Acute Toxicity Test with *Daphnia magna*: An Alternative to Mammals in the Prescreening of Chemical Toxicity?

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Guilhermino et al. 2000  
Ecotoxicol Environ Saf, 46: 357-362
Overview

• Background
• Objectives
• Material and Methods
• Results and Discussion
• Concluding remarks
• **Precise LD50 value** for mammals is **not required** for notification of new chemicals;

• Alternatives to the traditional acute oral toxicity test (*i.e.*, fixed dose procedure, acute toxic class method, up-and-down procedure);

• **Acute tests with invertebrates** (*i.e.*, earthworms, microcrustaceans) as **first screening methods** to assess lethal toxicity to mammals and humans;

• **Difference of biological organisation level** relative to mammals;

• Use as **prescreening methods significantly reduces the number of mammals required for toxicity testing**.
Objectives

- To study the relationship between LC50 values to *Daphnia magna* of 54 chemicals and the corresponding LD50 values for the rat (based on the EU classification of chemicals).

- To further investigate the possible use of invertebrate tests as prescreening methods for assessment of the toxicity of new chemicals for classification and labelling purposes.
**Materials and methods**

**D. magna acute toxicity tests**

15 chemicals (24- and 48-h LC50) oral LD50 values to the rat obtained from the specialised literature

Pearson correlation coefficient **strong relationship between species**

Sample expanded **54 cases** acute toxicity to *D. magna* and rat gathered from the literature

**Logistic regression** **cutpoint** in the 24-h LC50 values that could predict toxicity to the rat

**very toxic and toxic** chemicals (oral LD50 < 200 mg/kg)

**harmful or unclassified** chemicals (oral LD50 >= 200 mg/kg).
Studied compounds

Acetic acid  
Amitriptyline  
Amphetamine sulfate  
Aniline  
Arsenic trioxide  
Aspirin  
Cadmium chloride  
Caffeine  
Carbon tetrachloride  
Chloroform  
Chlorpyrifos  
Chromous chloride  
Copper chloride  
Copper sulfate  
Dodecyl benzyl sulfonate  
3,4-dichloroaniline  
Diazepan  
Diazinon  
Dichlorvos  
Digitoxin  
Disulfoton  
Endosulfan  
Ethanol  
Ethylene glycol  
Fenitrothion  
Ferrous chloride  
Ferrous sulfate  
Formaldehyde  
Hexachlorophene  
Isopropanol  
Lindan  
Malathion  
Mercurous chloride  
Methanol  
Methyl parathion  
Paraoxon  
Parathion  
p-chloroaniline  
p-cresol  
Pentachlorophenol  
Phenobarbital  
Phenol  
p-Nitrophenol  
Quinine sulfate  
Sodium dodecyl sulfate  
Sodium bromide  
Sodium chloride  
Sodium dichromate  
Sodium fluoride  
Stannous chloride  
Thallium sulfate  
Thiometon  
Toluene  
Zinc sulfate
<table>
<thead>
<tr>
<th>Chemical</th>
<th>D. magna LC50 (mg/L)</th>
<th>Rat oral LD50 (mg/kg)</th>
<th>24-h LC50, LD50</th>
<th>48-h LC50, LD50</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paraoxon</td>
<td>0.00055</td>
<td>0.00019</td>
<td>1.8</td>
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<tr>
<td>Parathion</td>
<td>0.00219</td>
<td>0.00216</td>
<td>13</td>
<td></td>
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<tr>
<td>3,4-dichloroaniline</td>
<td>0.271</td>
<td>0.100</td>
<td>648</td>
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<tr>
<td>Chlorpyrifos</td>
<td>*</td>
<td>0.344</td>
<td>145</td>
<td></td>
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<tr>
<td>Mercurous chloride</td>
<td>0.0027</td>
<td>0.002</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Cadmium chloride</td>
<td>0.071</td>
<td>0.017</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>Copper sulfate</td>
<td>0.399</td>
<td>0.0826</td>
<td>960</td>
<td></td>
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<tr>
<td>Zinc sulfate</td>
<td>35.403</td>
<td>4.029</td>
<td>2,150</td>
<td></td>
</tr>
<tr>
<td>Sodium dichromate</td>
<td>1.854</td>
<td>0.778</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>Chromous chloride</td>
<td>40.507</td>
<td>21.531</td>
<td>1,870</td>
<td></td>
</tr>
<tr>
<td>Sodium bromide</td>
<td>15,322</td>
<td>7,451</td>
<td>3,500</td>
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<tr>
<td>Ethanol</td>
<td>9,788</td>
<td>5,680</td>
<td>13,700</td>
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<tr>
<td>Methanol</td>
<td>4,816</td>
<td>3,289</td>
<td>13,000</td>
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<tr>
<td>SDS</td>
<td>45.898</td>
<td>19.129</td>
<td>1,288</td>
<td></td>
</tr>
<tr>
<td>DBS</td>
<td>38.514</td>
<td>9.546</td>
<td>2,000</td>
<td></td>
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24-h LC50, LD50 \( r = 0.93 \)

48-h LC50, LD50 \( r = 0.91 \)
Results

2 false positives (organophosphates)
11 false negatives (metals, organochlorines, organic compounds)

Logistic curve

\[
\ln(\text{LC50}) \text{ value: } -1.50
\]

'test' to predict the probability of toxicity to the rat
**Criterion validity of* D. magna* 24-h LC50 for prediction of chemical toxicity to the rat**

<table>
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<tr>
<th>Daphnia (test)</th>
<th>Rat</th>
<th>Toxic</th>
<th>Nontoxic</th>
<th>Total</th>
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<tbody>
<tr>
<td>LC50 &lt; 0.22 mg/L</td>
<td></td>
<td>10</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>LC50 ≥ 0.22 mg/L</td>
<td></td>
<td>11</td>
<td>31</td>
<td>42</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>21</td>
<td>33</td>
<td>54</td>
</tr>
</tbody>
</table>

**High specificity**
- Sensitivity (10/21) = 47.6%
- Specificity (31/33) = 93.9%

**Predictive value**
- Toxic Daphnia LC50 = 83.3%
- Nontoxic Daphnia LC50 = 73.8%
Criterion validity of *D. magna* 24-h LC50 for prediction of chemical toxicity to the rat

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Relative risk of toxicity to the rat = 3.2 (1.7-6.3)
Concluding remarks

- *D. magna* test is more specific than sensitive for an indication of the toxicity to the rat;

- Using the logistic regression model, the *D. magna* test seems to have a predictive capacity comparable to that of mammalian cytotoxicity tests;

- It is an *in vivo* test taking into account the biotransformation of toxicants and potential integrated effects that occur in the organism as a whole;

- It is thus preferable to *in vitro* methods that have been considered to evaluate human acute toxicity.
Concluding remarks

- Use of **D. magna** bioassays as prescreening methods may be advantageous at least in some situations allowing the **reduction of the number of mammals required for toxicity testing**;

- **Cost effectiveness**, given the high predictive value to the rat;

- **D. magna** is a standard organism in ecotoxicology; acute toxicity testing standardised by international organisations (OCDE, 1992; EPA, 1991).

- A considerable number of **LC50 values for a great variety of chemical agents already exist**.
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Thank you very much!