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COMPARISON OF TWO BIOASSAY TECHNIQUES FOR ASSESSING THE ACUTE TOXICITY OF PESTICIDES TO CHIRONOMID LARVAE (DIPTERA: CHIRONOMIDAE)

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ABSTRACT. Two container and substrate combinations were compared to determine which provided optimal survival of larvae of *Chironomus tepperi* and *Glyptotendipes paripes* in the absence of toxicants. Unfed final-stage larvae of *G. paripes* survived significantly ($P < 0.05$) better in waxed paper cups with sand substrate (92.8% after 3 days) than in glass tubes with a shredded paper substrate (85.3%). Survival of larvae of *C. tepperi* over the same period did not differ significantly in the 2 systems. Larvae of *C. tepperi* were bioassayed against 3 insecticides (technical and formulated imidacloprid, chlorpyrifos, and betacypermethrin) with both container and substrate combinations. Median lethal concentration values (24 h) obtained with waxed cups with sand were 1.8 times higher on average than those obtained with glass tubes with shredded paper (range 1.13–2.65 times). To determine the cause of this variability, solid-phase microextraction was used to measure changes in chlorpyrifos availability over time in the 2 bioassay systems. Chlorpyrifos concentrations in the waxed cups and sand system fell from 7.50 to 3.36 μg/liter over 24 h, probably as a consequence of chemical adsorption to the waxed surfaces. Chlorpyrifos concentrations in the glass tubes and paper system remained unchanged over this period. Excluding substrates from the containers had only a minor effect on chlorpyrifos availability. These results demonstrate that the behaviors of both test organisms and toxicants within bioassay systems need to be understood if the data generated with different systems are to be compared. Understanding how toxicant availability is affected by different container and substrate types is particularly important where bioassays are conducted with nominal concentration values rather than analytically determined exposure concentrations.

KEY WORDS Chironomidae, bioassays, solid-phase microextraction, insecticides

INTRODUCTION

The larvae of chironomid midges are among the most abundant and widely distributed invertebrates in aquatic environments (Cranston 1995). They are an important food source for other invertebrates, fish, and waterfowl (Armitage 1995), and play a significant role in nutrient cycling (Berg 1995).

Despite their ecological importance, chironomids also can be serious pests. Adult chironomids are important pests in Japan, Italy, and parts of the USA, where dense swarms, often emerging from eutrophic habitats, cause a range of nuisance, economic, and in some cases, medical problems. Midge swarms attracted to lights in urban areas can seriously impact tourism, and may limit outdoor activities. The nuisance and economic impacts of adult chironomid swarms have been reviewed by Ali (1995, 1996).

The larvae of some chironomid species are serious pests of aquatic crops, particularly rice (Stevens 1992, Surakarn and Yano 1995). In southern Australia, larvae of *Chironomus tepperi* Skuse feed on the roots of young rice plants, and can reach densities of more than 13,000/m^2 in small experimental rice bays. At such high densities, more than 85% of seedlings can be destroyed (Helliwell and Stevens 2000). Studies on the acute toxicity of chemicals to chironomids are frequently conducted to assess their effectiveness as larvicides, either to protect crops, or to reduce adult emergence in urban areas.

Larval chironomids are usually bioassayed in the presence of a sand or paper substrate, primarily to reduce cannibalism (Mulla and Khasawinah 1969, Robinson and Scott 1995, Ali et al. 1998), whereas mosquito larvae frequently are bioassayed without a substrate (Ali et al. 1998, Wirth 1998, Wirth and Georghiou 1999). Although many toxicological studies on aquatic dipterans are conducted with glass bioassay containers, several other container types also have been used in studies with synthetic pesticides. These include waxed paper cups (Mulla et al. 1982, Wirth 1998, Wirth and Georghiou 1999), polystyrene cups and bowls (Mulla and Darwazeh 1979, Estrada and Mulla 1986, Robinson and Scott 1995), and polyvinyl chloride scintillation tubes (Halpern et al. 1999).

Variations in bioassay methodology may or may not cause problems in the interpretation of experimental results. Individual pest management studies often are concerned more with the relative toxicity of different compounds or the relative response of different pest species or strains, rather than absolute measures of toxicity. Such studies often are conducted as precursors to field studies on efficacy, with the knowledge that laboratory lethal concentration (LC) values cannot be translated directly and reliably into effective field application rates, re-

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gardless of the choice of bioassay conditions. However, problems arise when the results of separate studies conducted with different bioassay techniques need to be compared. The influence of factors that differ among bioassay systems, and may affect the response of test organisms, needs to be understood before such comparisons are attempted. Physical variables, such as container and substrate type, may influence the behavior and survival of test organisms, and also influence pesticide bioavailability. Variations in bioassay temperature and exposure period also influence the response of organisms to pesticides, and may further complicate attempts to compare results generated with differing methodologies.

Mulla and Khasawinah (1969) developed a technique for assessing acute pesticide toxicity to chironomid larvae. Based on studies conducted with *Goeldichironomus holoprasinus* (Rempel) and an unidentified *Chironomus* species, they concluded that waxed paper cups containing sand and without supplementary aeration minimized both control mortality and cannibalism. This technique subsequently has been used by other workers to assess the toxicity of insecticides to chironomid larvae in both California and Florida (Ali and Mulla 1980, Ali 1981, Ali et al. 1998).

An alternative approach was developed by Treverrow (1985), who used capped 25-mm-diameter glass tubes to bioassay larvae of *C. tepperi* against *Bacillus thuringiensis israelensis.* His technique was modified by Stevens (1992) for the assessment of organophosphorus insecticides. In the modified technique, tubes are left uncapped to facilitate gas exchange, and a small quantity of thin tissue paper strips is added to provide an artificial larval substrate. This technique also has been used to determine the LC values of pyrethroids (Stevens 1993) and the phenyl pyrazole insecticide fipronil (Stevens et al. 1998) against larvae of *C. tepperi.*

This study was conducted to determine whether these 2 bioassay systems (waxed cups with sand and glass tubes with paper) affect the survival of chironomid larvae (*C. tepperi* and *Glyptotendipes paripes* Edwards) in the absence of toxicants, and whether the bioassay systems produce differing estimates of pesticide toxicity against *C. tepperi.* When it became apparent that the 2 systems were returning consistently different results for each pesticide evaluated, a study on the persistence of chlorpyrifos in the bioassay containers was initiated to explain the observed discrepancies.

MATERIALS AND METHODS

Rearing of larvae of C. tepperi*:* Larvae of *C. tepperi* were reared by using the technique of Stevens (1992). Glass aquaria containing 10 liters of 1× Martin's rearing solution (Martin et al. 1980) supplemented with thiamine hydrochloride at 1.2 mg/liter were lined with ethanol-washed paper tissues to provide dietary bulk and tunnel-building material. Fresh egg masses of *C. tepperi* were collected from rainwater pools at Yanco Agricultural Institute (34°37′S, 146°26′E) and added to the aquaria, along with a small quantity of K9® fish food (Carnation, Noble Park, Victoria, Australia). The aquaria were covered with plastic film to prevent contamination and to reduce evaporation, and were maintained in a controlled-environment room at 25 ± 1°C with a 15:9 h light : dark lighting cycle. Aeration was provided to each aquarium through a hypodermic needle attached to an aquarium aerator via plastic tubing.

Bioassay containers and substrates: Waxed paper cups (model S303, 100-ml capacity) were obtained from the Sweetheart Cup Company, Owings Mills, MD. River sand was washed for 20 min in tap water with constant agitation, oven dried, and passed through a 710-μm-mesh sieve onto a stainless steel tray. The sand was then sprayed with distilled water, covered, and autoclaved at 121°C for 1 h, oven dried at 60°C, and allowed to cool. Five grams of sand was used as a substrate in each cup.

Flat-bottomed glass specimen tubes, 100 mm in height and with an internal diameter of 25 mm, were obtained from Australian Entomological Supplies Pty. Ltd., Bangalow, New South Wales, Australia, and washed thoroughly before use. Paper tissues used as a substrate were cut into thin, short strips (ca. 3 × 15 mm), soaked in ethanol for 24 h, and then drained and air dried for at least 3 days before use. Four to 6 strips were used in each glass bioassay tube.

Survival of C. tepperi *and* G. paripes *in untreated bioassay containers:* This experiment was conducted to determine the suitability of the different containers and substrates for use in bioassays of up to 72-h duration. Thirty cups with sand and 30 tubes with paper were prepared as described previously. Twenty milliliters of 1× Martin's rearing solution with thiamine hydrochloride supplementation (1.2 mg/liter) was added to each of the tubes, and 80 ml was added to each of the cups. Ten laboratory-reared final-stage larvae of *C. tepperi* were then added to each cup and each tube. The cups and tubes were then randomly positioned on a stainless steel tray and placed in a controlled-environment room set to 25 ± 1°C with a 15:9 h light : dark lighting cycle. No food or aeration was provided. After 24 h, 10 tubes and 10 cups were randomly removed from the tray and larval mortality was assessed. Larvae were considered dead if unable to make a sustained, coordinated response when lightly grasped with a pair of fine forceps. Larval survival was assessed from further sets of 10 tubes and 10 cups at 48 and 72 h. Total survival across the 10 tubes or cups was recorded at each assessment. The experiment was replicated 6 times.

The study was repeated in Sanford, FL, with final-stage larvae of *G. paripes.* The experimental procedure was the same as for *C. tepperi*; however,

larvae of *G. paripes* were collected daily from Lake Monroe in central Florida (28°49′N, 81°17′W) for use in the experiment, and aged tap water was used as the culture solution.

Survival data were analyzed separately for *C. tepperi* and *G. paripes*. Percentage survival was transformed to $y' = \sqrt{(100 - y)}$, since all survival data were in the 80–100% range (Steel and Torrie 1980). Analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test were used to compare survival rates between treatments.

Bioassays of technical and formulated insecticides: Final-stage larvae of *C. tepperi* were bioassayed against 3 insecticides (imidacloprid, chlorpyrifos, and betacypermethrin). Bioassays were conducted with both technical-grade materials and commercial formulations, and with both container and substrate combinations (paper cups with sand and glass tubes with paper tissue). Imidacloprid (technical grade, 98.4% purity), and Confidor® 200SC (200 g active ingredient [AI]/liter) were obtained from Bayer Australia, Pymble, New South Wales, Australia. Betacypermethrin (technical grade, 97.8% purity), and an experimental 100EC formulation (100 g AI/liter) were obtained from Nufarm Australia, Laverton North, Victoria, Australia. Technical-grade chlorpyrifos (97.3% purity) was obtained from CropCare Australasia, Pinkenba, Queensland, Australia, whereas formulated material (Lorsban® 500EC [500 g AI/liter]) was obtained from Dow Chemical, French's Forest, New South Wales, Australia. Technical materials were made up as 10 g/liter stock solutions in acetone before use in bioassays.

Pesticide suspensions were prepared by serial dilution of either the technical-grade stock solutions or the formulated materials. Initial dilutions were made in distilled water, with the final dilution being made with 1× Martin's rearing solution. Preliminary bioassays were used to locate a range of rates for each pesticide and container combination that provided partial (10–90%) mortality. Twenty-one bioassay containers (either glass tubes with shredded paper or waxed cups containing sand, as described previously) were used in each bioassay. Three containers were maintained as untreated controls, whereas 3 containers were used for each of the 6 pesticide concentrations evaluated. In bioassays of technical materials, the controls were treated with acetone at a concentration equivalent to that present in the strongest test dilution. Suspension volumes used were 20 ml per glass tube and 80 ml per paper cup. After the suspensions were added to the containers, 10 final-stage larvae of *C. tepperi* were added to each tube or cup with a fine wire hook. The bioassay containers were placed in a controlled-environment room set to 25 ± 1°C with a 15:9 h light:dark lighting cycle. No food or aeration was provided during pesticide exposure. Larvae were assessed for mortality after 24 h of exposure, by using the same protocol used in the survival study. Each bioassay was replicated 4 times, exposing a total of 120 larvae to each concentration of pesticide. The results for the 4 replicates were pooled and analyzed by probit analysis (Finney 1971).

Persistence of chlorpyrifos in bioassay media: To explain the differences between the bioassay results obtained with the 2 systems, an experiment was conducted to determine how the bioassay containers and substrates might have affected the bioavailability of the pesticides. Technical chlorpyrifos was dissolved in acetone to provide a stock solution of 10 g/liter. Serial dilution with 1× Martin's rearing solution was used to prepare 4 liters of chlorpyrifos solution with a initial concentration of 7.50 μg/liter. A control sample was prepared with a 1-liter amber glass bottle, previously treated with Coatasil® glass treatment (APS Ajax Finechem, Auburn, New South Wales, Australia; 2% w/w dimethyldichlorosilane in 1,1-dichloro-1-fluoroethane) to minimize pesticide adsorption. The bottle was filled to within 20 ml of capacity with the chlorpyrifos solution, covered with aluminium foil, and capped.

A total of 36 experimental containers (18 waxed cups and 18 glass bioassay tubes, as described previously) were used for 4 separate treatments. Nine cups and 9 tubes were left without any substrate, whereas 5 g of sand was added to each of the remaining cups, and several strands of ethanol-sterilized paper tissue were added to each of the remaining tubes. Eighty milliliters of the remaining chlorpyrifos solution was then added to each of the cups, and 20 ml was added to each of the tubes. The 36 experimental containers and the bottled control sample were then placed in a controlled-temperature room and maintained at 25 ± 1°C with a 15:9 h light:dark photoperiod for the duration of the experiment.

Three aliquots of the control solution and the solutions in 3 of the experimental containers from each treatment were analyzed for available chlorpyrifos 24 h after the experiment commenced by using solid-phase microextraction (SPME) and gas chromatography (GC) with electron-capture detection. After analysis, the experimental containers were discarded. Additional aliquots of the control solution and the remaining sample sets were analyzed at 48 and 72 h.

Extractions were made at 25 ± 1°C with a Supelco field-sampler SPME assembly incorporating a 100-μm polydimethylsiloxane fiber. Extractions were performed on 20-ml aliquots of each sample that had been transferred to 25-ml glass scintillation vials previously treated with Coatasil to minimize surface adsorption. Vials were equipped with a stirring bar (10 × 4 mm) and constant stirring was maintained (ca. 300 rpm) during the 30 ± 0.5-sec sorption time. Chlorpyrifos (ChemService, West Chester, PA) standards ranging from 1 to 10 μg/liter were prepared from a 1,000-μg/liter stock

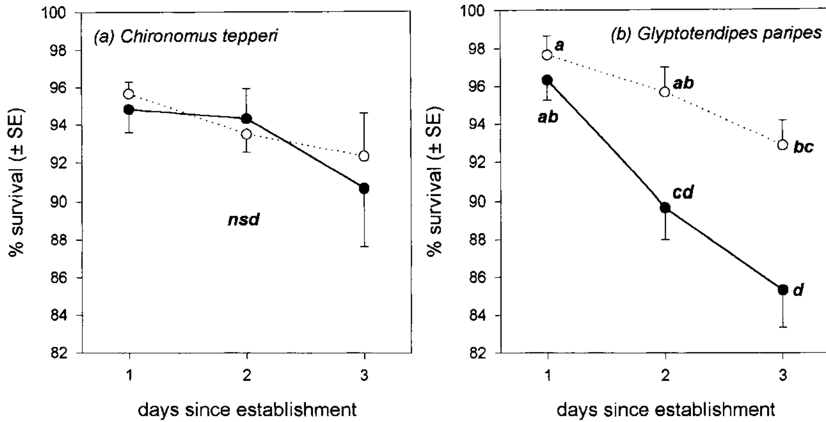

Fig. 1. Survival of final-stage larvae of (a) *Chironomus tepperi* (nsd, no significant differences; $P > 0.05$) and (b) *Glyptotendipes paripes* (points accompanied by different letters are significantly different; $P < 0.05$) in untreated bioassay systems. ——●——, glass tubes with shredded paper; – – –○– – –, waxed paper cups with sand.

chlorpyrifos standard in methanol. Standards were prepared in 1× Martin's rearing solution and exposed for the same period of time under the same conditions as the test samples. The use of Martin's rearing solution as the matrix was precautionary, because standards made up in distilled water were later shown to give similar GC responses.

Analysis was carried out on a Varian 3400 gas chromatograph (Varian Australia Pty. Ltd., Melbourne, Victoria, Australia). A Jade valve (Alltech Australia, Baulkham Hills, New South Wales, Australia) was fitted at the injection port, and electron-capture detection was used. A DB-5ms (J & W) capillary column (30 m × 0.25-mm inner diameter) (Alltech Australia) with a film thickness of 0.25 μm was fitted. The initial oven temperature was set at 200°C and was held for 1 min before ramping to 270°C at 10°C/min and holding for 10 min. The total run time was 18 min. Injector and detector temperatures were 275 and 300°C, respectively. After injection, the fiber was exposed in the splitless mode for 1 min and then allowed to desorb for a further 4 min to ensure minimal carryover. Data from the 4 treatments were analyzed by ANOVA and Tukey's HSD test.

RESULTS

Larval survival in untreated bioassay containers

Results from the survival study in untreated bioassay containers are shown in Fig. 1. Larvae of *C. tepperi* and *G. paripes* responded very differently to the different container and substrate combinations. In the case of *C. tepperi,* no significant differences in larval survival were found between the container and substrate combinations at any of the sampling intervals. Survival had fallen to 92.3 and 90.7% for cups with sand and tubes with paper, respectively, at the conclusion of the experiment. Survival of *G. paripes* remained in excess of 92% after 3 days in the waxed paper cups with sand substrate, but dropped rapidly in tubes with paper substrate to 85.3% at the conclusion of the experiment. Survival in the tubes was significantly ($P < 0.05$) lower on the 2nd and 3rd days.

Chironomus tepperi larval bioassays

Results from the bioassays of technical and formulated pesticides are given in Table 1. Median LC (LC_{50}) values obtained in bioassays with the waxed cups with sand substrate were, on average, 1.80 times higher (range 1.13–2.61 times) than those obtained with the glass tubes with paper substrate. The corresponding value for 90% LC (LC_{90}) estimates was 1.67 (range 1.00–2.65). Based on the nonoverlap of 95% fiducial limits, 5 of the 6 differences between LC_{50} estimates were significant. No clear relationship was found between the size of the discrepancies between container and substrate combinations for technical material bioassays and the octanol–water partition coefficient (K_{ow}) values of the active ingredients. The greatest discrepancies occurred with chlorpyrifos, which has a K_{ow} intermediate between those of imidacloprid and betacypermethrin (Tomlin 1994).

Persistence of chlorpyrifos in bioassay media

Results of the study on the persistence of chlorpyrifos in the bioassay containers with and without substrates are shown in Fig. 2. Chlorpyrifos concentrations in the glass tubes remained consistently close to those in the control sample throughout the experiment, and the paper substrate had no noticeable effect on concentrations until day 3, when concentrations in the tubes without paper were slightly but significantly ($P < 0.05$) lower than in the tubes with substrate. Chlorpyrifos concentrations in the

Table 1. Acute toxicity of formulated and technical-grade insecticides to final-stage larvae of *Chironomus tepperi* determined with 2 bioassay techniques. Numbers in parentheses next to median lethal concentration (LC_{50}) and 90% lethal concentration (LC_{90}) values are 95% fiducial limits.

Material	Technique	LC_{50} (μg/liter)	LC_{90} (μg/liter)	Slope	Chi-square (df)[1]
Imidacloprid (formulated)	Tubes with paper	1.60 (1.53–1.67)	2.33 (2.08–2.60)	7.6	3.6 (4)
	Waxed cups with sand	3.04 (2.89–3.19)	4.59 (4.15–5.07)	6.8	3.5 (4)
Imidacloprid (technical)	Tubes with paper	2.56 (2.35–2.79)	4.75 (3.93–5.74)	4.6	1.6 (4)
	Waxed cups with sand	2.90 (2.74–3.06)	4.76 (4.08–5.55)	5.7	2.8 (4)
Chlorpyrifos (formulated)	Tubes with paper	0.96 (0.91–1.02)	1.40 (1.31–1.50)	7.5	3.8 (4)
	Waxed cups with sand	2.51 (2.33–2.71)	3.71 (3.42–4.03)	7.2	1.8 (4)
Chlorpyrifos (technical)	Tubes with paper	1.34 (1.24–1.49)	2.28 (2.08–2.51)	5.4	2.2 (4)
	Waxed cups with sand	2.69 (2.50–2.90)	4.86 (4.25–5.54)	4.8	1.1 (4)
Betacypermethrin (formulated)	Tubes with paper	0.74 (0.65–0.84)	1.42 (1.04–1.96)	4.3	3.7 (4)
	Waxed cups with sand	1.01 (0.96–1.07)	1.65 (1.34–2.03)	5.8	1.4 (4)
Betacypermethrin (technical)	Tubes with paper	0.63 (0.49–0.80)	1.57 (1.21–2.04)	3.1	2.0 (4)
	Waxed cups with sand	1.12 (1.03–1.22)	1.77 (1.59–1.97)	6.2	2.0 (4)

[1] df, degrees of freedom.

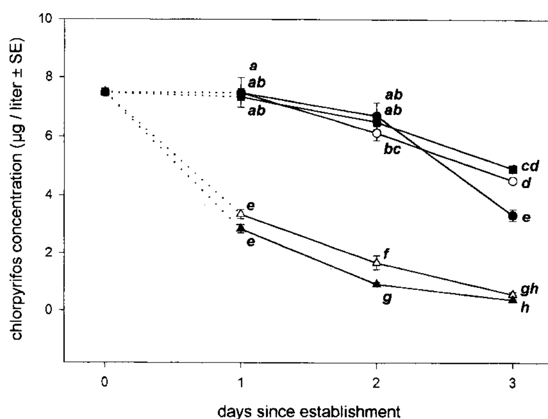

Fig. 2. Chlorpyrifos concentrations in waxed paper cups with and without sand substrate and glass tubes with and without shredded paper substrate. Initial concentration was 7.50 μg/liter in all containers. Control samples were taken from reference solution in sealed glass bottle. Points accompanied by different letters are significantly different ($P < 0.05$). ■, control sample; ○, glass tubes with paper; ●, glass tubes without paper; △, waxed paper cups with sand; ▲, waxed paper cups without sand.

waxed paper cups dropped rapidly in the 1st 24 h. Within this period, chlorpyrifos concentrations in the glass tubes (with paper substrate) did not decline below the initial concentration of 7.50 μg/liter, whereas in the cups (with sand substrate) the average concentration fell to 3.36 μg/liter. The presence of a sand substrate in the cups slowed the loss of chlorpyrifos from the test solution, with a significant ($P < 0.05$) difference compared to the concentration in the paper cups without substrate being detected after 2 days. Concentrations in the paper cups (with or without sand) were significantly ($P < 0.05$) lower than those in the tubes (with or without paper) on each day of the experiment.

DISCUSSION

Both bioassay systems allowed good control survival of unfed *C. tepperi* for at least 3 days. However, in the case of *G. paripes,* the paper cups with sand substrate allowed significantly better control survival when the bioassay period was extended to 48 h and beyond. Although *G. paripes* survival at 24 h did not differ significantly in the 2 systems, it is reasonable to assume that poor survival over longer periods reflects stresses on the larvae that start to take effect from the outset, and that these stresses may cause *G. paripes* to respond differently to toxicants even in short-term bioassays if an unsuitable substrate is used. Sand substrates are clearly preferable to paper for *G. paripes.*

When *C. tepperi* was bioassayed with both container and substrate combinations, the LC_{50} and LC_{90} estimates obtained from the waxed paper cups were consistently higher than those obtained from

the glass tubes. The chemical analysis results indicate that this is primarily a consequence of the container type, rather than the type of substrate. Chlorpyrifos concentrations in the cups were found to drop rapidly relative to those in the glass tubes, and this could have occurred as a result of 1 or more chemical processes: adsorption of chlorpyrifos to the waxed surfaces, the breakdown of chlorpyrifos via a reaction catalyzed by a component of the wax, or, because the cups are much wider than the tubes, losses through increased volatilization. Interestingly, chlorpyrifos concentrations dropped at a slightly slower rate in cups with sand than in cups without any substrate. Although this result was only statistically significant at 1 of the 3 sampling intervals, it strengthens the hypothesis that adsorption was the main cause of the rapid decline in chlorpyrifos concentrations. The sand substrate presumably slowed adsorption by partially covering the bottom of each cup, thereby reducing the area of waxed surface available for chemical exchange.

The substantial differences between the bioassay results obtained with the 2 different container and substrate combinations demonstrate that LC data obtained with 1 system is not directly comparable to that obtained with the other, although in this study the relative order of toxicity was largely maintained. The principle factor affecting chlorpyrifos bioavailability in this study was container type, and most chironomid and mosquito bioasssays of low-solubility organic pesticides conducted in waxed paper cups are likely to return higher LC values than those conducted in glass containers, assuming that all other factors are held constant.

Differences between bioassay systems assume particular significance when insecticide resistance is suspected. Results from this study demonstrate the importance of using consistent methodology in the conduct of bioassays with susceptible and potentially resistant populations if resistance levels are to be reliably quantified.

Our results clearly demonstrate that bioassay techniques need to be selected on the basis of both the biological characteristics of the test organism and the potential interactions between toxicants, containers, and where applicable, substrates. When information about substrate suitability for a particular species is available, conditions can be chosen that minimize stresses on the organism, other than those arising from the toxicant. Similarly, when the chemical behavior of toxicants in different bioassay systems is understood, informed decisions can be made about the validity of comparing the results obtained from each system. An appreciation of potential chemical interactions is particularly important in situations where bioassays are based on nominal starting concentrations, rather than on concentrations determined analytically across the full exposure period.

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REFERENCES CITED

Ali A. 1981. Laboratory evaluation of organophosphate and new synthetic pyrethroid insecticides against pestiferous chironomid midges of central Florida. *Mosq News* 41:157–161.

Ali A. 1995. Nuisance, economic impact and possibilities for control. In: Armitage PD, Cranston PS, Pinder LCV, eds. *The Chironomidae. Biology and ecology of non-biting midges* London, United Kingdom: Chapman and Hall. p 339–364.

Ali A. 1996. A concise review of chironomid midges (Diptera: Chironomidae) as pests and their management. *J Vector Ecol* 21:105–121.

Ali A, Mulla MS. 1980. Activity of organophosphate and synthetic pyrethroid insecticides against pestiferous midges in some southern California flood control channels. *Mosq News* 40:593–597.

Ali A, Nayar JK, Gu W-D. 1998. Toxicity of a phenyl pyrazole insecticide, fipronil, to mosquito and chironomid midge larvae in the laboratory. *J Am Mosq Control Assoc* 14:216–218.

Armitage PD. 1995. Chironomidae as food. In: Armitage PD, Cranston PS, Pinder LCV, eds. *The Chironomidae. Biology and ecology of non-biting midges* London, United Kingdom: Chapman and Hall. p 423–435.

Berg MB. 1995. Larval food and feeding behaviour. In: Armitage PD, Cranston PS, Pinder LCV, eds. *The Chironomidae. Biology and ecology of non-biting midges* London, United Kingdom: Chapman and Hall. p 136–168.

Cranston PS. 1995. Introduction. In: Armitage PD, Cranston PS, Pinder LCV, eds. *The Chironomidae. Biology and ecology of non-biting midges* London, United Kingdom: Chapman and Hall. p 1–7.

Estrada JG, Mulla MS. 1986. Evaluation of two new insect growth regulators against mosquitoes in the laboratory. *J Am Mosq Control Assoc* 2:57–60.

Finney DJ. 1971. *Probit analysis* 3rd ed. Cambridge, United Kingdom: Cambridge University Press.

Halpern M, Gasith A, Teltsch B, Porat R, Broza M. 1999. Chloramine and copper sulfate as control agents of planktonic larvae of *Chironomus luridus* in water supply systems. *J Am Mosq Control Assoc* 15:453–457.

Helliwell S, Stevens MM. 2000. Efficacy and environmental fate of alphacypermethrin applied to rice fields for the control of chironomid midge larvae (Diptera: Chironomidae). *Field Crops Res* 67:263–272.

Martin J, Kuvangkadilok C, Peart DH, Lee BTO. 1980. Multiple sex determining regions in a group of related *Chironomus* species (Diptera: Chironomidae). *Heredity* 44:367–382.

Mulla MS, Darwazeh HA. 1979. New insect growth regulators against flood and stagnant water mosquitoes—effects on non-target organisms. *Mosq News* 39:746–755.

Mulla MS, Darwazeh HA, Ede L. 1982. Evaluation of new pyrethroids against immature mosquitoes and their

effects on non-target organisms. *Mosq News* 42:583–590.

Mulla MS, Khasawinah AM. 1969. Laboratory and field evaluation of larvicides against chironomid midges. *J Econ Entomol* 62:37–41.

Robinson PW, Scott RR. 1995. The toxicity of cyromazine to *Chironomus zealandicus* (Chironomidae) and *Deleatidium* sp. (Leptophlebiidae). *Pestic Sci* 44:283–292.

Steel RGD, Torrie JH. 1980. *Principles and procedures of statistics. A biometrical approach* 2nd ed. New York: McGraw-Hill.

Stevens MM. 1992. Toxicity of organophosphorus insecticides to fourth-instar larvae of *Chironomus tepperi* Skuse (Diptera: Chironomidae). *J Aust Entomol Soc* 31: 335–337.

Stevens MM. 1993. Acute toxicity of synthetic pyrethroids to final instar larvae of the rice bloodworm, *Chironomus tepperi* Skuse (Diptera: Chironomidae). *Gen Appl Entomol* 25:61–64.

Stevens MM, Helliwell S, Warren GN. 1998. Fipronil seed treatments for the control of chironomid larvae (Diptera: Chironomidae) in aerially-sown rice crops. *Field Crops Res* 57:195–207.

Surakarn R, Yano K. 1995. Chironomidae (Diptera) recorded from paddy fields of the world: a review. *Makunagi/Acta Dipt* 18:1–20.

Tomlin C, ed. 1994. *The pesticide manual* 10th ed. Farnham, United Kingdom: British Crop Protection Council and the Royal Society of Chemistry.

Treverrow N. 1985. Susceptibility of *Chironomus tepperi* (Diptera: Chironomidae) to *Bacillus thuringiensis* serovar *israelensis*. *J Aust Entomol Soc* 24:303–304.

Wirth MC. 1998. Isolation and characterization of two novel organophosphate resistance mechanisms in *Culex pipiens* from Cyprus. *J Am Mosq Control Assoc* 14: 397–405.

Wirth MC, Georghiou GP. 1999. Selection and characterization of temephos resistance in a population of *Aedes aegypti* from Tortola, British Virgin Islands. *J Am Mosq Control Assoc* 15:315–320.