Nonclinical Studies –
What animal studies can (and can’t) tell us about drugs in milk

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FDA Lactation Workshop
27 – 28 April 2016
Disclosure

I am a regulatory toxicology consultant, advising pharmaceutical companies on non-clinical testing strategies during drug development.
Presentation Outline

• DART Testing Requirements
• Studies with lactational animals
• Extrapolation to human risk
  – Drugs in milk across species
• Concluding remarks
PLLRR Requirements

• Risk Summary – Lactation
  – Effects on milk production
  – Presence of drug in human milk
  – Effects on the breastfed child
  – Animal data not included if human data exist
  – Animal data, when included, should only state presence or absence of drug in milk
ICH S5(R2): Reprotox Testing Guidelines

Evaluate the entire reproductive life cycle, including the following….

A. **Premating to conception** - adult male and female reproductive functions, development and maturation of gametes, mating behavior, fertilization

B. **Conception to implantation** - adult female reproductive functions, preimplantation development, implantation

C. **Implantation to closure of the hard palate** - adult female reproductive functions, embryonic development, major organ formation

D. **Closure of the hard palate to the end of pregnancy** - adult female reproductive functions, fetal development and growth, organ development and growth

E. **Birth to weaning** - adult female reproductive functions, neonate adaptation to extrauterine life, preweaning development and growth

F. **Weaning to sexual maturity** - postweaning development & growth, adaptation to independent life, attainment of full sexual function
## Standard Study Designs ICH S5(R2)

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
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</thead>
<tbody>
<tr>
<td>Premating to Conception</td>
<td>Conception to Implantation</td>
<td>Implantation to Closure of Hard Palate</td>
<td>Hard Palate Closure to End of Pregnancy</td>
<td>Birth to Weaning</td>
<td>Weaning to Sexual Maturity</td>
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<tr>
<td><strong>Fertility Study - Rodent</strong></td>
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<td><strong>Embryo-Fetal Development Study (EFD)</strong>(2)</td>
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<td>Rodent, Rabbit (NHP)</td>
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<td><strong>Pre- and Postnatal Development Study</strong></td>
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Denotes Dosing Period
Pre-/Postnatal Development Study in the Rat

Group size = 24 females

Pregnant Females

Dosing Starts (Moms)

Parturition

Weaning

GD 0

GD 6

GD 22

PND 21

Exposure in utero

Exposure via milk

F₁ Survival, Growth, Behavior, Reproductive Capability

F₁ Adults
# PPN Endpoints

<table>
<thead>
<tr>
<th>$F_0$ Mothers</th>
<th>$F_1$ Offspring</th>
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<tbody>
<tr>
<td>Adult Toxicity</td>
<td>Survival</td>
</tr>
<tr>
<td>Gestation, Parturition</td>
<td>Growth</td>
</tr>
<tr>
<td>Lactation Process</td>
<td>Behavior</td>
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<tr>
<td></td>
<td>• motor activity</td>
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<td></td>
<td>• learning and memory</td>
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<td>• Reflex development</td>
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<tr>
<td>Reproduction</td>
<td>Systemic Exposure</td>
</tr>
<tr>
<td><em>(Drug in Milk)</em></td>
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</table>

- Drug in milk **not** typically measured
- Systemic exposure in nursing pups measured more often, but not universally
- Data in offspring confounded by pre- and postnatal exposure
Juvenile Toxicity Study

• Conducted to support pediatric clinical trials
• Direct dosing to juvenile animals
• Dosing can start as early as newborn
  – But – age of dosing will be determined by age of youngest children in clinical trials
• Toxicity and systemic exposure data collected
Juvenile Toxicity Study in the Rat
Direct Dosing of Pups

- Pregnant Females
- Parturition (Non-Dosed Moms)
- Direct Dosing of Pups (variable start day)
- Weaning

- GD 0
- PND 0
- PND 7
- PND 14
- PND 21
- PND 28

F₁ Survival, Growth, Behavior, Reproductive Capability

F₁ Adults
Juvenile Toxicity Endpoints

<table>
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<tr>
<th>Juvenile Animals</th>
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<td>Growth</td>
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<td>Systemic Exposure</td>
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Mothers not dosed – no ability to evaluated drug in milk
### Available Nonclinical Data

<table>
<thead>
<tr>
<th>Pre-Postnatal Study</th>
<th>Juvenile Toxicity Study</th>
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</thead>
<tbody>
<tr>
<td>Drug in milk not typically measured - <strong>but</strong> it could be</td>
<td>Drug levels in milk <strong>not available</strong></td>
</tr>
<tr>
<td>Offspring exposures are available</td>
<td>Offspring exposures are available</td>
</tr>
<tr>
<td>Juvenile toxicity data exists, <strong>but</strong> confounded by exposure period</td>
<td>Juvenile toxicity data exists, <strong>not</strong> confounded by in utero exposure</td>
</tr>
<tr>
<td></td>
<td><strong>But</strong> - age varies at start of dosing, not always applicable to breastfed baby</td>
</tr>
</tbody>
</table>

**No one perfect study to address lactation/ breastfed baby**

**But** – data are available!
PLL Requirements

- Risk Summary – Lactation
  - Effects on milk production
  - Presence of drug in human milk
  - Effects on the breastfed child
  - Animal data not included if human data exist
  - Animal data, when included, should only state presence or absence of drug in milk
  - “Due to species-specific differences in lactation physiology, animal lactation data typically do not reliably predict levels in human milk; however, animal lactation data can be helpful in predicting whether a drug and/or its active metabolite(s) will be present in human milk.”
# Drug Secretion into Milk

## Mechanisms of Drug Secretion

<table>
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<th>Mechanism</th>
<th>Description</th>
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<tbody>
<tr>
<td>Simple diffusion</td>
<td>Carrier-mediated diffusion</td>
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<tr>
<td>Pinocytosis</td>
<td>Reverse pinocytosis</td>
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<tr>
<td>Active transport</td>
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## Drug Characteristic

<table>
<thead>
<tr>
<th>Drug Characteristic</th>
<th>Milk/Plasma (M/P) Ratio</th>
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<tbody>
<tr>
<td>Highly lipid-soluble</td>
<td>~1</td>
</tr>
<tr>
<td>Small, water soluble drugs (MW&lt;200)</td>
<td>~1</td>
</tr>
<tr>
<td>Weak acids and bases</td>
<td>~1</td>
</tr>
<tr>
<td>Highly protein-bound in maternal serum</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Actively transported drugs</td>
<td>&gt;1 or &lt;1 (depends on direction)</td>
</tr>
</tbody>
</table>
Species Comparison for Lactation

• Lack of good information in the literature on species differences, in general
• Similarities in hormonal control of milk production
• Species differences:
  – Mammary gland anatomy
  – Storage and release of milk into ducts
  – Protein and fat composition of milk
• Not clear how these factor into drug levels in milk
• Drug concentration cross-species comparisons lacking
**Purpose:** Analyze concentrations of 27 drugs in mouse milk and compare to human milk concentrations

**Methods:**
- Dose mice with implanted micro-osmotic pumps
- Measure M/P ratio, and compare to human M/P ratio
- Compare actual concentrations with those predicted by models using physicochemical parameters of drug¹
- Measure lipid and protein-unbound fractions in mouse and human plasma and milk

¹ Koshimichi (2011) Drug Met Distr, 39:2370–2380
Results:

1. M/P ratio generally 2-fold higher in mice than humans
   – *Unbound* M/P ratio similar between mice and humans
   – Difference predictable based on differences in protein and lipid content in mouse vs. human milk

2. 18 of 27 drugs – concentrations close to values predicted by pH-partition model, when corrected for protein and lipid differences
   – *Suggests drug secretion mediated by diffusion*

3. 9 of 27 drugs – concentrations were not close to predicted values
   – *Suggests drug secretion mediated by active transport*
• Higher M/P than predicted:
  – Cimetidine, clindamycin, aclycovir, terbutaline
  – Suggests active transport – BCRP (ABC) or other
• Lower M/P than predicted:
  – Cefotaxime, trazodone, metformin, tripolidine, verapamil
  – Suggests absorptive reuptake transport
Ito et al., 2013, cont.

• Higher M/P than predicted:
  – Cimetidine\(^1\), clindamycin, aclycovir, terbutaline
  – Suggests active transport – BCRP or other

• Lower M/P than predicted:
  – Cefotaxime, trazodone, metformin, tripolidine, verapamil
  – Suggests absorptive reuptake transport

• Considerable species differences

\(^1\)Dostal 1990, McNamara 1992, Oo 1995
Conclusions – Animal to Human Extrapolation

• Animal studies can generate data on lactation, drugs in milk, and health of newborn
• Lactation – cross-species extrapolation possible
• Health of breast-fed child
  – Data can be generated on exposures and toxicity in offspring
  – Risk assessment based on animal data is possible
  – Human studies would be best, but could be difficult
  – Animal data might be useful in absence of clinical data
Conclusions, conc.

• Drug concentrations in milk
  – Gaps exist in our knowledge of cross-species concordance
  – Species differences exist in composition of milk, secretory processes, etc.
  – Drugs that diffuse – extrapolation possible (M/P ratio)
  – Drugs secreted by active transport – need more data
  – Biggest gap – how to tell which category the drug falls into
  – Increased urgency for human lactation studies
  – Closing data gaps could facilitate extrapolation in future

• PLLR – clarity needed around expectations for generating and using animal data in Lactation Section