Lethal and Sublethal Responses of an Aquatic Insect *Culex quinquefasciatus* (Diptera: Culicidae) Challenged with Individual and Joint Exposure to Dissolved Sodium Selenate and Methylmercury Chloride

Peter D. Jensen,[1] Mary A. Sorensen,[2] William E. Walton,[2] John T. Trumble[2]

[1]Department of Entomology, University of Maryland, College Park, Maryland, USA

[2]Department of Entomology, University of California, Riverside, California, USA

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ABSTRACT: Pollutants rarely occur alone in the natural environment, and few studies have focused on the potential interactions between metals or metalloids. In this study an aquatic insect, the southern house mosquito (*Culex quinquefasciatus*: Diptera), was used to test the individual and joint effects of dissolved sodium selenate (Se) and methyl mercury chloride (MeHg). We conducted ovipositional preference tests and 14-day chronic toxicity studies to determine lethal and sublethal responses of *C. quinquefasciatus* to a range of Se and MeHg concentrations and mixtures. No evidence was found for female ovipositional preference in field trials using artificial ponds. Larvae were more sensitive to MeHg than Se, with LC_{50} values of 30 μg/L (CI = 28–31 μg/L) and 11 mg/L (CI = 10–12 mg/L) respectively. In addition, larval survival was significantly reduced at concentrations as low as 25 μg/L of MeHg and 8 mg/L of Se. A synergistic interaction was observed in the toxicity of the Se-MeHg mixtures to *C. quinquefasciatus* larvae. Larval mosquito survival was significantly reduced at 7.5 μg/L MeHg + 2.75 mg/L Se and an LC_{50} value of 9 μg/L MeHg + 3.4 mg/L Se was determined for a fixed ratio mixture. The rate of growth of the larvae was analyzed using a Growth Index that provided a sensitive measure of the developmental effects of toxicant exposure. Sodium selenate at concentrations as low as 2 mg/L caused a significant decrease in growth between larvae in treatment versus control solutions after only 4 days. Similarly, MeHg at concentrations as low as 25 μg/L and a Se-MeHg mixture of 3 μg/L MeHg plus 1.1 mg/L Se caused significant growth reductions after only 2 and 3 days, respectively. These are the first reported survival and developmental data for an aquatic insect exposed to MeHg and Se-MeHg mixtures. Environ Toxicol 22: 287–294, 2007.

Keywords: selenium; mercury; mosquito; metal; mixture; synergism; insect

INTRODUCTION

Pollutants rarely occur alone in the natural environment. Many recent studies have evaluated the effects of several individual metals (Fountain and Hopkin, 2001, and references therein), but few of them have examined the potential toxic interactions between metals. In a review of research in aquatic systems, Hamilton (2002) pointed out that in the U.S.A. the national water criteria do not take into account potential interactions of metal species. These interactions

Correspondence to: P. Jensen; e-mail: pjensen@.umd.edu

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might be additive, antagonistic, or synergistic depending on the organisms studied.

Selenium and mercury are metals that have a well documented antagonistic relationship in fish (Ganther et al., 1972; Berlin, 1978; Satoh et al., 1985), birds (Elbegearmi et al., 1975; Welsh and Soares, 1975, 1976), and mammals (Lindh and Johansson, 1987; Lindh et al., 1996; Gailer et al., 2000; Wang et al., 2001; Watanabe, 2002), with a corresponding lack of information regarding the effects of the Se-Hg relationship on aquatic invertebrates. The alleviation of inorganic Hg toxicity by Se was reported as early as 1967, and a chemical mechanism of antagonism between sodium selenite and mercuric chloride has been described in mammalian tissue (Gailer et al., 2000). The mammalian detoxification mechanism consists of selenite absorption, reduction, and excretion as selenide by erythrocytes, which then reacts with albumin-bound mercuric mercury to form a Hg-Se-S species that binds to selenoprotein P and results in excretion. The interaction between organic mercury and Se was first reported by Ganther et al. (1972) and clearly showed the alleviating affects of sodium selenite on MeHg-induced toxicity in rats, although the mechanism was not known.

No information is available documenting whether a similar antagonistic relationship and/or alleviating mechanism occurs in insects. Insects respond to metal exposure in a similar fashion to vertebrates by initiating synthesis of metal-induced metallothionen-like proteins (Hopkin, 1989). However, this similarity between vertebrates and invertebrates may not readily extrapolate to toxicological or ecological effects. Understanding any potential interactions between mixture components is important both for elucidating physiological mechanisms of toxicity and for performing risk assessments in polluted ecosystems. Se and Hg were selected for our study because of their known relationships but also for their frequent co-occurrence. In the 2002-303(d) list, the California EPA reported over 200,000 acres of land/waterways contaminated by Se and Hg together in California alone. Mercury and Se also co-occur at 11 active Superfund sites in California (USEPA, 2002).

In many ecosystems, interactions involving insects and other arthropods are the primary routes of energy flow, with insect biomass exceeding that of vertebrates (Power et al., 1992; MacKenzie and Kaster, 2004). Disruption of insect populations can resonate throughout an ecosystem and affect organisms at all levels. Because toxicity studies on pollutant combinations are especially rare for insects, the information from such studies has the potential to not only provide insight into effects on aquatic ecosystems, but to add to the critical baseline data needed to craft scientifically sound legislation regarding potential joint effects of pollutants.

Considering the need for information on mixtures of pollutants on insect species the objectives for these experiments were to (1) establish whether larval exposure to selenium and methylmercury could occur in a contaminated environment based upon female insect oviposition choice, (2) determine the relative toxicity of selenate, methylmercury, and mixtures of selenate and methylmercury to a commonly occurring aquatic insect *Culex quinquefasciatus*, (3) evaluate the relationship between selenate and methylmercury in *C. quinquefasciatus*, and (4) determine the effects of selenium and methylmercury on the survival and growth of *C. quinquefasciatus*.

METHODS

Culex quinquefasciatus, the southern house mosquito, was chosen for this study because the larva is an aquatic collector-filter feeder in lentic habitats in tropical and temperate regions throughout the world (Walker and Newson, 1996). Members of the *Culex* complex readily breed in both clean and polluted ground pools (Goddard, 1993). These insects are algal and bacterial feeders, and are important as food for many organisms. In addition, this species is an important vector of several encephalitis viruses (James and Harwood, 1969) including the West Nile Virus now present throughout much of North America.

Rafts of *C. quinquefasciatus* eggs were obtained from a colony maintained in the UCR Department of Entomology. The eggs were allowed to hatch and develop to second instar before being used in treatment experiments. The larvae were reared at a constant 26 °C and a photoperiod of 16:8 L:D. They were fed a 3:1 (w/w) mixture of ground mouse chow (4% mouse/rat diet from Harlan/Teklad, Madison, WI) and brewer's yeast (MP Biochemicals, LLC, Aurora, OH). Eight grams of the diet were added to 100 mL of water that was then administered at a rate of two drops every second day. Treatment solutions were always created through serial dilution from stock solutions containing sodium selenate (Sigma-Aldrich, USA), methylmercury (II) chloride (Aldrich, Milwaukee, WI), or both, at concentrations calculated by mass to produce the desired treatment levels. Treatment concentrations were verified analytically and found to be within 5% of nominal levels (ICPMS, EPA method 200.8) at a certified laboratory (E.S. Babcock & Sons, Riverside, CA).

Pollutant Forms and Concentrations

Preliminary experiments with *C. quinquefasciatus* larvae showed no discernable toxic effects to larvae between concentrations of 1–10 μg/L Se which is generally accepted to be the toxic effects threshold for dissolved inorganic selenium in aquatic ecosystems (Lemly, 2002). In the interest of documenting a toxic range of dissolved selenate and characterizing the Se-MeHg toxicity interaction to *C. quinquefasciatus*, the range of test concentrations for selenate in our experiments varied from 2 to 32 mg/L.

Selenium has several different oxidation states including selenate (Se + 6), selenite (Se + 4), elemental selenium (Se0), and selenides or organic forms of Se (Se + 2), all of which can be found in the environment. However, in both

natural and Se-contaminated waters, selenate and selenite are by far the most prevalent forms (Presser and Ohlendorf, 1987). Dissolved organoselenium forms are also present in the water column, however, the chemical nature of these forms is not well known, and the concentrations are also much lower than those of the Se oxyanions (Fan et al., 2002). For this reason the selenium species chosen for all experiments was selenate.

Like Se, the chemical form of Hg determines its availability and toxicity to consumers. The major mercury species are elemental mercury (Hg^0), inorganic mercury (Hg^{2+}), and methylmercury (CH_3Hg^+). The methylation of this inorganic mercury is performed mainly by sulfate-reducing bacteria in aquatic systems (Compeau and Bartha, 1985). Methylmercury accumulates in aquatic organisms to a high degree (10,000–3,000,000 times water concentrations, Boening, 2000; Zillioux et al., 1993) and is the most common form found in plants and animals since it is absorbed most readily, reaching levels in excess of 1 μg/g (Burger et al., 2002). Therefore methylmercury was used in all trials, with a concentration range of from 10 to 40 μg/L.

Oviposition

Experimental "ponds" were created by filling 16 plastic containers (1.66 m by 1.0 m) with ~200 L of municipal water. Ten compressed alfalfa feed pellets (Sacate Pellet Mills, Phoenix, AZ) were added to each pond to provide volatile organic oviposition cues for naturally occurring mosquitoes. Four treatments were prepared with 4 replicates each: control, 30 mg/L selenate, 7 mg/L methylmercury, and a mixture of 30 mg/L selenate, and 7 mg/L methylmercury. The ponds were located at the UCR Aquatic Research Facility in a locked and shaded chain-link fence enclosure, and treatments were set out in a Latin square design. Water samples were taken weekly from each of the treatments and tested using ICPMS (EPA method 200.8) at a certified laboratory (E.S. Babcock & Sons, Riverside, CA) to monitor the concentrations of selenate or methylmercury in each pond. Finally, mosquito egg rafts were counted on the surface of each pond at the time of water sampling. The egg rafts were not counted twice as they hatch within 2 days of oviposition and disintegrate quickly thereafter. Spearman's rank correlation (Statview, 2001) was used to test the null hypothesis that there was no significant correlation between the concentration of each compound and the number of oviposited egg rafts. The average weekly counts for 4 weeks for each treatment were analyzed using ANOVA to determine if the female mosquitoes could detect the chemical treatments (Statview, 2001).

Survival and Development

Treatment solutions were created as described above using selenate concentrations of 2, 4, 8, 16, and 32 mg/L, methylmercury concentrations of 10, 15, 20, 25, 30, and 40 μg/L, and mixtures ranging from 5% (0.55 μg/g sodium selenate, 1.5 μg/L methylmercury) to 50% of the respective individual LC_{50} values (5.5 μg/g selenate, 15 μg/L methylmercury) of each component. Twenty-five second instars were introduced into each 100 mL glass jar containing food and treatment solutions, each replicated four times.

Survival was recorded daily along with instar until all insects had attained the adult stage or expired. This procedure allowed documentation of the larval development period, the total (larval and puparial) developmental time, and the number of individuals surviving to the adult stage. All development and survival data were analyzed using ANOVA (Statview, 2001). Percentage data were arcsine transformed prior to analysis to confer normality, and back-transformed for presentation. Data were not used from any experiments in which control mortality exceeded 20%, and Abbott's formula was used to correct for the control mortality that did occur (Tattersfield and Morris, 1924). As appropriate, ANOVA was followed by a post hoc analysis with Tukey's HSD to determine developmental or survival differences between individual concentrations within selenate, methylmercury, or mixture treatments. To measure the LC_{50} for each of the compounds mortality was recorded for each treatment and probit analysis (Minitab Statistical Software, 2000) was used to determine log dose probit lines and fiducial limits. Acute toxicity from Se is rarely a problem in either aquatic or terrestrial systems, rather it is the chronic toxicity that results from oral uptake and foodchain transfer which lead to elevated Se concentrations and ensuing toxicity (Saiki et al., 1993; Maier and Knight, 1994). Accordingly, we used 2-week exposure toxicity testing in our experiments to determine relative toxicity values.

Potential reductions in rate of growth were measured by determining the Relative Growth Index (RGI) (Zhang et al., 1993). This has been used successfully for previous studies on effects of toxicants on insects, including investigations of specific forms of selenium on the moth *Spodoptera exigua* (Trumble et al., 1998). GI (growth index) and RGI (relative growth index) values were calculated as described by Zhang et al. (1993), where:

$$\mathrm{GI} = \frac{\sum_{i=1}^{i_{\max}} \left[n_{(i)} \times i\right] + \sum_{i=1}^{i_{\max}} \left[n_{(i)} \times (i-1)\right]}{N \times i_{\max}}$$

where $i_{\max}$ = the highest attainable instar of the insect at complete development and n = the number of insects tested. RGI was determined as:

$$\mathrm{RGI} = \frac{\text{GI of the test group}}{\text{GI of the control group}}$$

The RGI values were calculated for each day of observations and plotted against the control group maximum GI for

the entire experiment to maintain continuity on the RGI plot. The RGI values were then tested using ANOVA followed by a post hoc analysis with Tukey's HSD to determine significant differences between treatment groups.

The joint toxicity of combinations of two chemicals was also studied. We used the LC_{25} concentrations (estimated from probit lines) and controls of each pollutant prepared as described in earlier experiments. Assuming that each component acts independently and has a different mode of toxic action, we defined an additive relationship as when the toxicity of the mixture could be predicted by adding the dose-response curve for each component (Bliss, 1939). When the sum of the activity of the components of a mixture was less than expected based upon the individual activity of each of the components, it was considered an antagonistic relationship. The opposite of antagonism was synergism, where the sum of the activity of the components of a mixture was greater than expected, with the special case of potentiation where one of the components was nontoxic at the concentration used in the treatments (Finney, 1971; Salama et al., 1984). Joint effects were described using Tabashnik's (1992) test for synergism, where the expected LC_{50} of the mixture is calculated:

$$LC_{50(mix)} = \left[r_a/LD_{50(a)} + r_b/LD_{50(b)}\right]^{-1}$$

In this formula, r is the relative proportion of a (r_a) and b (r_b) in the mixture. We compared the expected LC_{50} of the mixture to the observed mixture LC_{50} and the 95% confidence limits of the observed LC_{50} to test for synergism. Synergism was considered to be occurring if the observed LC_{50} was less than the expected LC_{50}. We also calculated the expected percentage mortality, given the null hypothesis of an additive effect, using the formula $[E = O_a + O_b(1 - O_a)]$ where E is the expected mortality from the mixture, O_a is the observed mortality from compound A alone, and O_b is observed mortality from compound B alone (Finney, 1971; Salama et al., 1984). A χ^2 test for homogeneity was performed on the observed mixture replicates to determine if they could be pooled followed by a χ^2 test to compare the observed mixture mortality with the calculated E value.

RESULTS

Oviposition

The concentration of mercury in the experiment ponds decreased rapidly from 23 to 2.2 μg/L over the first 12 days of the test and then stabilized within a range of 1.6–2.8 μg/L most likely because of the mercury volatilization and biotic uptake. The selenium concentration increased over the duration of the test because of the water evaporation, ranging from 4200 to 6000 μg/L. The mercury and selenium concentrations in the mixture treatment closely followed those in the individual treatments. The number of egg rafts in each treatment increased as the experiment progressed, with some variation on day 30 or 37 of the test. A Spearman rank correlation showed no significant relationship between the number of egg rafts and the concentration in the Se treatment ($P = 0.317$), Hg treatment ($P = 0.162$), or mixture treatment ($P = 0.294$). In addition, the control treatment average egg raft counts showed a similar pattern of increase and was not significantly different from any of the treatments on any of the sample days (ANOVA), indicating that it was most likely a location and/or organic volatile effect that caused the increase in oviposition, and not a decrease in the Hg concentration. Thus the female mosquitoes either cannot detect Hg and Se at the treatment levels or do not have a preference between treated and untreated water for oviposition sites.

Survival and Development

The chronic exposure trials showed that by day 14 larval survival was significantly reduced by selenate ($F_{5,18} = 56.66$, $P = 0.001$). Selenate significantly decreased larval survival by 27 ± 6% at 8 mg/L and by 83 ± 10% at 16 mg/L. Mercury also significantly reduced larval survival ($F_{6,21} = 18.96$, $P = 0.001$), by 31 ± 9% at 25 μg/L, by 51 ± 15% at 30 μg/L, and at the highest treatment (40 μg/L) by 90 ± 6%. The selenate-methylmercury mixture also significantly reduced larval survival ($F_{4,22} = 27.11$, $P = 0.001$) compared with controls because the 25% of the LC_{50} mixture (2.75 mg/L sodium selenate, 7.5 μg/L

TABLE I. Mixture component concentrations with corresponding observed percent mortality and calculated expected mortality for *Culex quinquefasciatus* exposed to Se-MeHg fixed-ratio mixtures for 11 days

Treatment	Se (6+) Conc. (mg/L)	MeHg Conc. (mg/L)	Expected LC_{50} (mg/L)	Expected Percent Mortality (E)	Observed Percent Mortality
LC_{50} Se (6+)	11.8			50	50 ± 9
LC_{50} MeHg		0.042		50	50 ± 7
50% of LC_{50} mix	5.5	0.015	5.5	86	100
25% of LC_{50} mix	2.75	0.0075	5.5	25	64 ± 11.5
10% of LC_{50} mix	1.1	0.003	5.5	6	52 ± 17.6
5% of LC_{50} mix	0.55	0.0015	5.5	8	49 ± 19.1

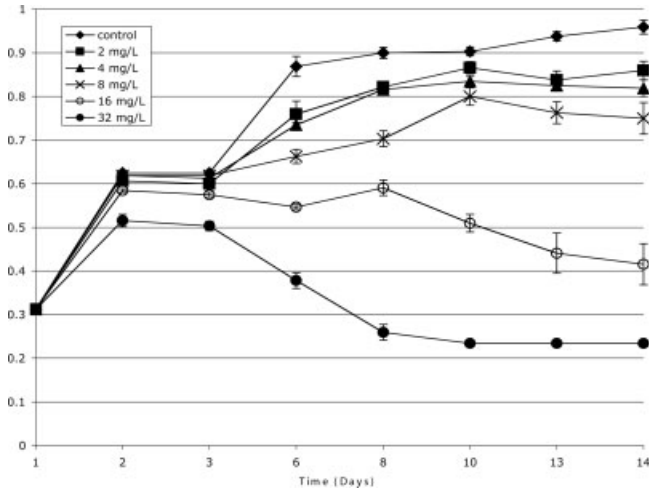

Fig. 1. Relative Growth Index of *C. quinquefasciatus* exposed to a range of sodium selenate concentrations over a 14-day period. Bars represent the standard error for each treatment on day of observation.

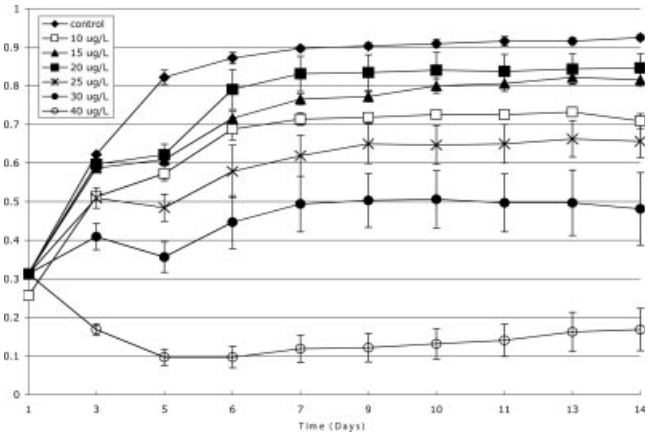

Fig. 2. Relative Growth Index of *C. quinquefasciatus* exposed to a range of methyl mercury chloride concentrations over a 14-day period. Bars represent the standard error for each treatment on day of observation.

methylmercury) and 50% of the LC_{50} mixture (5.5 mg/L sodium selenate, 15 μg/L methylmercury) caused 38 $\pm$ 13% and 93 $\pm$ 7% mortality, respectively (Table I).

The mean LC_{50} for selenate and methylmercury were determined for *C. quinquefasciatus*. The LC_{50} of selenate was 11 mg/L (95% Confidence Interval (CI) = 10–12 mg/L) and that of methylmercury was 30 μg/L (CI = 28–31 μg/L). The LC_{50} for the 13:1 mixture of sodium selenate to methylmercury was 3.4 mg/L (CI = 3.05–3.67 mg/L) and 9 μg/L methylmercury (CI = 8–11 μg/L).

The interaction between the components of the mixture was synergistic (Table I). The observed LC_{50} for the mixture (3.4 mg/L, CI = 3.05–3.67 mg/L) was less than the calculated expected LC_{50} value of 5.5 mg/L. In addition, after only 5 days exposure, expected mortality (E) from the mixture of selenate (1 mg/L) and methylmercury (4 μg/L) was calculated to be 4.96%. A χ^2 test for homogeneity indicated that the observed mixture results could be pooled (χ^2 = 3.69, 3 df, $P < 0.05$). After pooling the data, the observed mortality of the mixture (16 $\pm$ 1.0%, mean $\pm$ SE) was significantly greater than the E value of 4.96% ($\chi^2 = 19.09$, 1 df, $P < 0.05$), indicating that selenate and methylmercury interact and become more toxic than predicted by the action of either compound alone.

The Growth Indices and Relative Growth Indices were calculated for *C. quinquefasciatus* exposed to selenate, methylmercury, and a mixture of selenate and methylmercury (Fig. 1). The Growth Index was significantly different ($F_{5,18} = 36.97$, $P = 0.001$) between the control and all other treatments from day 4 to experiment termination. Even at the lowest concentration tested (2 mg/L), the larvae were negatively affected. In the mercury treatments (Fig. 2) the relative growth index was significantly different ($F_{6,21} = 33.25$, $P = 0.001$) between the control and the 25 to 40 μg/L concentrations from day 2 to experiment termination. Thus, even at the very low concentrations (25 μg/L), the RGI were significantly lower than for the controls after only 48 h exposure.

Growth indices were calculated for *C. quinquefasciatus* exposed to mixtures of selenate and methylmercury ranging from 0.55 mg/L Se and 1.5 μg/L MeHg to 5.52 mg/L Se and 15 μg/L MeHg. The relative growth indices of the various mixtures are shown in Figure 3. There was a significant difference ($F_{4,27} = 10.87$, $P = 0.001$) between the control RGI and the RGI for treatment levels as low as 1.1 mg/L Se plus 3 μg/L MeHg early in the experiment (day 3). After day 4, the variation in growth and mortality increased in each of the treatments and only the RGI of the two highest

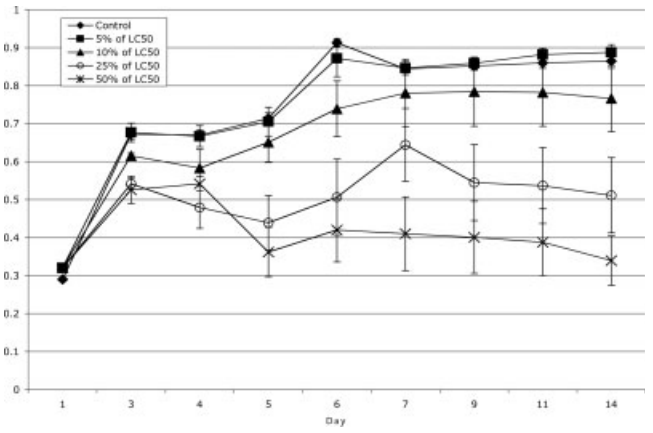

Fig. 3. Relative Growth Index of *C. quinquefasciatus* exposed to a range of sodium selenate and methyl mercury chloride fixed-ratio mixtures over a 14-day period. Bars represent the standard error for each treatment on day of observation.

treatment levels ($\geq$2.75 mg/L Se and $\geq$7.5 μg/L MeHg) remained significantly lower than the controls ($F_{4,27} = 3.78$, $P = 0.015$).

DISCUSSION

Oviposition is a critical stage in the mosquito life cycle and may determine population levels, distribution, biting behavior, pathogen transmission (Clements, 1999), and in this case metal exposure. Our finding that oviposition rates in treatment ponds were not significantly different from control treatments indicates that female *C. quinquefasciatus* would not distinguish between oviposition sites contaminated or uncontaminated with selenate or methylmercury individually or jointly. Therefore, larval exposure to selenium and mercury is likely in areas with such contamination. While ovipositional attractants and repellants are frequently investigated for *Culex* and other mosquito genera because of their roles as disease vectors (Millar et al., 1992), we were unable to find other research investigating the role of inorganic pollutants in mosquito ovipositional preferences. However, a similar inability to distinguish between contaminated, unsuitable larval food sources, and food sources acceptable to larvae was observed in the aquatic insect *Chironomous riparius* exposed to cadmium (Williams et al., 1987). A terrestrial insect, *Megaselia scalaris*, did not distinguish between food sources containing sodium selenate, methylmercury, and mixtures of the two compounds (Jensen et al., 2006) or hexavalent chromium (Trumble and Jensen, 2004). Thus, a pattern is emerging that suggests that many insect species have not evolved to avoid oviposition on substrates contaminated with metals and metalloids.

Mortality of *C. quinquefasciatus* larvae significantly increased depending upon the concentration of selenium and/or methylmercury in the water. The decrease in larval survival at concentrations greater than 8 mg Se/L and an LC_{50} of 11 mg Se/L after 14 days in this experiment was consistent with previously reported values for other dipterans. Ingersoll et al. (1990) reported selenate 48-h LC_{50} values for *Chironomus riparius* larvae of 10.5–16.2 mg/L and toxic effects from chronic exposure at 6 mg/L, while Maier and Knight (1993)reported a selenate 48-h LC_{50} of 23.7 mg/L for *Chironomus decorus* larvae. Although selenium can reach very high concentrations (8.3 mg/L) (Seiler et al., 1999), selenium concentrations in mosquito larval habitats such as natural waters average 1 μg/L (Saiki and Lowe, 1987) and agricultural evaporation ponds can contain selenate concentrations up to 2 mg/L (Thompson-Eagle and Frankenberger, 1990). Our results show that *C. quinquefasciatus* survival will not be directly affected at the 2 mg/L Se level. However, the consensus of research indicates that most of the selenium in fish tissues results from selenium in the diet rather than in the water (Maier and Knight, 1994). *Culex quinquefasciatus* could easily survive in contaminated evaporation ponds or natural systems as described and thereby provide a contaminated food source for invertebrate predators and fish. Any concentrations above 8 mg/L are likely to impact populations of mosquito larvae and potentially other insects, reducing food supplies for higher trophic levels.

The decrease in larval survival at methylmercury concentrations greater than 25 μg/L and the LC_{50} of 30 μg/L are the first reported values for an aquatic insect exposed to organic mercury. The concentrations of inorganic mercury acutely toxic to aquatic insects range from 2.0 to 1200 mg/L (Heliovaara and Vaisanen, 1993). Organic mercury often causes toxicity to aquatic organisms at levels 10 times lower than for inorganic mercury (Boening, 2000). Even with this extrapolation, *C. quinquefasciatus* exhibited the lowest observed effective concentration (LOEC) at an order of magnitude lower than would be predicted. Nonetheless, the reported MeHg concentrations in a variety of water bodies are much lower and range from 0.04 to 2.2 ng/L (Watras et al., 1995; Domagalski, 2001). Methylmercury is known to bioaccumulate in aquatic ecosystems at even extremely low water concentrations of MeHg (0.045–0.1 ng/L), resulting in zooplankton and phytoplankton containing from 4 to 56 ng/g of MeHg (Watras and Bloom, 1992). Determination of the toxicity of MeHg to *C. quinquefasciatus* following oral exposure would be of interest to compare the relative toxicity of MeHg-contamination in food *versus* water.

Relative growth rates over time revealed sublethal toxic effects at concentrations lower than evident from the survival experiments. The most dramatic results occurred in the selenate treatments. The Se growth index LOEC (2 mg/L) was four times lower than the Se LOEC from the survival experiments (8 mg/L) and was within reported Se concentrations in agricultural evaporation ponds (Thompson-Eagle and Frankenberger, 1990). The growth index results were also consistent with other reported results: Ingersoll et al. (1990) found that emergence time was delayed in *Chironomus riparius* at concentrations greater than 837 μg Se/L. In a terrestrial insect, *Megaselia scalaris*, Se delayed development by 25% or greater at concentrations of $\geq$100 μg/g in the diet (Jensen et al., 2006).

Methylmercury treatments also caused significantly reduced RGI values for larvae in treatments $\geq$25 μg/L from day two of the experiment until termination at day 14. While no other reports of MeHg effects on development were found for aquatic insects, growth inhibition of a freshwater alga *Poterioochromonas malhamensis* by MeHg was reported at 2 μg/L (Roderer, 1983), and a terrestrial insect *Megaselia scalaris* exhibited 35–60% delays in the completion of the larval period when exposed to diet treated with MeHg at levels as low as 100 μg/g (Jensen et al., 2006). For *C. quinquefasciatus*, the additional development time would increase exposure to predators and parasites, and

also serve to enhance the potential larval contribution to Se and/or MeHg bioaccumulation in higher trophic levels. These data also highlight the sensitivity and usefulness of nonsurvival metrics in toxicity testing.

The interaction between Se and MeHg on an aquatic insect did not follow the antagonistic pattern of Se-mediation of mercury toxicity generally seen in mammals, avians and fishes. Survival for *C. quinquefasciatus* larvae was decreased at 2.75 mg/L sodium selenate and 7.5 μg/L methylmercury and an LC_{50} of 3.4 mg/L sodium selenate and 9 μg/L methylmercury was determined. In addition, the Relative Growth Index of the larvae at levels as low as 1.1 mg/L Se and 3 μg/L MeHg was significantly lower than in the controls. These lethal and sublethal effects occurred at concentrations lower than those observed with the individual toxicants and the effects were greater than we would have predicted based on the individual dose response curves. Using Tabashnik's calculation for interactions (1992), the interaction can be classified as "synergism," with an effect greater than would be expected form the individual dose-response results. The greater than additive interaction between Se and MeHg on *C. quinquefasciatus* survival should be of interest to researchers and regulators alike. It is noteworthy that Heinz and Hoffman (1998) found antagonism between selenium and mercury in the diet of adult birds but synergism towards the developing embryos. This parallel between immature avian vertebrates and larval stages of an insect suggests that the ability to detoxify Se and Hg may be limited in the early developmental stages of many life forms.

While the concentrations used to determine the relationship in this experiment are elevated, this study provides additional evidence that Se and Hg are not always antagonistic. We speculate that the reason for the synergism rather than antagonism is due to the lack of erythrocytes in insects that provide the environment for Se-reduction and subsequent Se-Hg-S binding in vertebrate detoxification. Alternately, the difference in the Se-Hg relationship may be related to the lack of Se-dependent glutathione peroxidase in some insects (Simmons et al., 1989) that acts as an important component of the antioxidant system in mammals. These results suggest the need to more closely examine selenium and mercury detoxification in invertebrates as well as further investigation of other pollutant mixtures, and to perform these experiments on a variety of species in addition to the standard test organisms.

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