

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.

EPA	FDA (ICH)
Product Performance Test Guidelines	Quality Guidelines
Product Properties Test Guidelines	Efficacy Guidelines
Fate, Transport and Transformation Test Guidelines	Safety Guidelines
Spray Drift Test Guidelines	
Ecological Effects Test Guidelines	
Residue Chemistry Test Guidelines	
Health Effects Test Guidelines	
Occupational and Residential Exposure Test Guidelines	
Biochemicals Test Guidelines	
Microbial Pesticide Test Guidelines	
Endocrine Disruptor Screening Program Test Guidelines	

EPA: <http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm>

FDA (ICH): <http://www.ich.org/products/guidelines.html>

## 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**

FDA, 1987, 21 CFR Part 158.

USEPA (TSCA), 1989, 40 CFR Part 792.

USEPA (FIFRA), 1989, 40 CFR Part 160.

# 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)**

Federal Register, Vol. 72, No. 207, Oct. 26, 2007, pp.60975-76.

# 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

FDA (ICH) Safety Guidelines	
<b>Carcinogenicity Studies</b>	
S1A	Rodent Carcinogenicity Studies for Human Pharmaceuticals
S1B	Need for Carcinogenicity Studies of Pharmaceuticals
S1C(R2)	Testing for Carcinogenicity of Pharmaceuticals
<b>Genotoxicity Studies</b>	
S2(R1)	Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use
<b>Toxicokinetics and Pharmacokinetics</b>	
S3A	Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies
S3B	Pharmacokinetics: Guidance for Repeated Dose Tissue Distribution Studies
<b>Toxicity Testing</b>	
S4	Duration of Chronic Toxicity Testing in Animals (Rodent and Non Rodent Toxicity Testing)
<b>Reproductive Toxicology</b>	
S5(R2)	Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility
<b>Biotechnological Products</b>	
S6(R1)	Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals
<b>Pharmacology Studies</b>	
S7A	Safety Pharmacology Studies for Human Pharmaceuticals
S7B	The Non-Clinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Internal Prolongation) by Human Pharmaceuticals
<b>Immunotoxicity Studies</b>	
S8	Immunotoxicity Studies for Human Pharmaceuticals
<b>Nonclinical Evaluation for Anticancer Pharmaceuticals</b>	
S9	Nonclinical Evaluation for Anticancer Pharmaceuticals
<b>Photosafety Evaluation</b>	
S10	Photosafety Evaluation of Pharmaceuticals

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

# Regulatory Testing Guidelines

EPA Health Effects Guidelines	
<b>Group A - Acute Toxicity</b>	
870.1100	Acute Oral Toxicity
870.1200	Acute Dermal Toxicity
870.1300	Acute Inhalation Toxicity
870.2400	Acute Eye Irritation
870.2500	Acute Dermal Irritation
870.2600	Skin Sensitization
<b>Group B - Subchronic Toxicity</b>	
870.3050	Repeated Dose 28-Day Oral Toxicity Study in Rodents
870.3100	90-Day Oral Toxicity in Rodents
870.3150	90-Day Oral Toxicity in Nonrodents
870.3200	21/28-Day Dermal Toxicity
870.3250	90-Day Dermal Toxicity
870.3465	90-Day Inhalation Toxicity
870.3550	Reproduction/Developmental Toxicity Screening Test
870.3650	Combined Repeated Dose Toxicity with the Reproduction/Development Toxicity Screening Test
870.3700	Reproduction/Developmental Toxicity Study
870.3800	Reproduction and Fertility Effects
<b>Group C - Chronic Toxicity</b>	
870.4100	Chronic Toxicity
870.4200	Carcinogenicity
870.4300	Combined Chronic Toxicity/Carcinogenicity
<b>Group D - Genetic Toxicity</b>	
870.5100	Bacterial Reverse Mutation Test
870.5140	Gene Mutation in <i>Aspergillus nidulans</i>
870.5195	Mouse Biochemical Specific Locus Test
870.5200	Mouse Visible Specific Locus Test
870.5250	Gene Mutation in <i>Neurospora crassa</i>
870.5275	Sex-Linked Recessive Lethal Test in <i>Drosophila melanogaster</i>
870.5300	In Vitro Mammalian Cell Gene Mutation Test
870.5375	In Vitro Mammalian Chromosome Aberration Test
870.5380	Mammalian Spermatogonial Chromosomal Aberration Test
870.5385	Mammalian Bone Marrow Chromosomal Aberration Test
870.5395	Mammalian Erythrocyte Micronucleus Test
870.5450	Rodent Dominant Lethal Assay
870.5460	Rodent Heritable Translocation Assays
870.5500	Bacterial DNA Damage or Repair Tests
870.5550	Unscheduled DNA Synthesis in Mammalian Cells in Culture
870.5575	Mitotic Gene Conversion in <i>Saccharomyces cerevisiae</i>
870.5900	In Vitro Sister Chromatid Exchange Assay

EPA Health Effects Guidelines	
<b>Group E - Neurotoxicity</b>	
870.6100	Acute and 28-Day Delayed Neurotoxicity of Organophosphorus Substances
870.6200	Neurotoxicity Screening Battery
870.6300	Developmental Neurotoxicity Study
870.6500	Schedule-Controlled Operant Behavior
870.6850	Peripheral Nerve Function
870.6855	Neurophysiology: Sensory Evoked Potentials
<b>Group F - Special Studies</b>	
870.7200	Companion Animal Safety
870.7485	Metabolism and Pharmacokinetics
870.7600	Dermal Penetration
870.7800	Immunotoxicity
<b>Group G - Health Effects Chemical-Specific Test Guidelines</b>	
870.8300	Combined Chronic Toxicity-Carcinogenicity Testing of Respirable Fibrous Particles
<b>Endocrine Disruptor Screening Program - Tier 1</b>	
890.1400	Hershberger Assay (Rat)
890.1450	Female Pubertal Assay (Rat)
890.1550	Male Pubertal Assay (Rat)
890.1600	Uterotrophic Assay (Rat)

Detailed recommendations are provided for each study design.

# Most Guidelines Specify Evaluation of Both Sexes

## Repeated-Dose Mammalian Toxicity Studies

EPA Health Effects Guidelines		M	F
<b>Group B - Subchronic Toxicity</b>			
870.3050	Repeated Dose 28-Day Oral Toxicity Study in Rodents	X	X
870.3100	90-Day Oral Toxicity in Rodents	X	X
870.3150	90-Day Oral Toxicity in Nonrodents	X	X
870.3200	21/28-Day Dermal Toxicity	X	X
870.3250	90-Day Dermal Toxicity	X	X
870.3465	90-Day Inhalation Toxicity	X	X
870.3550	Reproduction/Developmental Toxicity Screening Test	X	X
870.3650	Combined Repeated Dose Toxicity with the Reproduction/Development Toxicity Screening Test	X	X
870.3700	Prenatal Developmental Toxicity Study		X
870.3800	Reproduction and Fertility Effects	X	X
<b>Group C - Chronic Toxicity</b>			
870.4100	Chronic Toxicity	X	X
870.4200	Carcinogenicity	X	X
870.4300	Combined Chronic Toxicity/Carcinogenicity	X	X
<b>Group E - Neurotoxicity</b>			
870.6200	Neurotoxicity Screening Battery	X	X
870.6300	Developmental Neurotoxicity Study	X	X
870.6500	Schedule-Controlled Operant Behavior	(X)	(X)
870.6850	Peripheral Nerve Function	(X)	(X)
870.6855	Neurophysiology: Sensory Evoked Potentials	(X)	(X)
<b>Group F - Special Studies</b>			
870.7200	Companion Animal Safety	X	X
870.7485	Metabolism and Pharmacokinetics	X	(X)
870.7600	Dermal Penetration	X	
870.7800	Immunotoxicity	(X)	(X)
<b>Group G - Health Effects Chemical-Specific Test Guidelines</b>			
870.8300	Combined Chronic Toxicity-Carcinogenicity Testing of Respirable Fibrous Particles	X	X
<b>Endocrine Disruptor Screening Program - Tier 1</b>			
890.1400	Hershberger Assay (Rat)	X	
890.1450	Female Pubertal Assay (Rat)		X
890.1550	Male Pubertal Assay (Rat)	X	
890.1600	Uterotrophic Assay (Rat)		X

(X) = Either sex can be used.

FDA (ICH) Safety Guidelines		M	F
<b>Carcinogenicity Studies</b>			
S1C(R2)	Testing for Carcinogenicity of Pharmaceuticals	NS	NS
<b>Toxicokinetics and Pharmacokinetics</b>			
S3A	Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies	X	X
<b>Toxicity Testing</b>			
S4	Duration of Chronic Toxicity Testing in Animals (Rodent and Non Rodent Toxicity Testing)	NS	NS
<b>Reproductive Toxicology</b>			
S5(R2)	Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility	X	X
<b>Biotechnological Products</b>			
S6(R1)	Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals	X	X
<b>Pharmacology Studies</b>			
S7A	Safety Pharmacology Studies for Human Pharmaceuticals	X	X
S7B	The Non-Clinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals	NS	NS
<b>Immunotoxicity Studies</b>			
S8	Immunotoxicity Studies for Human Pharmaceuticals	X	X
<b>Nonclinical Evaluation for Anticancer Pharmaceuticals</b>			
S9	Nonclinical Evaluation for Anticancer Pharmaceuticals	X	X
<b>Photosafety Evaluation</b>			
S10	Photosafety Evaluation of Pharmaceuticals	NS	NS

NS = Not specified.

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

3 Rs: {  
    **Replace** animals with alternative  
    **Reduce** the number of animals used  
    **Refine** the testing

- One sex has been shown to be more responsive to treatment
  - Toxicopharmacokinetics (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)

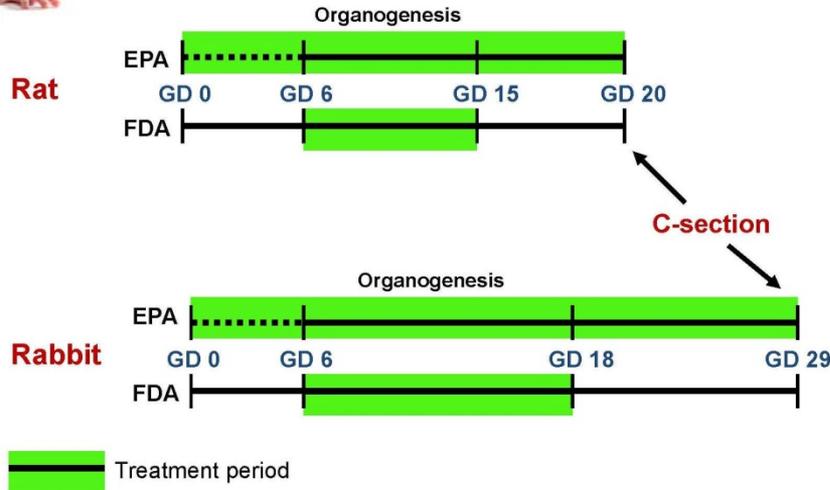
# Example of Study Dosing Only Females Subjects:

## Prenatal Developmental Toxicity Study

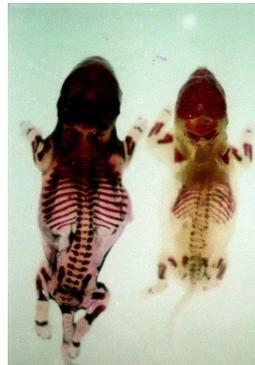
OPPTS 870.3700; OECD 414

## Embryo-Fetal Developmental Toxicity Study

ICH S5(R2)



If treatment-related preimplantation loss is demonstrated



- **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

## Example: Impact of Sex on Reference Value Derivation for Environmental Chemicals in the IRIS Database

- IRIS = Integrated Risk Information System ([www.epa.gov/iris](http://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:**  
 $RfV = NOAEL (BMDL) / UF$

# 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