After ovulation, the corpus luteum continues to produce progesterone and estrogen.
- Progesterone and estrogen feedback to the hypothalamus and pituitary to regulate menstrual cycle.

*In rats, mice, and hamsters, the menstrual cycle is negative/positive feedback regulated.

Diagram symbols:
- Blue arrows: Stimulation/secretion
- Green arrows: Inhibin
- Red arrows: Prolactin
- Dashed red arrows: Negative/positive feedback

* In rats, mice, hamster
Female Reproductive System

- Hypothalamus: receives input from the CNS and rest of the body; secretes GnRH to stimulate the pituitary.

- Pituitary: responds to GnRH by releasing LH and FSH.

- Ovary: responds to LH/FSH, releasing oocytes, secretes
  - Estradiol from follicular cells
  - Progesterone and inhibin from corpus luteum

- Oviduct, Uterus, Cervix, Vagina*: Oocyte “handling”, facilitate and enable egg and sperm transport, and maintain pregnancy.

* Functions of these organs regulated by estradiol and progesterone
Hormonal Changes During Human Menstrual Cycle and Pregnancy

- LH: luteinizing hormone
- FSH: follicle-stimulating hormone
- Progesterone
- Estradiol
- hCG: human chorionic gonadotropin

P. Foster with permission
Temporal Comparison of Menstrual vs. Estrous Cycles

**Human**
- Follicle
- Menses
  - Day 1

**Rat**
- Diestrus (48 hr)
  - Day 1
- Proestrus (18 hr)
  - 2
- Estrus (28 hr)
  - 3
- Metestrus (6 hr)
  - 4

**Corpus Luteum**
- 14
- 28

**Ovulation**
- 15
Reproductive Cycle & Reproductive Toxicity Studies

Segmented approach to evaluate reproductive toxicity for pharmaceuticals

Seg. I  Fertility & early embryonic development study

Seg. II  Embryo-fetal development study

Seg. III Pre- & postnatal development study
Fertility & Early Embryonic Development Study
Fertility & Early Embryonic Development Study

Purpose

- To assess effects of compound on fertility and early embryonic development when exposure occurred during gamete development, fertilization & before implantation

Timing of study during drug development [ICH M3(R2)]

- Required before Phase III for subjects with reproductive capabilities
- Not needed for inclusion of subjects with reproductive potential in Phase I/II if adequate microscopic exams were performed in reproductive organs in repeat-dose study of at least 2 weeks in duration
- Not needed for drugs intended to treat patients with advanced cancer [ICH S9]. Use general tox data to assess effects on reproductive organs
Fertility & Early Embryonic Development Study (Segment I)

- **Gamete Maturation**
  - **Mating**
  - **In-life Termination**
    - Termination:
      - Male: 2-10 weeks before cohabitation, during cohab. & 2-3 weeks post-mating
      - Female: 2 weeks before cohabitation, during cohab. & until GD 6/7 (monitor estrous cycle until successful mating)
    - Treatment Period: n=20/sex/group

- **Gamete in utero Development**
  - **F0**
  - **F1**

Evaluate effects on:
- F0 gamete maturation
- F0 mating behavior & fertility
- F1 implantation & pre-implantation embryos

GD: Gestation Day
Reproductive Endpoints for Males

- Fertility and fecundity (ability to produce litters, number of implants or embryos/litter)
- Mating behavior, time to mate, etc.
- Organ weights (testis and accessory organs)
- Sperm indices
  - Sperm count in testis and epididymis (sperm production)
  - Sperm motility (sperm function)
  - Sperm morphology (sperm quality)
- Histopathology (typically performed in repeat-dose study)
  - Effects on spermatogenesis (most sensitive endpoint in male)
Reproductive Endpoints for Females

- Successful fertilization, implantation, and gestation
- Estrous cyclicity (sensitive marker of hormonal changes)
- Number of implants, embryo/fetus, numbers pregnant per group
- Organ weights and histology
Fertility & Early Embryonic Development Study: Evaluation Endpoints

- Estrous cyclicity
- Precoital interval
- Mating and fertility indices
- Cesarean section parameters
- Organ weights
- Sperm parameters
- Histopathology
### Male Fertility Index (%)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Low Dose</th>
<th>Mid-Dose</th>
<th>High Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>100</td>
<td>92</td>
<td>46**</td>
<td></td>
</tr>
</tbody>
</table>

### % Motile Sperm

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Low Dose</th>
<th>Mid-Dose</th>
<th>High Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>88</td>
<td>66**</td>
<td>16**</td>
<td></td>
</tr>
</tbody>
</table>

### Epididymal Sperm Count (10^6/ g cauda epididymis)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Low Dose</th>
<th>Mid-Dose</th>
<th>High Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>510</td>
<td>508</td>
<td>440**</td>
<td>220**</td>
<td></td>
</tr>
</tbody>
</table>

** Statistically different from control value
Interpretation

- Male fertility affected at the high dose
- Sperm parameters affected at the mid- and high dose
  - Rodent has large sperm reserve and can tolerate some effects on sperm without effect on fertility
  - Same is not true for humans
  - For potential risk on sperm parameters in men, needs to evaluate exposure margin between mid-dose and therapeutic dose
Embryo-Fetal Development Study
Embryo-Fetal Development Study

Purpose

- To assess effects of compound on embryo-fetal development when exposure occurred *in utero*

Timing of study during drug development [ICH M3(R2)]

- Required before exposure to women of childbearing potential (WOCBP)
- Not needed for inclusion of WOCBP if
  - Short term trials (e.g., 2 weeks) with intensive control of pregnancy risk
  - Trials must include WOCBP, but with sufficient precautions to prevent pregnancy
- WOCBP (up to n=150, with adequate birth control) may be included in Phase I/II (up to 3 months) if data from preliminary embryo-fetal development studies (2 species) are available

For anticancer drug [ICH S9]

- Not needed if genotoxic or has class effect on developmental tox
- If required, complete by marketing application (NDA/MAA)
Overview of Human Development

Fertilization

<table>
<thead>
<tr>
<th>Implantation</th>
</tr>
</thead>
</table>

Period of Organogenesis

Modified from Moore 1988
Window of Sensitivity is Greatest during Organogenesis

Degree of Sensitivity

Fertilization
Implantation
Organogenesis
Birth

Rat Gestation (Days)

Period of Organogenesis = Treatment Period for Segment II Study

Modified from Wilson (1973)
**Stages of *in utero* Development**

<table>
<thead>
<tr>
<th></th>
<th>Implantation*</th>
<th>Organogenesis Ends*</th>
<th>Birth*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>5</td>
<td>15</td>
<td>19-20</td>
</tr>
<tr>
<td>Rat</td>
<td>5-6</td>
<td>16</td>
<td>21-22</td>
</tr>
<tr>
<td>Rabbit</td>
<td>6-7</td>
<td>19</td>
<td>30-32</td>
</tr>
<tr>
<td>Monkey</td>
<td>9**</td>
<td>44-45</td>
<td>166</td>
</tr>
<tr>
<td>Human</td>
<td>6-7</td>
<td>50-56</td>
<td>266</td>
</tr>
</tbody>
</table>

* Days from fertilization

** Segment II
** Treatment Period

- Embryonic Period
  - (Establishment of body form, Organogenesis)

- Fetal Period
  - (Growth/differentiation of organ systems)

** Dosing typically starts on GD 20 due to the need to confirm pregnancy by ultrasound on GD 18-20.
Embryo-Fetal Development Study (Segment II or Teratology*)

F0 Mating

♂

In-life Termination

♀

F1 in utero

Development

Treatment Period

* Study of Terata (Monster) or birth defects

Evaluate effects on
- Viability of F1 embryos & fetuses
- Growth of F1 embryos & fetuses
- Structural development of F1 embryos & fetuses
Objective: Assess test article toxicity on maternal gestation and embryonic and fetal development

- Conventionally one rodent species and a non-rodent species (rabbit)
- Exposure post-implantation to end of organogenesis
- Endpoints: evaluate maternal toxicity, embryo/fetal death, fetal external, soft tissue and skeletal alterations

Female

| GD 0 | Rat GD 6
Rabbit GD 6/7 | Rat GD 16
Rabbit GD 20 | Cesarean Section + Fetal Exams

GD 0=Gestation Day 0, day when mating is confirmed

* Blood samples are typically obtained from satellite animals for toxicokinetics

Range-finding study is usually performed to select doses for the definitive study: typically 4-5 dose groups + controls, n=5-6/group, limited fetal exam (external)
Objective: Assess drug toxicity on maternal gestation & embryonic and fetal development, in order to allow inclusion of women of childbearing potential (up to 150, using adequate birth control methods) for trials of up to 3 months [ICH M3(R2)]

- One rodent species and a non-rodent species (rabbit)
- Exposure from post-implantation to end of organogenesis
- Endpoints: evaluate maternal toxicity, embryo/fetal death, fetal external and soft tissue alterations

Female n=6-8/group

GD 0 = Gestation Day 0, day when mating is confirmed

Rat GD 6
Rabbit GD 6/7

Rat GD 16
Rabbit GD 20

Cesarean Section + Fetal Exams

Rat GD 20-21
Rabbit GD 29-30

Treatment Period
Endpoints in Embryo-Fetal Development Studies

- **Maternal endpoints**
  - Clinical signs, abortion (rabbit only)
  - Food consumption, body weights, body weight gains

- **Fetal endpoints**
  - Number, sex ratio, viability, weight
  - External examination (all live fetuses)
  - Rats
    - Half get examined for internal soft tissue structure, using conventional and commonly-accepted dissection methods
    - Other half get examined for hard tissue structure (Alizarin Red staining for calcified tissue, sometimes Alcian Blue staining for cartilage)
  - Rabbits: all animals get examined for soft tissue structures followed by hard tissue stains and examination
Endpoints of Embryo-Fetal Development

- Altered Survival (live/dead embryos or fetuses)
  - Pre-implantation loss
  - Post-implantation loss*

- Structural Changes (external, visceral, skeletal)
  - Variation*
  - Malformation*

- Developmental Delays – usually recoverable
  - Growth (body weight, size)*
  - Skeletal development (ossification)*

- Functional Deficits – not evaluated in Segment II
  - Biochemical
  - Behavioral

* Evaluated and used to assess risk of exposure
Malformations and Variations

- Malformation is a permanent structural change that may adversely affect survival, development or function.

- Variation is a divergence beyond the usual range of structural constitution that may not adversely affect survival or health.

Examples:
- Hydrocephaly
- Spina bifida
- 7th Cervical rib
- Incomplete centrum ossification
Data Interpretation

- **Litter is the statistical unit**
- Maternal toxicity can affect fetal development (e.g., low fetal weight, supernumerary ribs)
- Some changes are recoverable after birth (e.g., wavy ribs, delayed ossification)
- Read changes against concurrent controls and against the historical control database in the lab
- To tell if something is noise or really treatment-induced
  - Look for patterns
  - Dose-relationship
  - Greater number of dams affected (rather than many pups from 1 dam)
# Litter Mean Calculation

| Litter No. | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | Total | Mean |
|------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|------|------|
| No. affected fetuses | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 4  | 0  | 1  | 0  | 0  | 1  | 6  |      |
| No fetuses/litter     | 7  | 10 | 8  | 10 | 7  | 8  | 8  | 7  | 9  | 14 | 4  | 8  | 4  | 9  | 8  | 121  | 5%   |
| % affected fetuses/litter | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 28.6 | 0  | 12.5 | 0  | 0  | 12.5 | 53.6% | 3.6% |

Total no. affected fetuses = 6  
Total no. fetuses evaluated = 121  
\[ \frac{6}{121} \times 100 \approx 5\% \]  
Correct value  
\[ \frac{53.6}{15} \approx 3.6\% \]  
* Incorrect value because did not consider individual litter as an experimental unit, i.e., each litter contains different no. of fetuses.
Integrated Approach in Data Interpretation

- Spontaneous vs. treatment-related malformations/variations
- Dose-response relationship
- Presence/absence of maternal toxicity
- Cross-species concordance
- Similarities/differences in pharmacokinetics (test species vs. humans)
- Exposure ratio (test species/humans)
# Embryo-Fetal Development Study

## Case Study

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Low Dose</th>
<th>Mid-Dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal Body Weight Gain</strong></td>
<td>Normal</td>
<td>Normal</td>
<td>4% less than control value</td>
<td>18% less than control value**</td>
</tr>
<tr>
<td><strong>Mean Fetal Weight (g)</strong></td>
<td>3.5</td>
<td>3.8</td>
<td>3.4</td>
<td>2.9**</td>
</tr>
<tr>
<td><strong>% Fetus With Delayed Ossification</strong></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5**</td>
</tr>
</tbody>
</table>

** Statistically different from control value
Interpretation

- Maternal toxicity at the high dose
- Fetal effects observed at the high dose
  - Low fetal weight and delayed ossification are signs of growth retardation
- Fetal effects likely due to maternal toxicity (low weight gain) at the high dose
Pre- & Postnatal Development Study
Pre- and Postnatal Development Study

Purpose

- To assess effects of compound on pre- and postnatal development when exposure occurred \textit{in utero} and during postnatal development (via mother’s milk)

Timing of study during drug development [ICH M3(R2)]

- Required for marketing approval submission (NDA/MAA)
- Not needed for drugs intended to treat patients with advanced cancer [ICH S9]
Pre- & Postnatal Development Study (Segment III)

- **F0**
  - Development
  - in utero

- **F1**
  - Delivery

- **Treatment Period**
  - GD 6 – LD 20 (n=20/group)

- **In-life**
  - Sexual Maturation
  - Mating
  - Gestation

- **F1 GD 13**
- **F1 LD 4**

Evaluate effects on:
- Maternal gestation, parturition, nursing behavior & lactation
- F1 viability and growth
- F1 functional development & reproductive capability

GD = Gestation Day, day of mating confirmation is GD 0
LD = Lactation Day, day of delivery is LD 0
Endpoints of Pre-/Postnatal Development Study

Maternal (F0) parameters

- Clinical signs, food consumption, body weights, body weight gains
- Gestation length
- Parturition, nesting & nursing behavior
- Litter size
- Lactation (presence/absence of milk in the stomach of offspring)
Endpoints of Pre-/Postnatal Development Study

F1 Parameters

- Viability (usually culled to 4/sex/litter on Lactation Day 4)
- Growth (body weight)
- Physical development
  - Pinna detachment
  - Incisor eruption
  - Eyelid opening
  - Vaginal opening
  - Preputial separation
- Reflexological & Sensory Development
  - Righting reflex
  - Negative geotaxis
  - Pupillary reflex
  - Preyer reflex
Endpoints of Pre-/Postnatal Development Study

F1 Parameters (cont.)

- Motor activity functions
  - Open field test
  - Figure “8” maze
- Learning and memory tests
  - Passive avoidance
  - Water maze
- Reproductive performance
  - F1 Fertility
  - F1 Gestation
  - F2 viability
Data Interpretation

- Litter is the statistical unit (where appropriate)
- Maternal toxicity can affect growth and development of offspring
- Endpoints ranked by sensitivity
  - Viable litter size
  - Neonatal growth (body weight is the most sensitive indicator) & survival
  - Gestation length
  - Landmarks of sexual maturity
  - Functional maturation, learning & memory capability
- Evaluate all study data as a whole, not individual endpoints in isolation
- To tell if something is noise or really treatment-related
  - Dose-relationship
  - Greater number of dams affected
# Pre- and Postnatal Development Study
## Case Study

## Landmarks of Sexual Development

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Low Dose</th>
<th>Mid-dose</th>
<th>High Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preputial Separation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(PND)</td>
<td>43</td>
<td>44</td>
<td>47**</td>
<td>47**</td>
</tr>
<tr>
<td><strong>Body weight (g)</strong></td>
<td>236</td>
<td>235</td>
<td>218**</td>
<td>215**</td>
</tr>
</tbody>
</table>

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vaginal Opening</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(PND)</td>
<td>32</td>
<td>32</td>
<td>33</td>
<td>36**</td>
</tr>
<tr>
<td><strong>Body weight (g)</strong></td>
<td>111</td>
<td>108</td>
<td>115</td>
<td>95**</td>
</tr>
</tbody>
</table>

PND: Postnatal Day  
** Statistically different from control value
Pre- and Postnatal Development Study
Case Study

Interpretation

- Sexual maturation was delayed in males and females
- Body weights in the affected groups were correspondingly lower than controls
- Delay in sexual maturation was due to growth retardation
Range-finding Study

- Fertility & early embryonic development study
  - Range-finding study often not necessary
  - Typically use data from repeat-dose studies to select doses

- Embryo-fetal development studies
  - Rat: best to do a range-finding study
  - Rabbit: range-finding study a must

- Pre- & postnatal development study
  - Range-finding study often not necessary
  - Typically use data from embryo-fetal development study to select doses
Dose Selection

High dose

- Should produce maternal or parental toxicity
- In the absence of maternal toxicity, limit dose or maximum feasible dose may be used
  - Sufficient exposure should be demonstrated
- In the absence of parental toxicity or sufficient exposure, alternative vehicle, route, dosing regimen, or species should be considered

Low dose

- Should be the No-Observed-Adverse-Effect Level (NOAEL)
  - Exposure at multiples of clinical efficacious dose, if possible
Toxicokinetics in Reproductive Toxicity Studies

- Generally not determined in
  - Fertility and early embryonic development study
  - Pre- and postnatal development study

- Embryo-fetal development studies
  - Not a requirement but generally performed in dam/doe to determine maternal exposure
  - Useful for new drug, to provide exposure margin over human exposure (assessment of human risk in drug label)
  - Generally not determined in fetus due to
    - Technical difficulties in obtaining sufficient fetal blood samples
    - No comparative information in humans
Assessment of Risk in Reproduction & Pregnancy


Pregnancy Categories
A: Not teratogenic in humans
B: Not teratogenic in animal & no controlled human data
C: Teratogenic in animals & no controlled human data
D: Teratogenic in humans but benefit to mother may outweigh risk to fetus
X: Teratogenic in humans, risk of use during pregnancy outweighs any possible benefit

Pregnancy & Lactation Labeling Rule (P LLR)*

8.1 Pregnancy
- Pregnancy exposure registry
- Risk summary (required information)
- Clinical considerations
- Data

8.2 Lactation
- Risk summary (required information)
- Clinical considerations
- Data

8.3 Females and males of reproductive potential
- Pregnancy testing
- Contraception
- Infertility

* Final Rule: Content and Format of Labeling for Human Prescription Drug and Biological Products; Requirements for Pregnancy and Lactation Labeling (effective June 30, 2015)
Reproductive/Developmental Toxicity Studies for Chemicals
Reproductive/Developmental Toxicity Studies for Chemicals*

- Prenatal developmental toxicity study
  - Exposure from gestation day (GD) 6/7 through end of pregnancy (GD 20 in rats and GD 28/29 in rabbits)
  - C-section on the day before delivery, perform fetal exams similar to Segment II studies for drugs

- Reproduction and fertility effect study (2-generation reproduction study)
  - Exposure 10 weeks (males/females) before cohabitation, during cohabitation, pregnancy and lactation
  - Exposure continues for selected F1 offspring that are mated and allowed to deliver and maintain their (F2) pups to weaning
  - Assess effects on reproductive development for 2 generations

- Developmental neurotoxicity study
  - Exposure from GD 6 to postnatal day 10 or 21
  - Evaluate developing nervous system

* See guidelines listed in Slide No. 75.
Juvenile Animal Study
Juvenile Animal Study

Purpose

- To assess effects of compound on postnatal growth and development when (direct) exposure occurred from neonatal to pre-adult period

Timing of study during drug development [ICH M3(R2)]

- Prior to long-term pediatric study
- Generally not needed to support short-term pharmacokinetic studies in pediatric population (e.g., 1 to 3 doses)
Different Age Groups in Pediatric Population & Juvenile Animals

<table>
<thead>
<tr>
<th></th>
<th>Human</th>
<th>Rat</th>
<th>Dog</th>
<th>Cynomolgus Monkey</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neonate</strong></td>
<td>Birth – 1 month</td>
<td>Birth – PND 7</td>
<td>Birth – 3 weeks</td>
<td>Birth – 4 months</td>
</tr>
<tr>
<td><strong>Infant</strong></td>
<td>1 – 24 months</td>
<td>PND 7 – 21</td>
<td>3 – 6 weeks</td>
<td>4 – 6 months</td>
</tr>
<tr>
<td><strong>Child</strong></td>
<td>2 – 12 years</td>
<td>PND 21 – 35</td>
<td>6 – 20 weeks</td>
<td>6 – 36 months</td>
</tr>
<tr>
<td><strong>Adolescent</strong></td>
<td>12 – 16 years</td>
<td>PND 35 – 60</td>
<td>5 – 8 months</td>
<td>3 – 5 years</td>
</tr>
</tbody>
</table>

PND=Postnatal Day

Pediatric population has different subgroups, each showing a different stage/rate of growth and organ/system maturation.
## Physiology/Pharmacokinetics in Neonate/Infant Compared to Adult

<table>
<thead>
<tr>
<th>Neonate/ Infant</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GI Motility/ pH</strong></td>
<td>Lower GI motility (↑ GI absorption), higher pH (↑ absorption of basic molecule, ↓ absorption of acidic molecule)</td>
</tr>
<tr>
<td><strong>Protein Binding</strong></td>
<td>Typically lower, more free compound</td>
</tr>
<tr>
<td><strong>Total Water Content</strong></td>
<td>Higher, affects volume of distribution</td>
</tr>
<tr>
<td><strong>Metabolism</strong></td>
<td>Enzymes not fully developed, affecting pharmacokinetics</td>
</tr>
<tr>
<td><strong>Biliary Excretion</strong></td>
<td>Lower, may ↑ half-life</td>
</tr>
<tr>
<td><strong>Glomerular Filtration</strong></td>
<td>Lower, may ↑ half-life</td>
</tr>
</tbody>
</table>
Organ/System Maturation Period in Human

- **Urinary System**
- **Pulmonary/GI Systems**
- **Cardiovascular/Immune Systems**
- **Biotransformation Enzymes**
- **Central Nervous/Reproductive Systems**
- **Skeletal System**

Dosing period in juvenile animal study will be determined by target organs that are undergoing development during clinical exposure.
Non-Clinical Studies Performed to Support Pediatric Use

Animals should be treated throughout the stages of development that are comparable to the timing of exposure in the intended pediatric population.
**Adult vs. Juvenile Animal Study**

Evaluate drug’s effect on developmental stage and effect of developmental stage on drug

<table>
<thead>
<tr>
<th></th>
<th>Repeat-Dose Studies in Adult Animals</th>
<th>Juvenile Animal Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at 1st Dose</strong></td>
<td>Young adult</td>
<td>Varies, depending on pediatric regimen</td>
</tr>
<tr>
<td><strong>Dosing Period</strong></td>
<td>Typically standard, e.g., 4-, 13-week</td>
<td>Varies, depending on pediatric regimen</td>
</tr>
<tr>
<td><strong>Study Endpoints</strong></td>
<td>Typically standard</td>
<td>Study-specific</td>
</tr>
</tbody>
</table>
Factors Influencing Juvenile Animal Study Design

- Pediatric indication
- Pediatric population
  - Age range
  - Duration of exposure
- Toxicity of drug in adult human/animal
- Whether target organs identified in adults undergo significant postnatal development
- Differences in pharmacology, pharmacokinetics, and toxicity profiles between adult and pediatric population
Juvenile Animal Study Design (1)

Step-wise Approach in Designing Juvenile Animal Study

1. Determine dosing regimen in pediatric population
   - Dosing begins from age X
   - Dosing extends from age X to Y

2. Identify appropriate
   - Species
   - Target organs (observed in adult humans and animals)
   - Developing organs/systems in humans at age X to Y, and corresponding developing intervals for these and target organs in test species

3. Determine age at 1st dose and dosing period based on #1 and #2

4. Study should include evaluation of effects on
   - Organs/systems in #2
   - Growth, functional & structural development, as appropriate
Step-wise Approach in Designing Juvenile Animal Study

5. Dose route
   - Intended route of clinical exposure, as appropriate
   - Use labs whose technicians are competent in dosing young pups

6. Essential to perform pilot/range-finding study

7. Study should include
   - Toxicokinetic evaluation
   - Recovery assessment

Because the study design is usually complicated, uses many animals, takes a long time to complete and expensive, important to design the study appropriately

Where possible, get concurrence on study design from regulatory agencies
Summary

- Important to understand biology/timing of development
- Embryo/fetus depends on dams for growth & development
- Important to distinguish between direct fetal toxicity & fetal changes due to maternal toxicity
- Litter is the unit for evaluation/comparison
- Use integrated approach to evaluate reproductive toxicity
- Fetal/pup weight is the most sensitive indicator for growth & development
- For juvenile animal studies
  - Need to know the age range and duration of exposure, target organs, timing of organ/system development in animals vs. humans
Male Fertility Study

* Control and high-dose males mated twice, (1) after 11 weeks of dosing [for up to 3 weeks], (2) 2 weeks after the end of dosing (at the end of 1st mating period)
Low and mid-dose males sacrificed at Week 17-18, high-dose males sacrificed at the end of 2nd pairing
ICI 204,636: Segment I Studies in Alpk:ApfSK (Wistar-derived) Rats

Female Fertility Study

Mating

F0

2 weeks

F1 in utero Development

F1 males sacrificed at the end of mating

F1 growth/development

F1 females sacrificed on GD 21 or LD 22-24

Treatment Period

GD: Gestation Day
LD: Lactation Day

① C-section on GD 21 to perform uterine/fetal exam

② Dosing completed LD 22-24
ICI 204,636: Embryo-Fetal Development Studies

F0 Mating

Treatment Period

Alpk:ApfSK rats: GD 6-15
Dutch Belted rabbits: GD 6-18

GD: Gestation Day

F1

in utero

Development

In-life Termination

♀

♂
ICI 204,636: Peri- & Postnatal Development Study

Alpk:ApfSK (Wistar-derived) Rats

Treatment Period

GD: Gestation Day
LD: Lactation Day

♂

♀

F0

F1 in utero Development

GD 16

Delivery

Termination at LD 21

F1
Abbreviations

- **ABP**  Androgen-binding protein
- **CNS**  Central nervous system
- **DHT**  Dihydro-testosterone
- **FSH**  Follicle-stimulating hormone
- **GD**   Gestation day
- **GnRH** Gonadotropin-releasing hormone
- **LD**   Lactation day
- **LH**   Luteinizing hormone
- **MAA**  Marketing Authorisation Application
- **NDA**  New Drug Application
- **NHP**  Nonhuman primate
- **PND**  Postnatal day
- **WOCBP** Women of childbearing potential
References (1)

1. ICH M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals (June 2009).


References (2)


Reproductive Toxicity Testing Guidelines for Chemicals


Glossary of Reproductive Indices/Terms (1)

Precoital Intervals (Days) = Number of days to mate successfully

Male Mating Index (%) = \( \frac{\text{Number of } \delta \text{ with evidence of mating}}{\text{Total number of } \delta \text{ used for mating}} \) \times 100

Male Fertility Index (%) = \( \frac{\text{Number of } \delta \text{ siring at least 1 litter}}{\text{Total number of } \delta \text{ with evidence of mating}} \) \times 100

Female Mating Index (%) = \( \frac{\text{Number of } \varphi \text{ with evidence of mating}}{\text{Total number of } \varphi \text{ used for mating}} \) \times 100

Female Fertility Index (%) = \( \frac{\text{Number of } \varphi \text{ with confirmed pregnancy}}{\text{Total number of } \varphi \text{ with evidence of mating}} \) \times 100

Gestation Index (%) = \( \frac{\text{Number of } \varphi \text{ with live pup}}{\text{Number of } \varphi \text{ with evidence of pregnancy}} \) \times 100
Pre-implantation Loss (%): \[
\text{Pre-implantation Loss (\%)} = \frac{\text{Number of corpora lutea} - \text{Number of implants}}{\text{Total number of corpora lutea}} \times 100
\]

Post-implantation Loss (%): \[
\text{Post-implantation Loss (\%)} = \frac{\text{Number of implants} - \text{Number of viable embryos}}{\text{Total number of implants}} \times 100
\]

Live Birth Index (%): \[
\text{Live Birth Index (\%)} = \frac{\text{Number of pups born alive}}{\text{Total number of pups born}} \times 100
\]

Survival Index (%): \[
\text{Survival Index (\%)} = \frac{\text{Number of live pups (at a given time point)}}{\text{Total number of pups born}} \times 100
\]

Lactation Index (%): \[
\text{Lactation Index (\%)} = \frac{\text{Number of pups with evidence of milk in stomach}}{\text{Total number of pups born}} \times 100
\]
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