Are Daphnia Adequate Model Organisms for Chemical Testing?

by

Erica Geerdes

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The members of the Honors Thesis Committee appointed to examine the thesis of Erica Lynn Geerdes find it satisfactory and recommend that it be accepted.

______________________________________________
Dr. Jacob Kerby
Assistant Professor of Biology
Director of the Committee

______________________________________________
Dr. David Swanson
Professor of Biology

______________________________________________
Dr. Megan Porter
Assistant Professor of Biology
ABSTRACT

Are *Daphnia* Adequate Model Organisms for Chemical Testing?

Erica Lynn Geerdes

Director: Dr. Jacob Kerby, Ph.D

Model organisms provide a simplified method for determining relative chemical toxicities. It is necessary that the model organism chosen to represent a group of taxa accurately portrays the sensitivity of the taxa being analyzed. The Environmental Protection Agency widely uses *Daphnia* as model organisms for aquatic chemical testing. In order to decipher the relative sensitivity of *Daphnia* to different chemical categories, species sensitivity distributions (SSD’s) were built using acute toxicity data present in the EPA’s AQUIRE database. HC\textsubscript{50} values of a total of 21 aquatic taxa were compared across 29 chemical categories. The results for 19 of the 29 chemical categories revealed one or multiple taxa more highly sensitive than *Daphnia*, suggesting the use of alternative or secondary model organisms may be necessary.

KEYWORDS: *Daphnia*, species sensitivity distribution, model organism, EPA
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Introduction

Under the Toxic Substances Control Act (TSCA), the U.S Environmental Protection Agency has the power to require testing of chemical substances in order to regulate exposure to chemicals and set acceptable environmental concentrations (EPA, Chemical Testing Overview, 2013). For example, to specifically regulate allowable chemical exposures to aquatic environments, the EPA utilizes the Aquatic Life Criterion, which is the maximum concentration of a specific pollutant that does not cause significant harm to aquatic life. This criterion is utilized to protect aquatic organisms and is determined in part by acute toxicity tests (EPA, Aquatic Life Criteria, 2013). While the obvious best-case scenario would be thorough experimentation to determine the lowest chemical concentration harmful to any possibly affected species, this is impossible due to obvious budgets and time limitations. Instead, the EPA utilizes model organisms to determine the overall chemical toxicities.

Model organisms are essential to providing relative toxicities of chemicals when it is impractical to determine toxicities to every species that may be affected. Standardized protocols are used, which makes comparisons across chemicals possible. The distinct methods employed by the EPA for acute toxicity testing are detailed in the EPA publication *Methods for Aquatic Toxicity Identification Evaluation, Phase I-III* (NEPIS, 1999). Other desirable characteristics common in model organisms include ease of laboratory use. Short generation times, large numbers of offspring, and low maintenance make a typical model organism easy to raise in a laboratory setting. Model organisms often have sequenced genomes, allowing them to be manipulated or examined
at the genetic level. Due to their abundance, ease of laboratory care, and sequenced genomes, large amounts of toxicity data are available for model organisms.

*Daphnia* (Crustacea: Branchiopoda) are utilized by the EPA as a model organism for aquatic toxicity testing. *Daphnia* have many of the appealing attributes described previously, being easily cultured in a laboratory setting, requiring little space while producing large numbers of offspring with a short generation time. Other model organism assets provided by *Daphnia* species include the ability to switch between sexual and asexual reproduction in a process known as cyclical parthenogenesis. This provides an opportunity to study genetic responses to environmental stimuli while maintaining a constant genetic lineage through clonal reproduction (Miner et al., 2012). Additionally, in 2011, *Daphnia pulex* became the first crustacean to undergo genome sequencing. This known genome allows for studies of ecotoxicology and environmental stressors at the level of gene-environment interaction (Colbourne et al., 2011).

In addition to these advantages as a model organism, it is an implicit assumption that *Daphnia* are one of the more sensitive invertebrates, which has resulted in their widespread use in the determination of chemical toxicities. While *Daphnia* species have been found to be relatively sensitive (Morgan, 1972), there has yet to be a thorough analysis of how sensitive *Daphnia* are in comparison to other taxa. Studies have found evidence of other taxa that may be more sensitive than *Daphnia* in particular cases. For example, species of fish have been found to be more sensitive than *Daphnia* to chemicals such as phenols and endosulfans (DeGraeve et al., 1980 and Nebeker et al., 2009). To compensate for these discrepancies in sensitivity, the EPA uses the fathead minnow (*Pimephales promelas*) as an additional model organism for aquatic chemical regulations.
Given the clear differences in contaminant susceptibility among taxonomic groups and the widespread use of *Daphnia* as a model organism, it is of critical importance to understand the relative sensitivity of *Daphnia* to a variety of aquatic contaminants. As it has already been mentioned, it is highly improbable that *Daphnia* species are the most highly sensitive across all tested chemical categories, so it is necessary to determine the chemical categories for which *Daphnia* proves the most sensitive and the chemicals for which other groups are more sensitive. For those chemical categories where *Daphnia* species are not the most sensitive, perhaps there is a need to explicitly assign a more highly sensitive taxon as the model taxon. For instance, the fathead minnow has already been incorporated as a model organism for the testing of particular chemical categories, and with a comparison of *Daphnia* sensitivity to that of other taxa, additional species may prove useful and or necessary.

Data necessary for this comparison of chemical sensitivities among taxa can be obtained from the U.S. EPA’s Aquatic Toxicity Information Retrieval (AQUIRE) database, which contains toxicity data from thousands of published studies dating back nearly a century. This database contains valuable information regarding the toxicity of chemicals to an array of taxa. This study will examine these existing data for the purpose of providing a relative estimation of the sensitivity of *Daphnia* and other taxonomic groups to major contaminant groups.
Methods

Database

More than 110,000 acute toxicity studies were collected from the AQUIRE database (http://www.epa.gov) in April 2013. For each species tested by a chemical, a dose-response curve model was used to estimate the chemical concentration that is lethal to 50% of exposed individuals in a species ($LC_{50}$). $LC$ values provide a standard measure comparable across all taxa. Specifically, the $LC_{50}$ value is the most reliable point to estimate on the dose-response curve, and for this reason it is used to make comparisons among species (Newman and Unger, 2003). Exposure ranges of 24-96hrs were used, as this time frame included the majority of tests while still providing a small exposure time. Combined, these acute toxicity tests provide the $LC_{50}$ values for 24-96hr exposure ranges to 7228 species.

Compiling toxicity data

In order to generalize the findings from this analysis across chemicals and species, each was grouped into a larger category. From the toxicity tests, 4,895 chemicals were grouped into 29 chemical categories according to chemical structure and toxic mode of action based on previous assignments on this same dataset by www.pesticideinfo.org. Each species utilized in the study was subsequently grouped into taxonomic groups corresponding to their class. Overall we included 25 taxonomic classes in the study although not all classes were examined for each chemical category due to lack of sufficient toxicity data for some combinations. These groupings were then analyzed to determine generalized assessments of overall sensitivity.
Species sensitivity distributions

In order to compare the toxicity of chemical categories across taxonomic groups, we followed the methodology by Kerby et al. (2011) to build species sensitivity distributions (SSDs) (Posthuma et al. 2002) to compare the relative sensitivity among the different taxa tested in each chemical group. The SSD method mimics the LC$_{50}$ approach but provides a dose response curve of species across a taxonomic group rather than just of individuals across a species. As all species exhibit different sensitivities to a particular compound, it treats the average LC$_{50}$ values for each chemical in the chemical group as an individual value. This creates a cumulative curve of the relative sensitivities for each taxonomic group. These average species LC$_{50}$ values were calculated using the geometric mean in order to account for large differences in logarithmic values. Using this method, curves were built for each taxonomic class for each chemical group. In order to be included in the analysis, taxon SSD curves needed to include a minimum of seven species, with each data point representing the average LC$_{50}$ value for one species. While dose-response curves provide the species LC$_{50}$, SSDs provide an analogous hazardous concentration (HC$_{50}$), which represents the point at which 50% of species in a taxon exhibit 50% mortality. After the curves were made, least squares linear regressions were fit to the data to estimate HC$_{50}$ values for each SSD.

Statistical comparisons

Generalized linear models were then constructed to statistically compare each of the taxa to the HC$_{50}$ values calculated for Branchiopoda (*Daphnia*) in a standard Dunnet comparison for each chemical category. Nearly all Branchiopods included in the study
were *Daphnia* (*Daphnia pulex, Daphnia magna, Daphnia spp.*). Comparisons were completed excluding the few non-*Daphnia* species and there were no differences in the results and so they are not reported here. In instances where sensitivities appear lower but were not found significant, *Daphnia* species were considered among the most sensitive taxa. Statistics were performed in R statistical software v. 3.0.2 (R Foundation for Statistical Computing 2013).
Results

Overview

The final analysis included toxicity studies for a total of 1183 species across 1251 different chemicals. These data resulted in 21 taxonomic classes tested for sensitivity when summing the taxonomic classes among the 29 chemical categories. Of the 29 chemical categories analyzed, 19 contained toxicity data that revealed taxa significantly more sensitive to exposure than *Daphnia* (Table 1). Within the 19 chemical categories exhibiting taxa more sensitive than *Daphnia*, 9 groups had several taxa that were more sensitive (Figs.1-9) while the remaining 10 chemical categories revealed a single taxon more sensitive than *Daphnia* (Figs. 10-19). *Daphnia* species were among the most sensitive taxa in 10 of the 29 chemical categories analyzed (Figs. 20-29).

Species in the class Malacostraca (Anthropoda) followed *Daphnia* species in most commonly exhibiting the highest sensitivity, having the lowest HC$_{50}$ in five chemical categories, the organochlorine, pyrethroid, inorganic arsenic, anilide, and botanical chemical categories. Monogononta (Rotifera) provided the lowest HC$_{50}$ values for the inorganic, chlorinated phenol, and urea chemical categories. Actinopterygii (Chordata), Amphibia (Chordata), and Bivalvia (Mollusca) each accounted for the highest sensitivities in two chemical categories and Anthozoa (Cnidaria), Gastropoda (Mollusca), Insecta (Anthropoda), Maxillopoda (Anthropoda), and Ostracoda (Anthropoda) were each the most sensitive taxa for a single chemical group.

Regarding the total 29 chemical categories, *Daphnia* were among the least sensitive to exposure to five different categories, including the triazine, alcohol ether, organotin, soap, and chloroacetanilide categories.
Chemical categories revealing multiple taxa more sensitive than Daphnia

Nine chemical categories showed taxonomic classes being more highly sensitive than Daphnia. A staggering eight taxonomic classes were shown to be more sensitive than Daphnia to the inorganic chemical category, with species belonging to the Monogononta class having the lowest HC$_{50}$ value (Fig. 1). This placed Daphnia near the middle of the distribution, with five classes having lower sensitivities. Daphnia were the least sensitive class for the triazine category. In this category, seven classes were more sensitive with Gastropoda being the most sensitive class (Fig 2). Again, Daphnia provided only the seventh highest sensitivity for the chlorinated phenol category (Fig. 3). For this category, Monogononta was the most sensitive class. Five taxa were more sensitive than Daphnia to the organochlorine chemicals, and Malacostraca had the lowest HC$_{50}$ value (Fig. 4). Five taxa were also more sensitive than Daphnia to the alcohol ether and organotin chemical categories (Figs 4-6). Bivalvia and Maxillopoda were the most sensitive taxa for the alcohol ether and organotin categories, respectively, while Daphnia were the least sensitive. The combined studies for soap toxicity revealed that Daphnia were again the least sensitive of the taxonomic classes included, with four classes providing higher sensitivities, the highest of these belonging to the Bivalvia class (Fig. 7). Two classes were more sensitive than Daphnia to the chlorophenoxyacid/ester category, with Insecta being the most sensitive (Fig. 8). The final category revealing multiple taxa that were more highly sensitive than Daphnia was the pyrethroid category, for which the Monogononta class, one of two taxa more sensitive than Daphnia, had the lowest HC$_{50}$ value (Fig. 9). Of these nine categories for which multiple taxa were more sensitive than
*Daphnia*, four categories revealed *Daphnia* to be among the least sensitive to that category.

**Chemical categories revealing a single taxon more sensitive than Daphnia**

An additional ten chemical categories revealed a single taxon as more highly sensitive than *Daphnia* to that category. Malacostraca provided the highest sensitivities to the urea category (Fig. 10). The Anthozoa class was the most sensitive to the inorganic copper category, while ten classes showed lower sensitivities than *Daphnia* (Fig. 11). In addition to the triazine, alcohol ether, organotin, and soap categories, *Daphnia* were also among the least sensitive classes tested for the chloroacetanilide category. Amphibia provided the highest sensitivity of the four classes tested for the chloroacetanilide category (Fig. 12). Malacostraca had the lowest HC$_{50}$ value for both the inorganic arsenic, phenol, anilide, and botanical categories (Figs. 13-15 and 17). The Actinopterygii class was the most sensitive for the dinitrophenol derivative category (Fig. 16). The halogenated organic and organophosphorus categories also had one taxa more sensitive than Daphnia, with Amphibia and Ostracoda providing the lowest HC$_{50}$ values, respectively (Figs. 18-19).

**Chemical categories for which Daphnia were the most sensitive**

*Daphnia* were among the most sensitive to the remaining ten chemical categories. The inorganic mercury, carbamate, inorganic chromium (IV), and inorganic zinc categories revealed Daphnia to be the most sensitive while eight or more taxa were less sensitive (Figs. 23 and 27-29). Six taxa were less sensitive than *Daphnia* to the petroleum
derivative category (Fig. 22), and five taxa were less sensitive than Daphnia to the thiocarbamate category (Fig. 26). The bipyridylum, inorganic silver, and dithiocarbamate categories included four taxonomic classes that were less sensitive than *Daphnia* (Figs. 20-21 and 25). Daphnia were among the most sensitive taxa for the 2,6 dinitroaniline category while only one class showed a lower sensitivity (Fig. 24).
Discussion

My analyses revealed that *Daphnia* were typically not among the most sensitive taxonomic group. *Daphnia* were found to be the least sensitive in five chemical categories. Providing among the highest sensitivity in 10 of 29 chemical categories, *Daphnia* cover the broadest change of chemicals when choosing a single taxon on which to base chemical regulation. However, it is interesting to find that *Daphnia* have the highest sensitivity in only 34.5% of chemical groups analyzed in this study.

These results reveal the importance of a meta-analysis approach when analyzing toxicities. The vast majority of toxicity studies focus on specific toxicities of particular chemicals to particular organisms, while a meta-analysis is able to examine trends in data and compare toxicities among taxa. While few studies of this nature have been done, those that exist have provided important information to the field of ecotoxicology. The results of one such meta-analysis by Kerby et al. (2010), which compared acute toxicity data for amphibians to those of other taxonomic groups, disputed the widely accepted concept of amphibians as “canaries,” or indicator species for environmental contamination. Other meta-analyses have been conducted that also focus on multiple species but are narrower in scope, analyzing the effects of a particular chemical. Rohr and McCoy (2010), for example, have reviewed the effects of atrazine on amphibians. Missing from the literature are meta-analyses involving a wide scope of toxins as well as taxa. Comparing the toxicities of a wide scope of chemicals among a large number of taxa reveals trends in toxicity data and breaks down chemical sensitivities by taxa and chemical category. This allows the most appropriate model organism to be fit to particular chemical toxicity assessments.
Model organisms provide a simplified basis for determining chemical toxicities, and *Daphnia* were found to adequately provide this basis in more chemical categories than any other taxon for which data have been collected. It has been assumed that *Daphnia* are one of the more sensitive invertebrates (Morgan, 1972), and therefore their widespread use as a catch-all model organism has been accepted. Additionally, *Daphnia* provide ease of laboratory rearing and a recently sequenced genome, which allows an opportunity to investigate environment-gene interactions. Despite the desirable characteristics possessed by *Daphnia* and a reasonably high success rate for being among the most sensitive organisms, there were 19 out of 29 chemical categories for which *Daphnia* did not represent the highest sensitivity. Data such as these suggest the need for additional, secondary model organisms. These secondary model organisms could be used in cases where Daphnia were found to not adequately represent aquatic species sensitivities.

**Suggestions of alternate model organisms**

Species belonging to the class Malacostraca provided the highest sensitivities across five chemical groups examined. Among these five are the organochlorine category, for which *Daphnia* were less sensitive to exposure than five other taxa. Malacostraca were also the most sensitive taxonomic class for the pyrethroid, inorganic arsenic, anilide, and botanical categories, for which *Daphnia* were the second most highly sensitive. Potential secondary model organisms belonging to this class include the Marmorkrebs, marbled crayfish (*Procambarus fallax*), belonging to the order Decapoda (Martin et al., 2007). Reproducing parthenogenetically, this species provides many of the
same valuable attributes as *Daphnia* and shows a greater sensitivity for several chemical groups.

Monogononta species had the highest sensitivities of available data for three chemical categories. Included in these categories were the inorganic category, in which *Daphnia* were found to be less sensitive than eight other taxa. Monogononta species were also found to be the most sensitive to exposure to the chlorinated phenol and urea categories. Many species from the Rotifera order are easily maintained in a lab and multiply via parthenogenetic reproduction. Species such as *Euchlanis dilatata* and *Lecane inermis* are have been established as model organisms and could be used to represent aquatic species sensitivities for inorganic, chlorinated phenol, and urea chemicals (King, 1972 and Beata, 2013).

Amphibia and Actinopterygii were each the most sensitive class found for two chemical categories. Amphibia had the lowest HC\textsubscript{50} values for the chloroacetanilide and halogenated organic categories, and Actinopterygii had the lowest HC\textsubscript{50} values for the phenol and dinitrophenol derivative categories. The African clawed frog (*Xenopus laevis*) and zebrafish (*Danio rerio*) are widely used model organisms from the classes Amphibia and Actinopterygii.

Among the categories for which toxicity data revealed multiple taxa more sensitive than *Daphnia*, particularly pertinent are the triazines, for which the SSD revealed six taxa more highly sensitive than *Daphnia*, which were found to be the least sensitive taxonomic group (Fig.2). Triazines contain some of the most widely used pesticides in the United States, such as atrazine. The physiology as well as behavior of aquatic organisms can be impacted by atrazine contamination (Rohr and McCoy, 2010).
Given that up to 90,000 tons of atrazine may be used globally each year (Premazzi and Stecchi, 1990), the triazine chemical group is of particular importance when considering allowable environmental concentrations. While *Daphnia* is among the least sensitive taxa for which there is triazine toxicity data, species in the class Gastropoda had the highest sensitivity to this category.

Similar to Bivalvia, which was found to be the most sensitive taxa when exposed to the alcohol ether and soap categories, and Maxillopoda, Insecta, Anthozoa, and Ostracoda, which were each the most highly sensitive to exposure in one chemical category, no obvious model organism may exist for Gastropoda, but an expert familiar with each of these classes may be able to determine a suitable organism.

*Caveats*

Despite the obvious appeal of utilizing model organisms, it is important to be aware that even choosing a secondary model organism from a determined highly sensitive taxon does not ensure that organism will be representative of the sensitivities of a range of taxa to a specific chemical group or even the sensitivities within that specific taxon. This study compares a broad range of sensitivities, and while a taxon may be found to have the lowest HC\textsubscript{50} when exposed to a chemical group, the LC\textsubscript{50} values for species within that taxon may vary widely. Analysis of the organochlorine chemical category revealed that Malacostraca was the most sensitive class of organisms with an HC\textsubscript{50} value of 1.27µg/L and the class Ciliatea was the least sensitive, with an HC\textsubscript{50} value of 3.69µg/L. However, LC\textsubscript{50} values for acute toxicity tests involving species belonging to Malacostraca reach as high as 5.18µg/L, much higher than the HC\textsubscript{50} value of least
sensitive taxa, and those for Ciliatea were as low as 0.51µg/L, which is much lower than the HC$_{50}$ value of most highly sensitive taxa. This shows how although a certain taxa may highly resilient, species within that taxa may be susceptible and vice versa.

The AQUIRE database contains an exceptional number of toxicity tests containing valuable data pertaining to possible chemical contaminants. This analysis reviewed the LC$_{50}$ data available within this database. While data exists for other, nonlethal toxicity measures (EC, or effective concentration, values), these studies test more subjective values involving measures such as behavioral or reproductive effects, making these points difficult to compare, particularly across different taxa. Measures of lethality provide the simplest parameter to compare across taxa. Despite being difficult to compare, these sub-lethal measures are informative, making them important to consider in future studies. Additional measures of lethal concentrations also exist, but the LC$_{50}$ is the most reliable point to estimate on the dose-response curve, and for that reason it was chosen as the point of interest for this analysis (Newman and Unger, 2003). Finally, 24-96hr exposure ranges encompassed the majority of the data present in the database while still maintaining a narrow exposure window for comparison. This resulted in our use of LC$_{50}$ values for acute toxicity tests with 24-96hr exposures as the basis for our comparison.

**Conclusion**

With access to this amount of toxicity evidence, the EPA should be able to recognize the sensitivity differences across taxa and chemical groups using studies such as that which has been accomplished here. While Daphnia exhibit the highest sensitivity
to the largest number of the 29 chemical categories tested in this study, they nevertheless fail to represent the most sensitive taxa or taxon in 19 of these categories. While model organisms provide the benefits of cost effectiveness and efficiency, utilizing a single, primary model organism to represent such a broad array of chemicals and taxa leaves many organisms unrepresented and susceptible. The results of this analysis show the pertinence of a more specific approach to the testing and regulation of aquatic chemicals, suggesting the use of specific, secondary model organisms chosen from the classes shown to be the most highly sensitive to each chemical category.
APPENDIX
Figure 1. HC50 values of inorganic sensitivities across taxa. Error bars are ±1 SE and asterisks represent significant difference. *Daphnia* fall within the middle of the sensitivity ranges for the inorganic chemical category.
Figure 2. HC$_{50}$ values of triazine sensitivities across taxa. Error bars are ±1 SE and asterisks represent significant difference. *Daphnia* are the least sensitive to the triazine chemical category.

Figure 3. HC$_{50}$ values of chlorinated phenol sensitivities across taxa. Error bars are ±1 SE and asterisks represent significant difference. *Daphnia* fall within the middle of the sensitivity ranges for the chlorinated phenol chemical category.
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Figure 9. HC₅₀ values of pyrethroid sensitivities across taxa. Error bars are ±1 SE and asterisks represent significant difference. *Daphnia* fall within the middle of the sensitivity ranges for the pyrethroid chemical category.
**Figure 10.** HC$_{50}$ values of urea sensitivities across taxa. Error bars are ±1 SE and asterisks represent significant difference. One taxon is more highly sensitive to the urea chemical category than *Daphnia*.

**Figure 11.** HC$_{50}$ values of inorganic copper sensitivities across taxa. Error bars are ±1 SE and asterisks represent significant difference. One taxon is more highly sensitive to the inorganic copper chemical category than *Daphnia*. 

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Figure 12. HC₅₀ values of chloroacetanilide sensitivities across taxa. Error bars are ±1 SE and asterisks represent significant difference. One taxon is more highly sensitive to the chloroacetanilide chemical category than Daphnia.

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Figure 15. HC$_{50}$ values of anilide sensitivities across taxa. Error bars are ±1 SE and asterisks represent significant difference. One taxon is more highly sensitive to the anilide chemical category than *Daphnia*. 
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**Figure 17.** HC$_{50}$ values of botanical sensitivities across taxa. Error bars are ±1 SE and asterisks represent significant difference. One taxon is more highly sensitive to the botanical chemical category than *Daphnia*. 
Figure 18. HC$_{50}$ values of halogenated organic sensitivities across taxa. Error bars are ±1 SE and asterisks represent significant difference. One taxon is more highly sensitive to the halogenated organic chemical category than *Daphnia*.

![Halogenated Organic](image)

Figure 19. HC$_{50}$ values of organophosphorus sensitivities across taxa. Error bars are ±1 SE and asterisks represent significant difference. One taxon is more highly sensitive to the organophosphorus chemical category than *Daphnia*.

![Organophosphorus](image)
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**Figure 21.** HC₅₀ values of inorganic silver sensitivities across taxa. Error bars are ±1 SE and asterisks represent significant difference. *Daphnia* are among the most highly sensitive for the inorganic silver chemical category.
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Figure 24. HC$_{50}$ values of 2,6dinitroaniline sensitivities across taxa. Error bars are ±1 SE and asterisks represent significant difference. *Daphnia* are among the most highly sensitive for the 2,6dinitroaniline chemical category.

Figure 25. HC$_{50}$ values of dithiocarbamate sensitivities across taxa. Error bars are ±1 SE and asterisks represent significant difference. *Daphnia* are among the most highly sensitive for the dithiocarbamate chemical category.
**Figure 26.** HC$_{50}$ values of thiocarbamate sensitivities across taxa. Error bars are ±1 SE and asterisks represent significant difference. *Daphnia* are among the most highly sensitive for the thiocarbamate chemical category.

**Figure 27.** HC$_{50}$ values of carbamate sensitivities across taxa. Error bars are ±1 SE and asterisks represent significant difference. *Daphnia* are among the most highly sensitive for the carbamate chemical category.
**Figure 28.** HC$_{50}$ values of inorganic chromium (IV) sensitivities across taxa. Error bars are ±1 SE and asterisks represent significant difference. *Daphnia* are among the most highly sensitive for the inorganic chromium (IV) chemical category.

**Figure 29.** HC$_{50}$ values of inorganic zinc sensitivities across taxa. Error bars are ±1 SE and asterisks represent significant difference. *Daphnia* are among the most highly sensitive for the inorganic zinc chemical category.
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</tr>
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<td>2</td>
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</table>

**Table 1.** Comparison of HC$_{50}$ values for the most sensitive taxon for each chemical category and *Daphnia* (Branchiopoda).
REFERENCES


NEPIS. (1999). Methods for aquatic toxicity identification evaluations phase III toxicity confirmation procedures for samples exhibiting acute and chronic toxicity. EPA.

