GENDER SENSITIVITY OF XENOBIOTICS

Summary of the Literature

In order to conserve animals in acute toxicity testing, OECD experts have recommended the use of test animals of a single sex. Sex as a cause of differences in metabolism, transformation, and toxicity, have been reviewed by a number of authors. These authors have compiled available data on gender sensitivity to toxicants in rats, mice and humans. See, for example, Reviews by Salem, Trimbell, Sipes and Gandolpho, DeBethizy and Hayes, and Moser (1, 2, 3, 4, 5). However, we are not aware of systematic investigations into differences in sensitivity for lethality of xenobiotics of males and females across chemicals.

Surveys of the literature show that generally, the responses in male and female rats are similar. When differences in sensitivity occur, it is often the female that is more sensitive (Kedderis and Mugford, 6). Summarizing acute toxicity data on 766 chemicals, no significant sexual differences are noted in 711 cases, constituting 93% of the cases. When differences are noted, females are more sensitive in 42 cases, while males are more sensitive in 13 cases. (See Table 1.) In other tabulations, for 91 chemicals the female average LD50 value is slightly lower than that for males, while for 143 chemicals, the opposite is true. In some cases, dissimilarities in sensitivity between male and female rats can be significant. For example, in a comparison of male and female rat oral and dermal LD50 values for pesticides (EPA, 7), 14 out of 79 pesticides showed significant differences in sensitivity in male and female rats. In this report, difference in response was deemed to be significant if there was no overlap of the 95% confidence intervals characterizing each sex's response. As shown in Tables 1 and 2, for 11 cases, females were more sensitive and for 3 cases, males were more sensitive. Properties and structures for the chemicals in Table 2 are given in Table 2A. The three chemicals which showed greater sensitivity in the male rat were Landrin, a carbamate insecticide, Triflumizole, an imidazole fungicide, and vitamin D3, a steroidal pesticide. Additional disparities in sex sensitivity were seen for many of the rest of the chemicals in the pesticide data base, although for these chemicals, 95% confidence intervals overlapped to some extent. While these data suggest that the sexes are not equally sensitive to all of the chemicals tested, no clear cut generalizations about sex sensitivity could be made; although females were often more sensitive, this was not always true.

The published literature records cases when male rodents are more sensitive to xenobiotics than females. A detailed review of the metabolism of Chlorpyrifos can be found in Moser. Timbrell notes that Chlorpyrifos is more acutely toxic to male rats than to females. Differences in the way that vital organs react to toxins can also have a significant impact on overall toxicity. Chloroform induces nephrotoxicity in male mice, but not females; chloroform is converted to a reactive intermediate (phosgene) an order of magnitude faster by microsomes from male mouse kidneys than in those from female mice (Sipes and Gandolpho). Metabolic differences due to gender can also have an effect on sensitivity for acute effects. The insecticides aldrin and heptachlor are metabolized more rapidly to the toxic epoxide forms in male rats. These chemicals demonstrate a lower toxicity in the female rat (Trimbell).

Sensitivity Differences in Avian Species:

A. Rispin, H. Podall and W. Meyer – 04/03/2000
In a separate review, Elwood Hill (8) compared the toxicity of ten insecticides in birds (sex unspecified). The list contained both organophosphate and carbamate pesticides. (Tables 3 and 3A). The redwing blackbird has lower specific hepatic microsomal monooxygenase activity than most other animals (for example, rock dove, chukar, mallard, or ring-necked pheasant). By analogy to female rats with their lower biotransformation capacity, one would expect the redwing blackbird to have lower LD50 values for these insecticides than the other species. In fact, the redwing blackbird was more sensitive than the other avian species to seven chemicals. However, for two chemicals, chlorpyrifos and mexacarbate, the redwing blackbird was generally less sensitive than the other species.

Biotransformation and Differences in Sensitivity:

If gender differences are seen in toxic responses to xenobiotics, differences in biotransformation are the probable cause. Because male rats metabolize most foreign compounds faster than females, one would expect the biological half-life of most xenobiotics to be longer in the female than the male rat. However, if a metabolite or intermediate is responsible for the toxic response, male rats would be expected to show the greater susceptibility (Sipes and Gandolfo).

In general, CYP mediated reactions lead to detoxification and subsequent excretion of xenobiotics (phase I metabolism). For example, certain organophosphate pesticides are detoxified by glutathione S-transferases. However, CYP mediated metabolism can also cause formation of reactive metabolites. Female rats are known to have 10 - 30% less total CYP as compared with male rats. (Kedderis and Mugford).

Phase II conjugative enzymes, i.e. sulfotransferases, glutathione S-transferases, and glucuronyltransferases, also play a role in detoxification. Sex-dependent differences have also been found in expression of phase II enzymes. When such sex-dependent differences are seen, it is generally the male rats which have higher enzyme activities. For example, glutathione protects tissues against electrophilic attack by xenobiotics. DeBethizy and Hayes note that glutathione conjugating activity toward dichloronitrobenzene is two- to three-fold higher in male than female rats.

Biotransformation does not always lead to detoxification. Examples of activation of xenobiotics to their toxic forms by mixed function oxidase enzymes are:

- epoxidation of chlorobenzene and coumarin to generate hepatotoxic metabolites,
- oxidative group transfer of certain organophosphorous pesticides to the toxic organophosphate, e.g. conversion of parathion to paraoxon,
- reductive dechlorination of carbon tetrachloride to a trichloro methyl free radical,
- oxidative dechlorination of chloroform to phosgene,
- activation of ethyl carbamate to (urethan)
However, many of these same chemicals are also detoxified by cytochrome P450 by conversion to less toxic metabolites. In some cases, the same enzyme may catalyze activation and detoxification reactions for a given chemical. The resulting toxic effect of a xenobiotic chemical is thus due to a balance between metabolic activation and deactivation (Casarett and Doull, 9).

Although female rats generally have less total CYP activity than males, there are important exceptions. For example, microsomal 16-hydroxylase is male specific and is not expressed in females. Whereas steroid sulfate 15 hydroxylase occurs in higher concentrations in females. One could speculate that these differences may account for the fact that vitamin D3 is more toxic in males than females.

De Bethizy and Hayes also note that phase II conjugation of xenobiotics may not always lead to more rapid excretion of the conjugated metabolite. In fact, some compounds are toxic only after conjugation with glutathione. Glutathional conjugates which are implicated in nephrotoxicity would be likely to show greater toxicity in males than females.

Choice of Sex for Acute Toxicity Testing:

As noted above, fourteen pesticides, from a sample of 84, were found to exhibit significant differences in sensitivity between male and female rats (Table 2). When they occur, dissimilarities in sensitivity of male and female rats can also have important implications for regulation. In five of the fourteen cases, the disparity of response was such that had only one sex been tested, and it was the least sensitive sex, the chemical would have been assigned for classification to a less toxic class.

The revised test guideline #425 uses a single sex, usually females. If the investigator has a priori reasons to believe that males may be more sensitive than the other, then it may be used for testing. Female rats have a lower relative detoxification capacity for most chemicals, as measured by specific activity of their mixed function oxidase enzymes. Therefore, for chemicals which are direct acting in their toxic mechanism, females would generally be the most sensitive. However, if metabolic activation is required for a chemical’s toxicity, consideration must be given as to whether the preferred sex for testing is the male.
Table 1. LD50 sensitivity of the sexes

<table>
<thead>
<tr>
<th>Author</th>
<th>No. Chemicals</th>
<th>LD50 Average (mg/kg)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Females</td>
<td>Males</td>
<td></td>
</tr>
<tr>
<td>DePass et al., 1984</td>
<td>91</td>
<td>2130</td>
<td>2470</td>
<td></td>
</tr>
<tr>
<td>Weil et al., 1953</td>
<td>143</td>
<td>8960</td>
<td>8360</td>
<td></td>
</tr>
<tr>
<td><strong>Weighted Average</strong></td>
<td><strong>234</strong></td>
<td><strong>6313</strong></td>
<td><strong>6069</strong></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>LD50 Sensitivity of the Sexes</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sexes Same</td>
<td>Sex More Sensitive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>Bruce, 1985</td>
<td>48</td>
<td>35</td>
<td>13</td>
</tr>
<tr>
<td>EPA, 1991</td>
<td>79</td>
<td>65</td>
<td>11</td>
</tr>
<tr>
<td>HSE, 1999</td>
<td>449</td>
<td>446</td>
<td>1</td>
</tr>
<tr>
<td>Lipnick et al., 1995</td>
<td>20</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Muller &amp; Kley, 1982</td>
<td>170</td>
<td>147</td>
<td>17</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>766</strong></td>
<td><strong>711</strong></td>
<td>(93%)</td>
</tr>
</tbody>
</table>
### Table 2. Chemicals without overlapping male and female LD50 (95% confidence limits)

<table>
<thead>
<tr>
<th>CHEMICAL NAME</th>
<th>CHEMICAL CLASS</th>
<th>USE</th>
<th>MALE LD50 mg/kg</th>
<th>FEMALE mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isazofos technical (93+%)</td>
<td>Organophosphate</td>
<td>Insecticide</td>
<td>118.68</td>
<td>48.21</td>
</tr>
<tr>
<td>Trimethacarb</td>
<td>Carbamate</td>
<td>Insecticide</td>
<td>7.20</td>
<td>9.30</td>
</tr>
<tr>
<td>Flusilazole (97%)</td>
<td>Fluorophenyl triazole silane</td>
<td>Fungicide</td>
<td>1110.00</td>
<td>674.00</td>
</tr>
<tr>
<td>Cadusafos (94.9%)</td>
<td>Organophosphate</td>
<td>Insecticide</td>
<td>47.50</td>
<td>20.10</td>
</tr>
<tr>
<td>Cycloate technical (98%)</td>
<td>Carbamate</td>
<td>Herbicide</td>
<td>3200.00</td>
<td>2275.00</td>
</tr>
<tr>
<td>Clomazone (88.8% a.i.)</td>
<td>Chlorophenyl isoxazolidinone</td>
<td>Herbicide</td>
<td>2077.00</td>
<td>1369.00</td>
</tr>
<tr>
<td>Troysan polyphase (99%)</td>
<td>Iodo-acetylenic carbamate</td>
<td>Fungicide/wood preservative</td>
<td>1795.00</td>
<td>1065.00</td>
</tr>
<tr>
<td>Parathion technical (in corn oil)</td>
<td>Organophosphate</td>
<td>Insecticide</td>
<td>10.80</td>
<td>2.52</td>
</tr>
<tr>
<td>Chlorehoxyfos (86% a.i.)</td>
<td>Organophosphate</td>
<td>Insecticide</td>
<td>4.60</td>
<td>1.80</td>
</tr>
<tr>
<td>ASPON technical (90%); (inerts 10%)</td>
<td>Organophosphate</td>
<td>Insecticide</td>
<td>2800.00</td>
<td>740.00</td>
</tr>
<tr>
<td>Triflumizol technical</td>
<td>Imidazole</td>
<td>Fungicide</td>
<td>1057.00</td>
<td>1780.00</td>
</tr>
</tbody>
</table>
Table 2. Chemicals without overlapping male and female LD50 (95% Confidence limits) (cont’d.)

<table>
<thead>
<tr>
<th>CHEMICAL NAME</th>
<th>CHEMICAL CLASS</th>
<th>USE</th>
<th>MALE LD50 mg/kg</th>
<th>FEMALE mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 Thiodicarb (in methyl cellulose)</td>
<td>Carbamate</td>
<td>Insecticide</td>
<td>129.00</td>
<td>59.10</td>
</tr>
<tr>
<td>13. Vitamin D3 technical</td>
<td>Steroid</td>
<td>Antirachitic</td>
<td>352.00</td>
<td>619.00</td>
</tr>
</tbody>
</table>
Table 2A. Identification of Chemicals in Table 2

1) **CGA-123 technical**
   - This substance is identified in the MRID as CGA 12223 from Ciba, Ltd.
   - According to the Farm Chemicals Handbook (FCH), vol.86 (2000), the following information was obtained:
     - **Common Name:** Isazofos
     - **Chemical Name:** O -5-chloro- 1-isopropyl-1H-1,2,4-triazol-3-yl-O,O-diethyl-phosphorothioate
     - **CAS No.** 42509-80-8
     - **Chemical Class:** organophosphate
     - **Use:** Insecticide
   - **Structure:**

     ![Chemical Structure of CGA-123](image)

   - **Empirical Formula:** C9 H17 N3 P O3 S Cl
   - **Molecular Weight:** 313.5

2) **El-919**
   - **Trade name (of Shell):** Landrin
   - **Common Name:** Trimethacarb
   - **Chemical Name:** 3,4,5-trimethylphenyl methylcarbamate
   - **CAS No.** 2655-15-4
   - **Chemical Class:** carbamate
   - **Use:** Insecticide
   - **Structure:**

     ![Chemical Structures of El-919](image)

   - **(Note: The pesticide is a mixture of both forms, 3,4,5- and 2,3,5- trimethylphenyl methylcarbamate)**

   - **Empirical Formula:** C11 H15 O2 N
   - **Molecular Weight:** 182
3) 1-[(bis (4-fluorophenyl) methylsilyl) methyl]-1H-1,2,4-triazole
CAS No. 85509-19-9
Common Name: Flusilazole
Tradename: Nustar
Chemical Class: fluorophenyl triazole silane
Use: Fungicide
Structure:

Empirical Formula: C16 H15 F2 N3 Si
Molecular Weight: 315.4

4) FMC 67825
Tradename: Rugby ; Apache
Common Name: Cadusafos
Chemical Name: O- ethyl-S,S- di-sec-butyl phosphorodithioate
Chemical Class: organophosphate
Use: Insecticide
Structure:

Empirical Formula: C10 H23 P O2 S2
Molecular Weight: 270
5) Cycloate technical
   Chemical Name: S-ethyl cyclohexyl (ethyl) thiocarbamate
   CAS No. 1134-23-2
   Chemical Class: carbamate
   Use: Herbicide
   Structure:

   ![Structure](image1)

   Empirical Formula: C11 H21 N O S
   Molecular Weight: 204

6) FMC 57020
   Tradename: Command
   Common Name: Clomazone
   Chemical Name: 2- [(2-chlorophenyl) methyl]-4,4-dimethyl -3-isoxazolidinone
   Chemical Class: chlorophenyl isoxazolidinone
   CAS No. 81777-89-1
   Use: Herbicide
   Structure:

   ![Structure](image2)

   Empirical Formula: C12 H14 N O2 Cl
   Molecular Weight: 239.5
7) 3-iodo-2-propynyl butylcarbamate  
   Complete Chemical Name: 3-iodo-2-propynyl N-n-butyl carbamate  
   Tradename: Troysan polyphase  
   Chemical Class: iodo-acetylenic carbamate  
   Use: fungicide/ wood preservative  
   Structure:

   ![Structure of 3-iodo-2-propynyl butylcarbamate]

   Empirical Formula: C₈ H₁₂ O₂ N I  
   Molecular Weight: 281

8) Parathion technical  
   Chemical Name: O, O-diethyl- O-(4-nitrophenyl) phosphorothioate  
   CAS No. 56-38-2  
   Tradename: Thiophos  
   Chemical Class: organophosphate  
   Use: Insecticide  
   Structure:

   ![Structure of Parathion]

   Empirical Formula: C₁₀ H₁₄ N P O₅ S  
   Molecular Weight: 291
9) Fortress (tradename-Dupont)
   Common Name: Chlorethoxyfos
   Chemical Name: O,O-diethyl-O-(1,2,2,2-tetrachloroethyl) phosphorothioate
   Chemical Class: organophosphate
   Use: Insecticide
   Structure:

   ![Structure of Fortress](image)

   Empirical Formula: C6 H11 P O3 S Cl4
   Molecular Weight: 336

10) O,O,O,O-tetrapropyl dithiopyrophosphate
    CAS No. 3244-90-4
    Tradename: ASPON technical (Stauffer Chemical Co.)-- discontinued 1987 by Stauffer.
    Chemical Class: Organophosphate
    Use: Insecticide
    Structure:

   ![Structure of O,O,O,O-tetrapropyl dithiopyrophosphate](image)

   Empirical Formula: C12 H28 O5 P2 S2
   Molecular Weight: 378
11) Triflumizole
Chemical Name: (E)- 4-chloro-aaa- trifluoro-N-(1-imidazole)-1 yl- 2-propoxy-ethylidene-o-toluidine
CAS No. 99387-89-0
Chemical Class: Imidazole
Use: Fungicide
Structure:

Empirical Formula: C15 H15 N3 O Cl F3
Molecular Weight: 345.5

12) Larvin (tradename / Rhone-Poulenc)
Common Name: Thiodicarb
Chemical Name: dimethyl N,N-(thiobis (methylimino) carbonyloxy) bis-ethanimidothioate)
CAS No. 59669-26-0
Chemical Class: Carbamate
Use: Insecticide
Structure:

Empirical Formula: C10 H18 N4 S3 O4
Molecular Weight: 354
13) Vitamin D3
Chemical Names: (3b,5Z,7E)-9,10-secocholesta-5,7,10-(19)-trien-3-ol; or activated 7-dehydro-cholesterol; or cholecalciferol
Use (Merck Index, p.1711): antirachitic
Structure:

Empirical Formula: C27 H44 O
Molecular Weight: 385

* References:

Table 3. Most sensitive cases.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Red-winged blackbird</th>
<th>Other avian species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocrotophos</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Dicrotophos</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Parathion</td>
<td></td>
<td>Mallard</td>
</tr>
<tr>
<td>EPN</td>
<td></td>
<td>Ring-necked pheasant</td>
</tr>
<tr>
<td>Propoxur</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td></td>
<td>European starling</td>
</tr>
<tr>
<td>Fenthion</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Temephos</td>
<td>X</td>
<td>Ring-necked pheasant*</td>
</tr>
<tr>
<td>Landrin</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Mexacarbate</td>
<td></td>
<td>Ring-necked pheasant,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chukar, Rock dove</td>
</tr>
</tbody>
</table>

* Red-winged black bird and Ring-necked pheasant are very close in sensitivity.
Table 3A. Identification of Chemicals in Table 3 *

1) Monocrotophos (common name)
   Chemical Name: dimethyl (E)-1-methyl-2-(methylcarbamoyl) vinylphosphate
   CAS No. 6923-22-4
   Chemical Class: Organophosphate
   Use: Insecticide
   Structure:
   ![Chemical Structure]

   Empirical Formula: C7 H14 P O5 N
   Molecular Weight: 223

2) Dicrotophos (common name)
   Chemical Name: (E)-2-dimethylcarbamoyl - 1- methylvinyl dimethylphosphate
   CAS No. 141-66-2
   Chemical Class: Organophosphate
   Use: Insecticide
   Structure:
   ![Chemical Structure]

   Empirical Formula: C8 H16 P O5 N
   Molecular Weight: 237

3) Parathion ------(same as 8 in Table 2A)
4) EPN (common name)  
   Chemical Name: O-ethyl-O-4-nitrophenyl phenylphosphonothioate  
   CAS No. 2104-64-5  
   Chemical Class: Organophosphate  
   Use: Insecticide  
   Structure:

   ![Structure of EPN](image_url)

   Empirical Formula: C14 H14 N O4 P S  
   Molecular Weight: 323

5) Propoxur (common name)  
   Chemical Name: 2-(1-methylethoxy) phenyl nethylcarbamate  
   CAS No. 114-26-1  
   Chemical Class: Carbamate  
   Use: Insecticide  
   Structure:

   ![Structure of Propoxur](image_url)

   Empirical Formula: C11 H15 N O3  
   Molecular Weight: 209
6) Chlorpyrifos (common name)
   Chemical Name: O,O-diethyl- O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate
   CAS No. 2921-88-2
   Chemical Class: Organophosphate
   Use: Insecticide
   Structure:
   [Diagram of Chlorpyrifos]

   Empirical Formula: C9 H11 Cl3 N P O3 S
   Molecular Weight: 350.6

7) Fenthion (common name)
   Chemical Name: O,O- dimethyl-O- [3-methyl-4-(methylthio) phenyl] phosphorothioate
   CAS No. 55-38-9
   Chemical Class: Organophosphate
   Use: Insecticide
   Structure:
   [Diagram of Fenthion]

   Empirical Formula: C10 H15 P O3 S2
   Molecular Weight: 278
8) Temephos (common name)
   Chemical Name: O,O-thiodo-4,1-phenylene- O,O,O',O'-tetramethyl-
   phosphorothioate
   CAS No. 3383-96-8
   Chemical Class: Organophosphate
   Use: Insecticide
   Structure:

   ![Temephos Structure](image)

   Empirical Formula: C16 H20 P2 S3 O6
   Molecular Weight: 466

9) Landrin (tradename of Shell) - discontinued by Shell
   Common Name: trimethacarb
   Chemical Name: 3,4,5- trimethylphenyl methyl carbamate
   CAS No. 2655-15-4
   Chemical Class: Carbamate
   Use: Insecticide
   Structure:

   ![Landrin Structure](image)

   (Note: The pesticide is a mixture of both forms, 3,4,5- and 2,3,5- trimethylphenyl methylcarbamate)
   Empirical Formula: C11 H15 O2 N
   Molecular Weight: 193
10) Mexacarbate ; Zectram  
   Chemical Name: 4- dimethylamino-3,5-xylyl methylcarbamate  
   Chemical Class: Carbamate  
   Use: Insecticide  
   Structure:

   \[
   \begin{align*}
   &\text{Empirical Formula: } C_{12}H_{18}N_2O_2 \\
   &\text{Molecular Weight: } 222.3
   \end{align*}
   \]

* References:
Literature.


