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Effect of Agrochemical Exposure on *Schistosoma mansoni* Cercariae Survival and Activity

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Abstract

Singular use of activity assays or staining dyes to assess pathogen agrochemical tolerance can underestimate tolerance if pesticides cause sublethal effects. We exposed *Schistosoma mansoni* cercariae, the aquatic life stage of this trematode that infects humans, to 4 insecticides at 5 concentrations using a 24-h time-to-death assay. We used Trypan blue dye, which stains dead tissue, and activity assays simultaneously to discriminate dead from live but paralyzed individuals. Whereas cypermethrin, deltamethrin, and dimethoate exposure did not affect cercariae at any ecologically relevant concentrations, methamidophos exposure increased survival of cercariae compared with those in the controls. This was because methamidophos-induced paralysis reduced cercarial activity and thus energy expenditures, extending the lifespan of this short-lived parasite that causes human schistosomiasis. These findings highlight that sublethal effects should be considered when pesticide effects on disease are under investigation.

Keywords

Ecotoxicology; Parasite; Organophosphate; Pyrethroid; Trematode

INTRODUCTION

The rate of production and use of synthetic chemicals, such as pesticides, has outpaced other human drivers of global environmental change (Bernhardt et al. 2017), which has resulted in the contamination of ecosystems worldwide (Vorosmarty et al. 2010; Stehle and Schulz 2015). Exposure to pesticides can cause direct, lethal effects on sensitive species or sublethal effects on an organism's behavior, physiology, and/or morphology (Fleege et al. 2003; Relyea and Hoverman 2006; Rohr et al. 2006; Clements and Rohr 2009; Kohler and Triebkorn 2013; Halstead et al. 2014). Organophosphate and pyrethroid insecticides, for example, target important esterases and nerve cell gates leading to ionic imbalances and

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uncontrollable convulsions and tremors before paralysis and eventual death (Sanchez-Bayo 2012; Antwi and Reddy 2015). Given that pesticide production and trade are estimated to increase drastically by 2050 (Tilman et al. 2001; Rohr et al. 2019), there is a growing need to understand how agrochemical contamination impacts human and wildlife health.

Freshwater ecosystems, which are vital for global economies, societal well-being, and maintaining human health (Baron et al. 2002; Strayer and Dudgeon 2010; Costanza et al. 2014), are threatened by agricultural activities and agrochemical use (Vorosmarty et al. 2010; Malaj et al. 2014; Ippolito et al. 2015). For instance, more than 40% of the global land area is at risk of producing insecticide runoff to lotic systems (Ippolito et al. 2015). Moreover, agricultural runoff can carry microorganisms including bacterial, viral, fungal, and helminth pathogens into adjacent aquatic environments that can lead to disease outbreaks or modify transmission dynamics of endemic pathogens (Patz et al. 2000, 2004; Rizak and Hrudey 2008). In developing countries, such as those in sub-Saharan Africa, increased land conversion for agriculture (Tilman et al. 2001; Alexandratos and Bruinsma 2012) combined with water management and development for irrigation (Steinmann et al. 2006; Sokolow et al. 2017) has led to an increase in human exposure to agrochemicals and waterborne pathogens (Southgate 1997; Jepson et al. 2014; Lapworth et al. 2017). Understanding the effects of agrochemical contamination on waterborne pathogens is vital because the density of human settlements near managed water systems and the demand for agricultural output are increasing (Lambin et al. 2003; Steinmann et al. 2006; Rohr et al. 2019).

Schistosomiasis is one example of a waterborne, neglected tropical disease that is affected by agriculture (Halstead et al. 2018). Schistosomiasis afflicts more than 200 million people annually, of which over 90% reside in sub-Saharan Africa, and is caused by aquatic parasitic *Schistosoma* trematodes (World Health Organization 2010; Fenwick 2012). Free-swimming *Schistosoma* cercariae are released from intermediate freshwater snail hosts and penetrate the skin of definitive human hosts while in the water. *Schistosoma* eggs, produced by matured worms, leave the human host via feces or urine, and hatch in aquatic environments where the next free-living stage, miracidia, penetrate the snail intermediate host to complete the life cycle. Cercariae and miracidia are short-lived organisms, generally only having enough reserves to live for approximately 24 h (Olivier 1966; Purnell 1966). In an outdoor mesocosm experiment, populations of intermediate snail hosts were shown to increase following bottom-up and top-down indirect effects caused by exposure to the herbicide atrazine and the insecticide chlorpyrifos, respectively (Halstead et al. 2018); whereas herbicide exposure increased algal food resources for snails, insecticide exposure reduced snail predator densities. Thus, human infection risk was predicted to increase following pesticide contamination of aquatic systems due to an increase in intermediate host densities. Indeed, Becker et al. (2020) reported increased intermediate snail host densities among sampled areas of Lake Victoria in Kenya characterized by pesticide pollution and eutrophication. However, it remains unknown how pesticide exposure influences the aquatic *Schistosoma* life stages. As insecticide runoff potential is high in developing African nations where schistosomiasis is prevalent (World Health Organization 2010; Stensgaard et al. 2013; Ippolito et al. 2015), investigating the direct lethal and sublethal effects agrochemical exposure has on the aquatic life stages of *Schistosoma* is of great importance.

Schistosoma miracidia and cercariae might be sensitive to environmental contaminants given their occurrence in freshwater environments during the transition between intermediate and definitive hosts (Pietrock and Marcogliese 2003). Surprisingly, previous studies have not reported significant lethal effects of pesticide exposure on either life stage of *Schistosoma* (Halstead et al. 2018). In contrast, cercariae of trematode species found in North American snails are known to be sensitive to the commonly applied herbicides atrazine (Koprivnikar et al. 2006; Rohr et al. 2008) and glyphosate (Monte et al. 2016), as well as organophosphate, pyrethroid, and neonicotinoid insecticides (Hua et al. 2016). Interestingly, the method of assigning cercarial survival differed among these studies. Survival can be assessed with 1) general swimming or climbing movement (Koprivnikar et al. 2006), 2) movement following stimuli (Reddy et al. 2004; Rohr et al. 2008; Raffel et al. 2009; Hua et al. 2016), or 3) Trypan blue staining (Halstead et al. 2018). Complicating the use of either activity or dyes to examine effects of pesticide exposure is that many insecticides cause paralysis; thus, paralyzed cercariae cannot respond to stimuli but can excrete Trypan blue, which is absorbed by dead cells and excreted by living cells (Strober 2001; McMahon and Rohr 2014). It is possible that pesticide toxicity is then underestimated if paralysis is mistaken for true mortality events (i.e., false positives). Moreover, if exposure to insecticides that target the nervous system reduces activity or causes paralysis, this could reduce energy consumption of short-lived, free-living, aquatic organisms, such as cercariae, potentially extending their lifespan. This could explain some of the variability in cercarial responses to insecticides.

To assess this hypothesis, we exposed *Schistosoma mansoni* cercariae, the trematode species responsible for intestinal schistosomiasis, to 5 different concentrations of 4 insecticides from 2 different pesticide classes (organophosphate and pyrethroid) using a 24-h time-to-death assay. To reduce potential false positives, we simultaneously employed activity assays and Trypan blue dye to distinguish among live and active, live and paralyzed, and dead cercariae (Supplemental Data, S1 Dataset). We predicted that cercarial exposure to the insecticides would cause paralysis given the need for both acetylcholine esterase (organophosphate target) and voltage-gated ion channel (pyrethroid target) function in *Schistosoma* cercarial movement (Bruckner and Vage 1974; Salvador-Recatala and Greenberg 2012), thus reducing activity. We also hypothesized that insecticide-induced paralysis would increase cercarial survival compared with cercariae not exposed to insecticides that were actively swimming, consuming limited energetic resources.

MATERIALS AND METHODS

Pesticide background

We chose 4 insecticides commonly used and detected in sub-Saharan African countries (Williamson et al. 2008; Jepson et al. 2014; Donald et al. 2016). Dimethoate (CAS 60-51-5; Sigma-Aldrich batch number: BCBS9338, purity: 99.8%) and methamidophos (CAS 10265-92-6; Sigma-Aldrich batch number: BCBT1513, purity: 99.1%) are broad-spectrum organophosphate insecticides that are acetylcholinesterase inhibitors used to protect crops such as grapes, tobacco, and potatoes. Deltamethrin (CAS 52918-63-5; Sigma-Aldrich batch number: BCBS3148V, purity: 99.7%) and cypermethrin (CAS 52315-07-8; Sigma-Aldrich batch number: BCBW1786, purity: 98.4%) are type-II pyrethroid insecticides that mimic

natural pyrethrins by interfering with sodium ion channels of nerve cells. Deltamethrin and cypermethrin are applied to numerous agricultural crops, such as corn, cotton, and rice, and play a vital role in integrated pest management strategies to reduce vector populations. Although estimated use records of each pesticide are scarce for African countries, some records are listed by the Pesticide Action Network Africa (2020). Moreover, previous research has reported human exposure to each pesticide, and has found residues of each in the air, water, soil, and produce of African countries (Ntow et al. 2006; Aktar et al. 2009; Kosikowska and Biziuk 2010; Donald et al. 2016).

Study organisms

We obtained 50 infected *Biomphalaria glabrata* snails on 11 September 2018 that had been exposed to *S. mansoni* (NMRI strain) on 5 September 2018 from the National Institute of Allergy and Infectious Disease Schistosomiasis Resource Center of the Biomedical Research Institute (Rockville, MD, USA). Snails were held individually in 200-mL containers filled with 200 mL high-hardness COMBO (HH COMBO; Oasis) water (Baer et al. 1999) and fed ad libitum a ration of ground fish flakes (Tetramin®) and spirulina (NOW FOODS®) suspended in agar (Fisher BioReagents®). Snails were held under laboratory conditions (25.5 °C, 12:12-h light:dark), and full water exchanges were conducted biweekly.

Time-to-death assay design

We examined the effects of the 4 pesticides on the survival and behavior of free-swimming cercariae of *S. mansoni* using a 24-h time-to-death assay on 19 November 2018. We conducted the time-to-death assay using 24-well tissue culture plates (Falcon® #353047; Corning). We tested 5 concentrations of each insecticide for a total of 20 pesticide treatments. To these treatments, we added a water control and an ethanol vehicle control. A vehicle control was included in the experimental design because pyrethroid insecticides are insoluble in water. We included 2 replicates of each control treatment and 1 replicate of each pesticide treatment on each 24-well plate and used 5 plates for a total of 120 wells.

To obtain *Schistosoma* cercariae, 8 infected snails were transferred to 50-mL glass beakers filled with 15 mL of oxygenated HH COMBO water and were held under direct artificial light for 1.5 h. Snails were then returned to their respective husbandry containers, the 15-mL HH COMBO solutions containing shed cercariae were homogenized, and we dispensed 250 µL of cercariae slurry to each well. On average, this resulted in 5.05 ± 0.43 (mean $\pm$ 1 standard error [SE]) cercariae/well.

We created our pesticide treatments by first making stock solutions of each chemical. Organophosphate insecticides were dissolved directly in HH COMBO water (5mg/mL), whereas pyrethroid insecticides were dissolved using ethanol (0.05 mg active ingredient/mL). We then added an aliquot of each stock solution to 10 mL of HH COMBO water to create an intermediate solution for each targeted concentration (20 intermediate solutions). Prior to addition of stock solutions, we removed the same volume of HH COMBO water from the 10-mL intermediate vial that we would be adding to correct for total volume. We added 100 µL of each intermediate solution to their respective wells to obtain the nominal concentrations of 10, 30, 50, 70, and 100 µg/L for pyrethroids and 100, 200, 300,

400, 500 mg/L for organophosphates. Although the chosen nominal pesticide concentrations fall above expected environmental concentrations (Table 1), they were selected following a series of pilot studies with the aim of causing increased cercariae mortality (see the Supplemental Data). The goal of our pilot studies was to identify concentrations for each insecticide class in which exposure caused a range of mortality. We initially employed 0.1, 0.5, 1.0, 2.0, and 10.0 µg active ingredient/L for pyrethroids and 5, 10, 35, 75, and 100 mg active ingredient/L for organophosphates. However, we did not observe significant death at these lower concentrations compared with the water control. Thus, we raised concentrations for both pesticide classes for the present experiment above the highest concentrations of the pilot studies. The ethanol vehicle control was created by adding 101 µL of ethanol (95%) to 9.899 mL of HH COMBO water to match the ethanol concentration in the highest volume of pyrethroid stock solution being transferred to the intermediate solution. To create our water controls, we instead added 100 µL of HH COMBO water to each respective well. We then added 15 µL Corning™ Trypan blue dye (Cat. no. MT25900CI; Fisher Scientific) to each well for cercarial staining. We conducted a 24-h time-to-death assay to compare survival of cercariae exposed to Trypan blue stain with that of cercariae in water controls and found no effect of staining on survival ($p = 0.44$). Lastly, we added 135 µL of HH COMBO water to each well to bring the total volume to 500 µL.

We assessed survival and activity of shed cercariae (~1.5-h old) using a 24-h toxicity test conducted under laboratory conditions (22 °C; 12:12-h light:dark cycle). Because survival and infectivity have been reported up to 24 h postemergence (Purnell 1966), we observed cercariae every 2 h for the first 12 h, and then every 6 h for the second 12 h. To assess survival, we counted the number of unstained (alive) and stained (dead) individuals. We simultaneously assessed activity of alive, unstained cercariae by recording the number of active individuals; an individual was recorded as active if it was actively swimming, crawling, or moving vertically or horizontally in the water column. We did not conduct water exchanges or renew pesticide concentrations during the 24-h exposure period. After 24 h, we added 20 µL Lugol's iodine solution to each well to euthanize and stain surviving cercariae. Lugol's iodine solution was used to determine the total number of cercariae/well because we used a standardized volume of shed cercariae in favor of separating individuals to reduce handling time of cercariae.

Statistical analysis

To examine the direct toxic effects of the 4 insecticides on *S. mansoni* cercariae, we analyzed cercarial survival over time using Cox's proportional hazard models (Cox 1972). We first conducted an analysis comparing survival of cercariae exposed to the ethanol vehicle control and the water control to assess any effect of the vehicle. We did not find any difference between the 2 treatments ($p = 0.94$; Table 2). We thus pooled the ethanol vehicle and water controls for all subsequent survival analyses. We first used Cox's proportional hazards model to investigate the main effect of pesticide treatment to assess whether cercarial survival in a pesticide treatment differed from survival in the pooled controls. We then conducted 4 independent survival analyses, one for each insecticide, to examine the effect of concentration (continuous variable) on cercarial survival. The pooled control treatment served as a 0.0 µg/L concentration in each model. Following a significant effect of

concentration, we then compared the survival of cercariae in each insecticide concentration (categorical variable) with the survival of cercariae in the pooled control treatment. We included “experimental well” as a random effect in each model. Cox's proportional hazards model were employed using RStudio Ver 1.1.453 (R Development Core Team 2016) and the *survival* and *coxme* packages. In addition, we used the *drc* package in RStudio to estimate the effective dose at 10, 50, and 90% (ED10, ED50, and ED90) for pesticides that induced significant concentration effects on cercarial survival. We first used the *drm* function to examine the effect of $\log_{10}$ -transformed methamidophos concentration (+1) on the occurrence of cercarial death, and then backcalculated estimated effective doses ($10^X - 1$).

To test whether cercarial activity over time was affected by insecticide exposure, we employed generalized linear mixed-effects models. We first examined whether activity over time differed between cercariae exposed to the water and to the ethanol vehicle controls. We examined whether the interactive effects of control treatment and time (independent variables) influenced the activity of cercariae, represented by the binomial response of the number of active and inactive cercariae within each experimental well. We found no difference in the activity of cercariae in the 2 control treatments ($\chi^2_{(1)} = 1.97$, $p = 0.161$), so we pooled the water and ethanol vehicle controls. We first investigated the overall effect of pesticide treatment and time (independent variables) on cercarial activity. For each insecticide, we then investigated the interactive effects of concentration (continuous) and time (independent variables) on cercarial activity. If we observed a significant effect of concentration, we then conducted a subsequent model that investigated the main and interactive effects of pesticide concentration (categorical) and time on the activity of cercariae and conducted Tukey's post hoc pairwise comparisons. We included “experimental well” as a random effect term within each model. Model analyses were conducted using RStudio and the *car*, *lme4*, and *multcomp* packages.

RESULTS

Time-to-death assays

Cox's proportional hazard models revealed that survival of cercariae exposed to cypermethrin, deltamethrin, and dimethoate did not differ from cercarial survival in the pooled controls ($p = 0.31$; Table 2). Survival of cercariae exposed to methamidophos was significantly higher than survival in the pooled controls ($p < 0.001$). Unsurprisingly, we found there was no effect of concentration on the survival of cercariae exposed to cypermethrin, deltamethrin, or dimethoate ($p = 0.64$; Table 2 and Supplemental Data S1 Dataset). In contrast, we did find a significant effect of methamidophos concentration on cercarial survival ($b = -0.003$, $p = 0.002$). Exposure to 100, 200, 300, and 400 mg/L methamidophos increased cercarial survival relative to the pooled controls ($p = 0.031$; Figure 1 and Table 2). Survival of cercariae exposed to 500 mg/L methamidophos did not differ from survival of cercariae in the pooled controls ($p = 0.12$). After 24 h of exposure, survival of cercariae in the pooled control was 41.3% compared with more than 73% for cercariae exposed to any methamidophos concentration.

To examine the toxicity of methamidophos to *S. mansoni* cercariae, we calculated the 24-h effective dose. The slope (b ; $p = 0.4517$) and the median lethal dose (e ; $p = 0.4380$) parameter estimates from the 2-parameter log-logistic model with fixed lower and upper limits were not different from 0. The estimated 24-h ED10, ED50, and ED90 values ($\pm$ SE) for methamidophos were 0.61 (± 3.68), 7.06 (± 13.75), and 9770.92 mg/L (± 628.13), respectively.

Activity assay

To examine the influence of insecticide exposure on cercarial activity, we used generalized linear mixed-effects models. Pesticide treatment ($p < 0.001$) and time ($p < 0.001$), but not their interaction ($p = 0.185$), affected cercarial survival. Cercarial activity was negatively associated with time across all pesticide treatments. Post hoc multiple comparison tests revealed that cercarial activity following methamidophos exposure was significantly reduced compared with all other treatments ($p < 0.001$). Cercarial activity did not differ between any other pesticide treatments and the pooled controls ($p = 0.679$). Although we did not find an effect of concentration ($p = 0.149$) or a concentration-by-time interaction ($p = 0.366$) for dimethoate, cypermethrin, or deltamethrin, activity declined with time for all 3 insecticides ($p < 0.001$). For methamidophos, concentration ($\chi^2_{(1)} = 37.48$, $p < 0.001$) and time ($\chi^2_{(1)} = 46.40$, $p < 0.001$), but not their interaction ($\chi^2_{(1)} = 0.534$, $p = 0.4649$), influenced cercarial activity (Figure 2 and Supplemental Data, S2 Dataset). Post hoc multiple comparison tests (Tukey) revealed that the mean activity of cercariae exposed to 300, 400, and 500 mg/L methamidophos was lower than the activity in the pooled controls ($p = 0.047$; Figure 2A). Activity of cercariae exposed to 100 and 200 mg/L methamidophos did not differ from that in the pooled controls ($p = 0.076$). Excluding the 0 mg/L control, activity of cercariae did not differ among methamidophos concentrations ($p = 0.071$). Mean cercarial activity declined over time (Figure 2B).

DISCUSSION

In the present study, we sought to understand how free-swimming *Schistosoma* cercariae respond to 4 insecticides from 2 chemical classes commonly used in agricultural practices in developing regions endemic to schistosomiasis. We found no significant effect of exposure to cypermethrin, deltamethrin, and dimethoate on the survival and activity of *S. mansoni* cercariae over 24 h. Surprisingly, exposure to methamidophos resulted in increased cercarial survival compared with the pooled control treatments. Moreover, the use of activity assays in combination with Trypan blue staining allowed us to observe that this increased survival appeared to be caused by the reduced activity of cercariae exposed to methamidophos.

Understanding the influence of common-use pesticides on waterborne pathogens is vital to protecting human health in developing regions. Our results suggest that cercariae are highly tolerant to the direct toxic effects of cypermethrin, deltamethrin, dimethoate, and methamidophos contamination. Given that the concentrations of insecticides reported in samples from developing regions all fall below concentrations used in the present study (Amoah et al. 2006; Aktar et al. 2009; Anderson et al. 2014; Diop et al. 2016; Donald et al. 2016), it is unlikely that *Schistosoma* cercariae suffer direct mortality from

insecticide exposure. Previous research has also reported no influence of chlorpyrifos (organophosphate) or atrazine (triazine) exposure at environmentally relevant concentrations on *S. mansoni* survival over 12 h (Halstead et al. 2018). Although the indirect effects of agrochemicals have been shown to potentially propagate schistosomiasis by increasing intermediate snail host densities (Halstead et al. 2018; Becker et al. 2020), the results of the present study suggest that the direct toxicity of pesticides is not an apparent counteractive or mitigating factor for disease risk due to the substantial tolerance of cercarial life stage. Given the complex life cycle of *S. mansoni*, future studies should investigate the insecticide tolerance of other life stages such as miracidia, encysted individuals, and their intermediate snail hosts (Becker et al. 2020) to fully understand how pesticide exposure will influence disease transmission. Because it is unlikely that cercariae in surface waters of natural systems are exposed to only a single chemical compound at a time, future research should also investigate the effects of pesticide mixtures on cercarial longevity and infectivity (Halstead et al. 2014). Lastly, investigation into the direct toxic effects of other pesticide classes, such as organochlorines, will be useful because older, more toxic pesticides are still used in developing regions due to availability and low cost (Ecobichon 2001).

Exposure to pesticides can also cause sublethal changes in behavior and physiology that can alter metabolic processes and energy use. We observed decreased activity of *S. mansoni* cercariae exposed to methamidophos, which was likely caused by full or partial paralysis. The life span (24–48 h) of *S. mansoni* cercariae is dependent on finite glycogen and fat reserves, and we hypothesized that the paralyzed cercariae might have prolonged longevity because of lower rates of energy consumption (Krakower 1940; Ginetsinskaya 1968). Indeed, we observed reduced cercarial activity among individuals exposed to methamidophos relative to the activity of cercariae in all other treatments. Other pesticides and naturally occurring chemicals have also been reported to reduce mobility of nematodes and trematodes, including *S. mansoni* (Hara and Kaya 1983; Koprivnikar et al. 2006; Gao et al. 2019). The data suggest that the exposure to methamidophos caused a true paralytic effect through acetylcholinesterase inhibition in affected individuals, because past research has shown that certain cholinergic agents exert an inhibitory effect on muscular activity of *S. mansoni* and other parasites (Bueding 1962; Barker et al. 1966).

Although methamidophos-exposed cercariae lived longer than cercariae in the pooled controls, they were “functionally dead” because their immobility prevents them from searching for and infecting definitive hosts (Gao et al. 2019). Methamidophos-induced paralysis and increased survival were consistent results found in both our preliminary work (see the Supplemental Data) and the present study, which were conducted on cercariae from separate snails. Thus, these results suggest that *S. mansoni* responses to methamidophos are conserved across cercariae shed by different intermediate hosts. Therefore, it is possible that methamidophos-induced paralysis could reduce disease transmission and thus negative impacts on humans. In contrast, if exposure is short-lived, because of either environmental breakdown or clearance by flowing water, we may overestimate the acute toxicological effects of methamidophos on the reduction of disease transmission. Furthermore, it is possible that the continuous daily release of thousands of cercariae by infected snail hosts in natural systems (Combes et al. 1994) will minimize the influence of pesticide-induced cercarial paralysis on disease dynamics. Infection assays in the future should seek to

confirm whether the cercarial paralysis observed in the present study provides protection to human hosts (Gao et al. 2019). Lastly, the methamidophos-induced paralysis may be strain-, species-, and life stage-specific, providing many questions for future researchers including the comparison of tolerance between laboratory-reared and field-collected *Schistosoma* cercariae, and whether tolerance varies among free-swimming cercariae or miracidia and encysted individuals. Future research that examines the complex relationship among trematode life stage, infected snail hosts, timing and frequency of pesticide exposure, and environmental conditions (e.g., flow, temperature) could reveal how each factor contributes to the transmission dynamics of schistosomiasis (sensu Halstead et al. 2018).

The combined use of Trypan blue staining and activity assays employed in the present study not only allowed us to discriminate whether cercariae were active versus inactive, but also whether they were paralyzed versus truly dead. This could have implications for the interpretation of previous toxicological assays of free-swimming trematode life stages that estimated lethal concentration values. For instance, previous research using activity to assign mortality to paralyzed cercariae (i.e., false positives) might underestimate the actual tolerance of trematodes and overestimate the toxicity of pesticides (Rohr et al. 2008).

Freshwater systems are increasingly threatened by numerous anthropogenic activities (Steinmann et al. 2006; Strayer and Dudgeon 2010; Stehle and Schulz 2015). Understanding the effects of contaminants on waterborne pathogens is of utmost importance in developing nations where water scarcity and increased agricultural activity might threaten human health (Rohr et al. 2019; Becker et al. 2020). Given the increased risk of agricultural runoff in these nations (Ippolito et al. 2015), it will be important for future studies to investigate the acute lethal and chronic sublethal effects of contaminants on aquatic pathogens. The combined use of activity assays with staining dye will better elucidate the lethal and sublethal effects of contaminants on waterborne pathogens, both improving toxicological estimates and more accurately predicting effects of pesticide exposure on disease dynamics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability Statement—

Data are included in the Supplemental Data as S1 Dataset and S2 Dataset.

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FIGURE 1: Survival of *Schistosoma mansoni* cercariae following exposure to one of 6 methamidophos concentrations. Cercariae were exposed to 0, 100, 200, 300, 400, or 500 mg/L methamidophos for 24 h using a time-to-death assay. The pooled control treatment represents the combined survival of cercariae in the water and vehicle control treatments.

FIGURE 2:

Mean cercarial activity (%) of *Schistosoma mansoni* following exposure to one of 6 methamidophos concentrations. We recorded the number of cercariae active over 24 h following exposure to 0, 100, 200, 300, 400, or 500 mg/L methamidophos. We calculated cercarial activity (%) by dividing the number of trematodes observed moving by the total number of individuals in the well. We observed the main effect of methamidophos concentration (A) and time (B) on cercarial activity. Data points represents overall treatment mean values ± 1 standard error.

TABLE 1:Peak estimated environmental concentrations (EECs) for each insecticide^a

Pesticide	Crop	Water body	Peak EEC (ppb; µg/L)
Methamidophos	Potato	Pond	6.05
		Reservoir	13.9
Dimethoate	Corn	Pond	8.17
		Reservoir	19.2
Cypermethrin	Cotton	Pond	0.859
		Reservoir	2.03
Deltamethrin	Corn	Pond	0.0036
		Reservoir	0.0086

^aWe used the US Environmental Protection Agency Pesticide in Water Calculator (PWC; Ver 1.52) to calculate EEC values for pond and reservoir surface waters. Following previously described methods (Rumschlag et al. 2019), we extracted pesticide parameters from the Pesticide Properties DataBase of the University of Hertfordshire (2020), the Pesticide Action Network, North America Pesticide Database (2019), and the Hazardous Substances Data Bank (National Library of Medicine 2019). We then selected the maximum EEC value generated by the PWC calculator.

TABLE 2:

Results of Cox's proportional hazard models

	<i>b</i>	<i>p</i> -value
Control treatment survival		
Water control ^a	—	—
Ethanol vehicle control	−0.023	0.94
Effect of pesticide treatment on survival		
Pooled control ^a	—	—
Methamidophos	−1.376	<0.001
Dimethoate	0.078	0.72
Cypermethrin	0.077	0.73
Deltamethrin	0.225	0.31
Effect of concentration ^b on survival		
Methamidophos conc.	−0.003	0.002 *
Dimethoate conc.	<0.001	0.8
Cypermethrin conc.	<0.001	0.98
Deltamethrin conc.	0.001	0.64
Effect of methamidophos concentration on survival		
Pooled control ^a	—	—
100 mg/L	−1.404	0.013 *
200 mg/L	−1.063	0.031 *
300 mg/L	−1.722	0.028 *
400 mg/L	−1.434	0.012 *
500 mg/L	−1.013	0.120

^aEach treatment was compared to the control survival in each model.^bPooled control survival was used as a 0.0 mg/L pesticide concentration in each model.* $p < 0.05$