INFLUENCE OF DEVELOPMENTAL STAGE ON SUSCEPTIBILITIES AND SENSITIVITIES OF *SIMULIUM VITTATUM* IS-7 AND *SIMULIUM VITTATUM* III-L-1 (DIPTERA: SIMULIIDAE) TO CHLORPYRIFOS

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Abstract—Determination of the most sensitive and susceptible organismal life stage is important for use of the organism in toxicity assessments of environmental pollutants. In the present study, the sensitivities and susceptibilities of larval developmental stages of two black fly sibling species, *Simulium vittatum* Zetterstedt cytospecies IS-7 and *S. vittatum* Zetterstedt cytospecies III-L-1, were determined using the organophosphate-insecticide chlorpyrifos. Differences in sensitivity and susceptibility were determined through analysis of slopes and median lethal concentration values produced from 24-h orbital shaker toxicity tests, respectively. The results showed no difference in sensitivity or susceptibility between sibling species. However, early instar (second and third) as well as mid-instar (fourth and fifth) groupings of *S. vittatum* IS-7 were significantly more susceptible than late-instar (sixth and seventh) larval groupings. Likewise, mid-instar groupings of *S. vittatum* III-L-1 were more susceptible than late-instar larval groupings. However, neither species showed significant differences in sensitivity among instar groupings. The results of the present study indicate that the mid-instar groupings of black fly larvae are the best choice for use in toxicity tests, both because of their increased susceptibility compared to late-instar groupings and because of their ease in handling and manipulating in the laboratory compared to early instar groupings.

Keywords—Susceptibility  Sensitivity  Life stage  *Simulium vittatum*  Black fly

INTRODUCTION

Black fly larvae (Diptera: Simuliidae) are filter-feeding organisms inhabiting lotic systems throughout the world. Black fly larvae have been used for many years in bioassays and toxicity tests to evaluate insecticides for use in controlling these organisms because of their nuisance biting and disease-vectoring capabilities as adults. However, simulids have been used only recently in toxicity tests and as a bioindicator organism for evaluating the environmental effects of contaminants [1,2].

Black flies possess several attributes that make them excellent organisms for use in environmental assessments and toxicity tests: They are the best-known group of aquatic macroinvertebrates at the species level [3], are nearly ubiquitous in running waters [3], have large polytene chromosomes that can be used in genetic assessments [4,5], have a larval head capsule morphology that can be used to assess contaminant effects [2], can be reared in large numbers in the laboratory [6], and at least regarding certain species, are highly susceptible to environmental contaminants [1].

As with any organism used in toxicity tests, it is important to determine the most susceptible life stage of development. Holometabolous insects, such as black flies, have four life stages: Egg, larva, pupa, and adult. An insect larva develops through a series of instars before pupation and emergence as an adult. Typically, the early life stages of organisms are the most susceptible to contaminants [7–10]. Thus, we would expect early instar black fly larvae to be more susceptible than late-instar larvae. However, Collyard et al. [11] showed that mortality in the amphipod *Hyallela azteca*, after exposure to several toxicants individually, was not related to age (or size), indicating that not all organisms display an inverse relationship between age and toxicity of a chemical.

In the present study, our objective was to determine if a significant difference existed in the sensitivity (the increment of increase in mortality for a unit increase in toxicant concentration; i.e., the slope) and the susceptibility (the concentration of a toxicant needed to kill a desired percentage of the organisms; i.e., the median lethal concentration [LC50]) among instars of two black fly sibling species, *Simulium vittatum* Zetterstedt cytospecies IS-7 and *S. vittatum* Zetterstedt cytospecies III-L-1. Instar sensitivity and susceptibility were assessed with the organophosphate-insecticide chlorpyrifos (O,O-diethyl O-[3,5,6-trichloro-2-pyridyl]-phosphorothioate), a common contaminant of streams and rivers in agricultural and urban watersheds [12].

MATERIALS AND METHODS

Organisms

*Simulium vittatum* IS-7. Larvae of *S. vittatum* IS-7 were obtained from a colony maintained in our laboratory. Larvae were reared as described by Gray and Noblet [6].

*Simulium vittatum* III-L-1. Eggs of *S. vittatum* III-L-1 were collected from a tributary of the South Fork Holston River (36°33’N, 82°33’W; Kingsport, Sullivan County, TN, USA). This tributary contains effluent from several industrial sources, including Willamet Paper Plant, Holston Army Ammunitions Plant, and the Eastman Chemical Company facilities (R. Strang, Eastman-Kodak, Kingsport, TN, USA, personal communication). Other sources of effluent impacting this river include Kingsport’s treated wastewater and the lake water of

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the Fort Patrick Henry Dam, built north of Kingsport, to produce hydroelectricity (R. Strang, personal communication).

Eggs of *S. vittatum* III-1 were collected from emergent substrates and transported in containers on ice to the laboratory. The eggs were sterilized according to the methods described by Fredeen [13], with modifications by Hoffman [14]. Eggs were allowed to hatch, and the larvae were reared following the aforementioned laboratory-colony protocol. However, after testing, tanks used for rearing this species were disassembled to prevent maturation of this species to adulthood in the laboratory.

**Larval sex and instar determination**

From each trial, 10 larvae were randomly collected from control flasks to determine larval sex and instar. The sex of each larva was determined by observing the shape of the gonads through the dorsal side of the abdomen. Male larva possess spherical gonads; females have elongated gonads [15]. If the gonads were not visible through the abdominal skin, then the larvae were dissected. After sex determination, larval instar was determined by examining the head-capule postgenal length (mandibular phragmata to the postgena) and the head-capule width (oculus to ocellus) [16–18]. All measurements were made using a Wild dissecting microscope (Heerbrugg, Switzerland) fitted with an ocular micrometer at ×50 magnification.

**Testing procedure**

Moderately hard test water was prepared following American Society for Testing and Materials methods [19]. The measured mean water chemistry parameters were as follows: Dissolved oxygen, 68%; alkalinity, 64.8 mg CaCO₃/L; hardness, 86.8 mg CaCO₃/L; pH, 7.6; and temperature, 19°C.

The sensitivity and susceptibility of different larval instars of *S. vittatum* IS-7 and III-1 were determined using an orbital shaker toxicity test. An orbital shaker was used in assessing insecticides to black flies, because the shaker produces a current inside the flask that simulates flowing water. The vast majority of black fly species, including *S. vittatum* IS-7, inhabit flowing waters because of food delivery and oxygen requirements. Thus, the orbital shaker testing protocol provides a suitable environment that meets their needs with respect to these basic requirements.

For each toxicity test, sections of nylon-screen substrate with several hundred larvae attached were removed from the runway of the rearing tank and placed into enamel pans with 1 L of culture water. Fifteen uniform larvae were transferred (Corning, NY, USA) flat-bottom extraction flasks containing 145 ml of test water. Flasks were placed on a New Brunswick Scientific G-10 Gy- ratory® shaker (Edison, NJ, USA), and the larvae were allowed to acclimate for 10 min at 100 rpm, 10 min at 125 rpm, and 40 min at 150 rpm (the final speed for the duration of the experiment) for a total acclimation period of 1 h. For the early instar trials, modifications were made to the shaker speeds used for acclimation and testing. These speeds were 75 rpm for 10 min, 100 rpm for 10 min, 125 rpm for 10 min, and 135 rpm for the rest of the trial exposure period. Shaker speed was reduced for testing the early instars to decrease control mortality to less than 10%. This acclimation period allows the larvae to choose a preferred attachment site and to position themselves in the optimal flow pattern within the flasks. After the acclimation period, the flasks were treated with 5 ml of

for each experiment. Replicate samples came from three different trials to establish the statistical significance of each trial. Standards were prepared and preserved for analysis.

Analyses of the chlorpyrifos concentration in the water from each trial were conducted using gas chromatography with an electron-capture detector for determination of halogenates (i.e., the chlorines in chlorpyrifos) [20]. Chlorpyrifos was recovered from water samples using solid-phase extraction. Solid-phase extraction C-18 cartridges (500 mg; Burdick and Jackson, Muskegon, MI, USA) were used for this experiment. All subsequent dosing solutions were made from the stock by adding a predetermined aliquot of the stock to moderately hard water, bringing the dosing solution up to at least 100 ml. For the early instar groupings (i.e., second and third instars), chlorpyrifos concentrations ranged from 0.008 to 0.128 μg/L, with an acetone control, culture water control, and moderately hard water control. For mid-instar groupings (i.e., fourth and fifth instars), chlorpyrifos concentrations ranged from 0.08 to 1.28 μg/L, with an acetone control, culture water control, and moderately hard water control. Finally, chlorpyrifos concentrations for the late-instar groupings (i.e., sixth and seventh instars) ranged from 0.2 to 3.2 μg/L, with an acetone control, culture water control, and moderately hard water control.

Acetone controls were made at the highest concentration tested to eliminate mortality caused only by acetone, because it was the solvent carrier for the insoluble chlorpyrifos. Duplications of both concentrations and controls were averaged within each concentration or control. Three trials were conducted for each instar grouping.

**Chemical analysis**

Water samples for chlorpyrifos validation were collected and stored at 9°C as the dosing solutions were prepared for each experiment. Duplicate samples came from three different trials to establish the statistical significance of each trial. Standards were prepared and preserved for analysis.

Analyses of the chlorpyrifos concentration in the water from each trial were conducted using gas chromatography with an electron-capture detector for determination of halogenates (i.e., the chlorines in chlorpyrifos) [20]. Chlorpyrifos was recovered from water samples using solid-phase extraction. Solid-phase extraction C-18 cartridges (500 mg; Burdick and Jackson, Muskegon, MI, USA) with the first filter removed to reduce overbinding of the compound (M. Riley, Clemson University, Clemson, SC, USA, personal communication) were activated, and 150-ml aliquots were pulled under vacuum through the cartridges. Chlorpyrifos was eluted from the cartridges with 2 ml of acetone (Optima, Fisher Scientific, Fair Lawn, NJ, USA). The acetone-suspended samples were then filtered through sodium sulfate and a 0.2-μm, nylon Acrodisc® filter (Pall, Ann Arbor, MI, USA) into screw-cap autosampler vials (National Scientific, Duluth, GA, USA).

Samples were analyzed on a Hewlett-Packard 8580 gas chromatograph (Palo Alto, CA, USA) with an electron-capture detector. The gas chromatograph column was a DB-5 (length,
In the first analysis comparing the two black fly sibling species, only the mid- and late-instar groupings were examined. The ANOVA performed on the LC50 values indicated no significance of the factor species or of the interaction of species with age. The sum of squares of the insignificant interaction term was pooled with the overall sum-of-squares error term to determine significant main effect factors [23,25]. Significance of mean LC50 values was found with respect to the factor of age (α = 0.01) [23,24] (Table 1). Tukey’s multiple-comparison test was performed to determine significant differences among the instars [23,24]. Mean slopes were not significantly different from one another using the same main effects and interaction as in the analysis of the mean LC50 values. The sum of squares of the insignificant interaction term was pooled with the overall sum-of-squares error term to determine significant main effect factors [23,25]. The mid-instar groupings of both species were more susceptible to chlorpyrifos than the late instars (Table 1). The mid-instar groupings were not more sensitive than the late instars, however, because the slopes were very similar among all instar groupings (Table 1). The power of the test was 0.92 [25].

In the second analysis, susceptibility and sensitivity to chlorpyrifos were examined in three larval instar groupings of S. vittatum IS-7. An ANOVA performed on the LC50 values indicated significance of instar (α = 0.01) [23,24]. Tukey’s pairwise-comparison test was performed to determine significant differences among the instars (α = 0.01) [23,24]. Mean slopes were also examined as a function of the main effect (instar) while blocking for tank of development [23,24]. Significance of mean slopes with respect to instar was examined further with Tukey’s pairwise-comparison tests (α = 0.01).

The early instar grouping was not significantly different in susceptibility from the mid-instar grouping (Table 1). However, the early instar and mid-instar groupings were both significantly more susceptible to chlorpyrifos than the late-instar grouping. All instar groupings were similar in sensitivity to chlorpyrifos, because the slopes were not significantly different (Table 1). The power of the test was 0.89 [25].

**Comparison of instar groupings**

In the final analysis comparing the two black fly sibling species, only the mid- and late-instar groupings were examined. The ANOVA performed on the LC50 values indicated no significance of the factor species or of the interaction of species with age. The sum of squares of the insignificant interaction term was pooled with the overall sum-of-squares error term to determine significant main effect factors [23,25]. Significance of mean LC50 values was found with respect to the factor of age (α = 0.01) [23,24] (Table 1). Tukey’s multiple-comparison test was performed to determine significant differences among the instars [23,24]. Mean slopes were not significantly different from one another using the same main effects and interaction as in the analysis of the mean LC50 values. The sum of squares of the insignificant interaction term was pooled with the overall sum-of-squares error term to determine significant main effect factors [23,25]. The mid-instar groupings of both species were more susceptible to chlorpyrifos than the late instars (Table 1). The mid-instar groupings were not more sensitive than the late instars, however, because the slopes were very similar among all instar groupings (Table 1). The power of the test was 0.92 [25].

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**Post-trial instar and sex determination**

Linear measurements of head-capscule width and postgenal length as determined by Ross [17] and Hudson [18] were used to examine the stage of development for each selected larve.
Post-trial analyses of head-capule measurements from randomly collected larvae of the designated instar grouping for each species (total, 150 larvae) confirmed that instars used for each 24-h orbital shaker toxicity test could be effectively grouped in the early, mid-, or late-instar grouping [17,18] (Table 2). Through use of ANOVA including factors of species, instar, and interaction of species and instar while blocking for tank of development, only instar was significant [23,24]. The sum of squares of the insignificant interaction term was pooled with the overall sum-of-squares error term to determine significant main effect factors [23,25]. Mean postgenal measurements and mean head-capule widths compared with Tukey’s pairwise-comparison test were significantly different for each instar, increasing with development [23,24] (Table 2). The postgenal lengths were statistically analyzed independently of the head-capule widths.

Post-trial analyses of sex ratios in randomly collected larvae indicated a predominance of males used in the trials (Table 3). Of the 150 randomly selected larvae, only eight were classified as unknown and were not used in any further analyses. Goodness-of-fit analysis of the sexed larvae resulted in rejection of the null hypothesis that 50% of the organisms used in testing would be male and the other 50% would be female ($\chi^2 > 6.63$).

**DISCUSSION**

**Instar determination**

Variable numbers of larval instars occur in species within the family Simuliidae; hence, extrapolation from species to species is ill-advised [16,26–28]. However, instar number has been determined for species complexes, before elucidation of cytospecies [29]. For example, the *S. vittatum* complex, consisting of *S. vittatum* IS-7 and *S. vittatum* III-L-1, was found to have a variable instar number, with the most accepted number of instars being seven [29]. Therefore, head-capule measurements of *S. vittatum* IS-7 larvae made by Hudson [18], in combination with metrics determined by Ross [17] of *S. vittatum* larvae (cytospecies unknown), were used to establish the mean number of larval instars of the cultured black flies in the present study.

Each study has its own variables, such as water temperature, water velocity, concentrations of food in water, and light cycles, that can cause changes in larval instar size and, thus, determination [29,30]. All environmental parameters in the laboratory were monitored and adjusted on at least a daily basis; therefore, the conditions of development for each species were similar. Linear estimates of larval development were used as approximations of probable instar number.

**Sex determination**

Selection of uniform-sized larvae for the toxicity tests with chlorpyrifos resulted in the predominant selection of males. Colbo and Porter [31] determined a shorter development time from larval to adult stages for *S. vittatum* (cytospecies unknown) males than for females, and the overall size of male larvae was smaller than that of female larvae. Thus, at the time of the experiments, more males may have been at the appropriate size for use in the various instar-grouping experiments. This trend could possibly confound the generated toxicity data because of physiological differences between males and females. However, most invertebrate toxicity tests do not take into consideration the gender of the test organisms because of the inability to distinguish gender for the life stage used. Thus, the ability to determine the sex of *S. vittatum* IS-7 may be useful for data interpretation or gender-based studies.

**Comparison of instar groupings**

The study of the responses by groups of larval instars to toxicants is not a new idea; however, the idea is unique with respect to black flies and chlorpyrifos. In the first analysis comparing the susceptibilities of the two black fly sibling species, it was determined that the susceptibility of the *S. vittatum* III-L-1 mid-instar grouping to chlorpyrifos was not significantly different from that of the *S. vittatum* IS-7 mid-instar grouping. The late instars also exhibited no significant difference in susceptibility from one another. However, the mid-instars of both

### Table 2. Mean head-capule measurements (± standard error) of *Simuliium vittatum* IS-7 and *S. vittatum* III-L-1 for instar determination and grouping$^a$

<table>
<thead>
<tr>
<th>Species</th>
<th>Instar grouping</th>
<th>N</th>
<th>n</th>
<th>Postgenal length (µm)</th>
<th>Head-capule width (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. vittatum</em> IS-7</td>
<td>Early (second and third instars)</td>
<td>3</td>
<td>30</td>
<td>157.1 ± 24.6 A</td>
<td>268.3 ± 25.5 A</td>
</tr>
<tr>
<td></td>
<td>Mid (fourth and fifth instars)</td>
<td>3</td>
<td>30</td>
<td>408.8 ± 24.3 B</td>
<td>477.3 ± 26.3 B</td>
</tr>
<tr>
<td></td>
<td>Late (sixth and seventh instars)</td>
<td>3</td>
<td>30</td>
<td>475.5 ± 24.5 C</td>
<td>569.9 ± 25.8 C</td>
</tr>
<tr>
<td><em>S. vittatum</em> III-L-1</td>
<td>Mid (fourth and fifth instars)</td>
<td>3</td>
<td>30</td>
<td>362.2 ± 26.3 B</td>
<td>392.2 ± 24.8 B</td>
</tr>
<tr>
<td></td>
<td>Late (sixth and seventh instars)</td>
<td>3</td>
<td>30</td>
<td>471.8 ± 25.5 C</td>
<td>560.9 ± 25.9 C</td>
</tr>
</tbody>
</table>

$^a$ Means with the same letter within each column are not significantly different ($p > 0.01$).

$^b$ N = number of replicates; n = sample size.

### Table 3. Sex ratios of *Simuliium vittatum* IS-7 and *S. vittatum* III-L-1 larvae used in the orbital shaker toxicity test$^a$

<table>
<thead>
<tr>
<th>Species</th>
<th>Instar grouping</th>
<th>N</th>
<th>n</th>
<th>Sex ratio (%)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. vittatum</em> IS-7</td>
<td>Early (second and third instars)</td>
<td>3</td>
<td>30</td>
<td>90:10</td>
</tr>
<tr>
<td></td>
<td>Mid (fourth and fifth instars)</td>
<td>3</td>
<td>30</td>
<td>77:23</td>
</tr>
<tr>
<td></td>
<td>Late (sixth and seventh instars)</td>
<td>3</td>
<td>30</td>
<td>79:21</td>
</tr>
<tr>
<td><em>S. vittatum</em> III-L-1</td>
<td>Mid (fourth and fifth instars)</td>
<td>3</td>
<td>30</td>
<td>80:20</td>
</tr>
<tr>
<td></td>
<td>Late (sixth and seventh instars)</td>
<td>3</td>
<td>30</td>
<td>81:19</td>
</tr>
</tbody>
</table>

$^a$ N = number of replicates; n = sample size.

$^b$ Male:female.
species were significantly more susceptible to chlorpyrifos than the late instars. This indicates that stage of development may play a greater role in dose–response relationships of black fly species than differences in size among the species themselves, considering that *S. vittatum* IS-7 larvae typically were larger than the *S. vittatum* III-1 larvae.

Of the two species, *S. vittatum* III-1 typically exists in systems that are highly loaded organically [32]. However, eutrophic systems are not synonymous with pesticide-polluted systems. Therefore, even though *S. vittatum* III-1 may tolerate warmer, more nutrient-rich systems, it is not necessarily more tolerant to chlorpyrifos in laboratory trials, even at low levels. Similar results were also observed by Sloof [33], who showed that pollution-tolerant organisms can be susceptible to chemical pollutants in laboratory toxicity tests.

In the second analysis of *S. vittatum* IS-7 susceptibility among instar groupings, similar results were obtained, with the early instars and mid-instar showing greater susceptibility to chlorpyrifos than the late instars. However, susceptibility in the early instar groupings was not significantly greater than that in the mid-instar groups. In addition, no significant difference was found in the sensitivities of the three instar groupings. Thus, no greater sensitivity or susceptibility was achieved by using the early instars. The early instars also were much more difficult to work with because of their small size, and testing protocols had to be modified to keep control mortality at less than 10%. Based on the data provided in these experiments, it is recommended that mid-instar larvae be used in toxicity tests because of their apparent high sensitivity and susceptibility to chemicals, ease of handling, and hardness for use in toxicity tests.

**Chlorpyrifos as the toxicant**

Chlorpyrifos is highly lipophilic and is taken up by *S. vittatum* larvae through processes of dermal absorption (i.e., direct contact) and ingestion (i.e., via encapsulation of the pesticide) [34]. Without encapsulation, it is highly probable that chlorpyrifos within the water column would become attached to bacteria and fecal matter within the water and, thus, could be taken up by ingestion [35].

Muirhead-Thomson [36] and Rodrigues et al. [34] found that microencapsulated formulations of chlorpyrifos-methyl resulted in late-instar *S. vittatum* larval detachment and subsequent death, with LC50 values of the most effective formulations being 5.5 and 24 μg/L [34]. These formulations consisted of a proteinaceous/poly saccharide-complexed capsule of chlorpyrifos-methyl, a less toxic form of chlorpyrifos [34]. The mode of uptake is via direct dermal contact or ingestion, which suggests that the lipophilic nature of chlorpyrifos causes it to be readily taken into black flies [34]. With respect to age, increasing fat bodies in older larvae may provide a repository for chlorpyrifos, making it less available for metabolism leading to subsequent death. Thus, fat might decrease black fly susceptibility and sensitivity, making older instars less suitable for standard comparative toxicological means.

In a study on drifting behavior, Wallace et al. [37] noted that after treatments with the organochlorine-compound methoxychlor, drifting larvae tended to increase in size during the drift period. This observation indicated that the younger instars were more susceptible overall [37]. Similarly, the inverse relationship between susceptibility and age has been recorded for larvae of *S. vittatum* with respect to the pesticide diflubenzuron [38].

Wallace et al. [37] suggested that differences in susceptibility of simulid instars to insecticides may result, in part, from different feeding rates (decrease with development) and physiological tolerances (increase with development) among instars. Chance [39] found no significant differences in the sizes of particles ingested by medium-sized and large larvae with respect to labral fan structure. Therefore, the inverse age–susceptibility relationship is unlikely to be a function of differences in labral fan structure (i.e., capture of particles).

### Table 4. Comparison of 24-h median lethal concentrations (LC50) among species of aquatic invertebrates to the insecticide chlorpyrifos

<table>
<thead>
<tr>
<th>Class</th>
<th>Order</th>
<th>Species</th>
<th>LC50 (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insecta</td>
<td>Diptera</td>
<td><em>S. vittatum</em> IS-7</td>
<td>0.06–0.68</td>
</tr>
<tr>
<td>Branchiopoda</td>
<td>Cladocera</td>
<td><em>Ceriodaphnia dubia</em></td>
<td>0.10</td>
</tr>
<tr>
<td>Insecta</td>
<td>Diptera</td>
<td><em>S. vittatum</em> III-1</td>
<td>0.13–0.91</td>
</tr>
<tr>
<td>Branchiopoda</td>
<td>Cladocera</td>
<td><em>Daphnia pulex</em></td>
<td>0.17</td>
</tr>
<tr>
<td>Insecta</td>
<td>Diptera</td>
<td><em>Aedes aegypti</em></td>
<td>0.30–2.31</td>
</tr>
<tr>
<td>Insecta</td>
<td>Diptera</td>
<td><em>Culex pipiens</em></td>
<td>0.50</td>
</tr>
<tr>
<td>Malacostraca</td>
<td>Amphipoda</td>
<td><em>Hyalella azteca</em></td>
<td>0.64</td>
</tr>
<tr>
<td>Insecta</td>
<td>Ephemeroptera</td>
<td><em>Cloeon dipterum</em></td>
<td>0.7</td>
</tr>
<tr>
<td>Malacostraca</td>
<td>Amphipoda</td>
<td><em>Gammarus lacustris</em></td>
<td>0.76</td>
</tr>
<tr>
<td>Insecta</td>
<td>Diptera</td>
<td><em>Anopheles gambiae</em></td>
<td>1.40–6.90</td>
</tr>
<tr>
<td>Branchiopoda</td>
<td>Cladocera</td>
<td><em>D. magna</em></td>
<td>3.7</td>
</tr>
<tr>
<td>Insecta</td>
<td>Plectoptera</td>
<td><em>Pteronarcella badia</em></td>
<td>4.2</td>
</tr>
<tr>
<td>Insecta</td>
<td>Coleoptera</td>
<td><em>Chaoborus punctipennis</em></td>
<td>5.40</td>
</tr>
<tr>
<td>Insecta</td>
<td>Coleoptera</td>
<td><em>Thermonecetus basilaris</em></td>
<td>6.00</td>
</tr>
<tr>
<td>Insecta</td>
<td>Coleoptera</td>
<td><em>Tropisternus lateralis</em></td>
<td>8.00</td>
</tr>
<tr>
<td>Insecta</td>
<td>Coleoptera</td>
<td><em>Claassenia sabulosa</em></td>
<td>8.2</td>
</tr>
<tr>
<td>Insecta</td>
<td>Coleoptera</td>
<td><em>Berosus styleurus</em></td>
<td>9.00</td>
</tr>
<tr>
<td>Insecta</td>
<td>Coleoptera</td>
<td><em>Belostoma sp.</em></td>
<td>15.00</td>
</tr>
<tr>
<td>Insecta</td>
<td>Coleoptera</td>
<td><em>Hydrometra triangularis</em></td>
<td>20.00</td>
</tr>
<tr>
<td>Insecta</td>
<td>Coleoptera</td>
<td><em>Hygrorus sp.</em></td>
<td>40.00</td>
</tr>
<tr>
<td>Insecta</td>
<td>Diptera</td>
<td><em>Chironomus decorus</em></td>
<td>45.00</td>
</tr>
<tr>
<td>Insecta</td>
<td>Plectoptera</td>
<td><em>Pteronarcys californicus</em></td>
<td>50.00</td>
</tr>
<tr>
<td>Insecta</td>
<td>Diptera</td>
<td><em>Procladius sp.</em></td>
<td>71.00</td>
</tr>
</tbody>
</table>

* Data from the U.S. Environmental Protection Agency Ecotox database.
Because *S. vittatum* IS-7 is an organism not typically used in toxicity testing or environmental assessments, it is of interest to compare its susceptibility to chlorpyrifos with those of other aquatic insect species and invertebrates. In comparison, *S. vittatum* IS-7 and *S. vittatum* III-L-1 rank among the most sensitive to chlorpyrifos among aquatic invertebrates (Table 4). Thus, toxicity data obtained using *S. vittatum* IS-7 may be useful for determining safe concentrations of insecticides to nontarget insects and other invertebrates in aquatic systems.

**CONCLUSION**

The susceptibility and sensitivity of the two black fly sibling species, *S. vittatum* IS-7 and *S. vittatum* III-L-1, to chlorpyrifos were not significantly different even though *S. vittatum* species, *S. vittatum* —We would like to thank Peter Adler, Thomas La Point, Ernest Smith, Melissa Riley, and John McCreadie for their advice on various aspects of the present study. We would also like to thank Elmer Gray and Rick Willey for help with the experiments. The present study was partially funded through the U.S. Department of Defense Environmental Fellowship Program (30-7296). Although the present study was partially funded through the U.S. Department of Defense Environmental Fellowship Program (30-7296), the results were not reviewed by the U.S. Department of Defense and, therefore, do not necessarily reflect the views of the department and no official endorsement should be inferred.

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