ToXXicologY: The Importance of Incorporating the Sex Variable in Research Studies

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Learning Objectives

1. Define the terms “sex” and “gender” and their influence on human health

2. Understand the implications of including the sex and gender variables in research studies

3. Identify strategies to incorporate sex as a biological variable in toxicology studies

4. Describe best practices to consider sex differences in experimental designs
**Sex vs. Gender**

**Sex:**
The biological characteristics that distinguish males and females.

**Gender:**
The socially constructed and enacted roles and behaviors that occur in a historical and cultural context and vary across societies and over time.
NIH Policy on Sex as a Biological Variable

Consider
Design studies that take sex into account, or explain why it isn’t incorporated

Collect
Tabulate sex-based data

Characterize
Analyze sex-based data

Communicate
Report and publish sex-based data
SABV Across the Biomedical Research Continuum

- Basic
  - Cell studies, sample of human or animal tissues, computer-assisted models, animal models

- Preclinical

- Clinical Research & Trials

- Regulatory Review

- Clinical Practice
  - Optimal health for everyone at every age and every stage
Why Is It Important?
### Table 1. CYP450 Enzymes and Their Sex-Dependent Activity

<table>
<thead>
<tr>
<th>CYP Enzyme</th>
<th>Enzyme Activity</th>
<th>Example Drugs</th>
<th>Other Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>M &gt; W</td>
<td>Clozapine, olanzapine</td>
<td>Suppressed activity during pregnancy</td>
</tr>
<tr>
<td>2A6</td>
<td>W &gt; M</td>
<td>Nicotine, coumarin</td>
<td>Increased activity in female users of oral contraceptives</td>
</tr>
<tr>
<td>2B6</td>
<td>W &gt; M</td>
<td>Bupropion, tamoxifen</td>
<td>Activity: Hispanic women &gt; Caucasian or African-American women</td>
</tr>
<tr>
<td>2C9</td>
<td>M = W</td>
<td>Imipramine, phenytoin</td>
<td>Increased activity during pregnancy</td>
</tr>
<tr>
<td>2C19</td>
<td>M = W</td>
<td>Imipramine, topiramate</td>
<td>Decreased activity during pregnancy or use of oral contraceptives</td>
</tr>
<tr>
<td>2D6</td>
<td>Mostly W &gt; M</td>
<td>Codeine, fluoxetine, haloperidol</td>
<td>Increased activity during pregnancy</td>
</tr>
<tr>
<td>3A4</td>
<td>Mostly W &gt; M</td>
<td>Cyclosporine, erythromycin, nimodipine</td>
<td>Increased activity during pregnancy</td>
</tr>
</tbody>
</table>


### Table 2. Phase II Enzymes and Their Sex-Dependent Activity

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Enzyme Activity</th>
<th>Example Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDP-glucuronosyltransferases (UGTs)</td>
<td>M &gt; W</td>
<td>Oxazepam, acetaminophen</td>
</tr>
<tr>
<td>Sulfotransferases</td>
<td>M &gt; W</td>
<td>Acetaminophen</td>
</tr>
<tr>
<td>N-acetyltransferases</td>
<td>M &lt; W</td>
<td>Isoniazid, hydralazine</td>
</tr>
<tr>
<td>Methyltransferases</td>
<td>M &gt; W</td>
<td>L-dopa, azathioprine</td>
</tr>
</tbody>
</table>

*UDP: uridine diphosphate; M: men; W: women. Source: References 16, 34, 37.*
Are Drug Side Effects Always Different in Men and Women?
Adverse drug reactions are significantly higher in women
80% of severe adverse drug reactions are reported in women!!
Bias in Evidence-Based Medicine May Be the Reason
Do Most Papers in Your Field . . .

- Analyze data by sex?
- Have design to test SABV?
- Report the sex of cells/animals used?
- Inform sex-specific conclusions?
Sexes compared (118)
No sex difference (35)
Sex difference was a minor finding (42)
Sex difference was a major finding (41)
Sexes not compared (29)

Animal studies

% papers

https://doi.org/10.1089/jwh.2019.8114

DOI: https://doi.org/10.7554/eLife.70817
The Sex Variable in Toxicology Studies
Studying Males and Females
Example: Ozone Exposure Effects

*C57BL/6 mice (8 weeks old)*

- Males
- Females

Ozone (2ppm, 3 hours) or Filtered Air (3 hours)

- Lung gene expression
- BALF inflammation
Example:
Ozone Exposure Effects

Cabello et al. 2015
Example: Ozone Exposure Effects

- **Group animals**
  - M/D – luteal phase
  - P/E – follicular phase

- **Estrous cycle determination**
  - 2-3 weeks

- **Ozone/FA exposure**
  - 11am
  - 2pm

- **4h timepoint**
  - Harvest lung tissue
  - Blood collection
  - 6pm

- **24h timepoint**
  - Lung function testing
  - BALF assessment
  - 11am

Fuentes et al., 2018
Example: Ozone Exposure Effects
How to Integrate Your Variable(s) of Interest AND the Sex Variable?
## Factorial Designs

<table>
<thead>
<tr>
<th>Independent Variable 1</th>
<th>Independent Variable 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>Dependent Variable 1</td>
</tr>
<tr>
<td></td>
<td>Dependent Variable 2</td>
</tr>
<tr>
<td>Level 2</td>
<td>Dependent Variable 1</td>
</tr>
<tr>
<td></td>
<td>Dependent Variable 2</td>
</tr>
</tbody>
</table>
Conclusions

• Sex and gender can influence health in different ways.

• Biological sex can alter drug metabolism and the body’s response to a variety of toxicants.

• Incorporating the sex variable in preclinical toxicology studies can reduce the chances of observing negative side effects in women/men.

• Using appropriate research designs can help us identify sex-specific effects, as well as interactions between sex and other variables.
Questions?