Environmental Chemistry

EFFECTS OF METAL AND ORGANOPHOSPHATE MIXTURES ON CERIODAPHNIA DUBIA SURVIVAL AND REPRODUCTION

AMY M. MAHAR and MARY C. WATZIN*
Rubenstein School of Environment and Natural Resources, Rubenstein Ecosystem Science Laboratory, University of Vermont, Burlington, Vermont 05405, USA

(Received 21 April 2004; Accepted 17 December 2004)

Abstract—The toxicity of mixtures of copper, zinc, and diazinon were determined for Ceriodaphnia dubia using 7-d survival and reproduction tests. Fifteen treatments, including combinations of the chemicals at 0, 25, 50, 75, and 100% of their individual median lethal concentrations, adding up to one toxic unit (TU) were tested. The TU was then used to classify each mixture response as additive, greater than additive, or less than additive. For survival, additive responses occurred in the 75% zinc plus 25% diazinon and the 50% copper plus 25% zinc plus 25% diazinon treatments. For reproduction, additive responses occurred in the 75% copper plus 25% zinc, 75% copper plus 25% diazinon, and 75% zinc plus 25% diazinon treatments. Copper and zinc played a greater role in toxicity than diazinon did. Less-than-additive interactions were found in all remaining mixtures, perhaps because of differences in mode of action between diazinon and metals. Consideration of dose–response curves can help to explain inconsistencies regarding toxic response in treatments with different ratios of the same chemicals. As TU percentages changed, mixture components were taken from different locations on differently shaped dose–response curves. Because most responses were less than additive, however, water-quality criteria based on individual concentrations probably are protective for most metal–organophosphate mixtures.

Keywords—Mixture toxicity Ceriodaphnia dubia Metals Organophosphates Toxic units

INTRODUCTION

Aquatic ecosystems often contain mixtures of a great variety of pollutants, including trace metals and pesticides, which may interact and mutually influence toxicity. Copper and zinc, primarily from automobile operation and construction [1,2], and the organophosphate insecticide diazinon (O,O-diethyl-O-[2-isopropyl-4-methyl-6 pyridimyl] phosphorothioate: CAS 333-41-5), from household, lawn, and garden applications [3,4], have been widely detected in urban stormwater runoff and adjacent surface waters at levels often exceeding the U.S. Environmental Protection Agency (U.S. EPA) [5] and International Joint Commission [6] chronic freshwater criteria [7–9]. Water-quality standards based on criteria for single toxicants often do not provide adequate protection for aquatic life in receiving waters when the effluent contains multiple pollutants [10–14]. Mixture-toxicity experiments, which more accurately represent environmentally realistic conditions, can assist in the determination of ecologically relevant water-quality criteria.

The basis of most models used to investigate the nature of mixture toxicity is concentration-additive toxicity [15–17]. Concentration additivity occurs when the toxicity of the mixture is equal to the toxicity that would be expected if the proportional, independent contributions of each toxicant (and of the breakdown products, although these are not considered separately), were simply added. The advantage of additive-toxicity models is that they can be used to classify organism response to the chemical mixtures [15]. The response may be concentration additive, less than concentration additive, or greater than concentration additive (potentiation) [16,17].

Environmentally relevant mixtures of metals and organophosphates are of great interest because the two chemical groups have very different modes of action within the exposed organism [18]. Some trace metals, such as copper and zinc, are involved in many metabolic pathways but become toxic when optimum levels are exceeded [19]. The primary mode of action for organophosphates, such as diazinon, is through their inhibition of the enzyme acetylcholinesterase, which is essential in neural transmissions [20]. However, despite the fact that both these toxicants commonly occur in urban stormwater runoff and urban surface waters at levels greater than their effect concentrations [7–9], the majority of published mixture literature has focused on interactions between toxicants from similar chemical classes [12,21–24].

Some research has been conducted to evaluate the toxicity associated with mixtures of toxicants exhibiting different modes of action [25–28], but only two examples evaluating the combined toxicity of metals and organophosphates have been identified. In the first, copper and diazinon exhibited less-than-additive effects on the survival of mayfly (Ephoron virgo) larvae [29]. In the second, mixtures of both copper and malathion and of cadmium and malathion had pronounced, more-than-additive lethal effects on the marine microcrustacean Ti
griopus bevicornis [30]. In neither study were common and highly sensitive [31,32] daphnid species represented. Because the types of toxic responses to mixtures vary, depending on the species exposed [12,21,22,25], the effects that these mixtures have on suspended filter-feeding daphnids also should be evaluated. In addition, both previous mixture studies focused primarily on lethal endpoints. Other mixture studies indicate that different types or degrees of response can be observed, depending on the sensitivity of the endpoints evaluated [11,23,27]. For this reason, reproductive success, which is essential for species success, is an important endpoint (in addition to survival) to monitor during mixture experiments.

In previous studies of mixtures that consisted of a large...
number of metals or organic chemicals, the effects on daphnid survival, growth, and rate of population increase most often have been additive; however, to our knowledge, no general trend has been seen when combinations of just two or three chemicals have been examined [11,13,16]. Therefore, a careful evaluation of toxicant interactions among smaller mixed groups of chemical constituents in storm water through experimentation is warranted.

The purpose of the present study was to measure the effects of metal–organophosphate combinations on both the 50% lethal concentration (LC50) and the 50% reproduction inhibition concentration of the freshwater cladoceran Ceriodaphnia dubia and to classify whether the interactions between and among these chemicals are additive, less than additive, or greater than additive.

MATERIALS AND METHODS

Test organism and culture conditions

Ceriodaphnia dubia was selected as the test organism, because it is very sensitive to many toxicants. It also is widely used in studies of pollution impacts, and the 7-d survival and reproduction test is one of several toxicity tests accepted by the U.S. EPA for estimating both acute and chronic toxicity in freshwater [31]. By establishing toxicant levels that protect this organism, other less sensitive organisms, such as certain minnow, amphipod, and midge species [33], also will be protected.

Ceriodaphnia dubia originally were obtained from the U.S. Geological Survey Columbia Environmental Research Center’s stock cultures (Columbia, MO) and cultured in house for two years. Ceriodaphnia dubia stock cultures were maintained in 1-L glass beakers of reconstituted, moderately hard water and exposed to a 16:8-h light:dark photoperiod. Reconstituted water was prepared according to U.S. EPA specifications [31] by adding reagent-grade salts to type I reagent-grade water (Aries High Purity Loop, West Berlin, NJ, USA). Nominal concentrations for total hardness, alkalinity, and pH were determined for each batch of reconstituted water and maintained between 88 and 96 mg/L as CaCO₃ for hardness, 60 to 68 mg/L as CaCO₃ for alkalinity, and pH 8.24 to 8.31. Water was stored in polyethylene carboys and aerated continuously. Temperature was maintained at 25 ± 1°C. Stock cultures were fed 1 ml of yeast (cerophyll) and trout chow (YCT) and 1 ml of Selenastrum capricornutum suspension daily (specific cell densities reported below). Stock water was renewed weekly, at which time cultures were thinned to 40 individuals. Brood organisms were kept separately in 30-ml polystyrene cups. Water was renewed every 48 h, and brood organisms were fed 0.1 ml of YCT and 0.1 ml of S. capricornutum suspension daily. Survival and reproduction of brood organisms were recorded and tracked for multiple generations. Neonates were collected from adults producing at least eight neonates in their third brood. Neonates used in the toxicity tests were all between 16 and 23 h of age at test initiation.

Toxic units

Mixture toxicology employs a dimensionless ratio, the toxic unit (TU) [15]. Each toxicant concentration is considered as a fraction of its individual toxicity, which most commonly is expressed in terms of its LC50. The total TU of the mixture is a sum of the individual fractions [12]. For example, if we wanted a mixture that contained equally toxic portions of contaminant A, with a LC50 of 20 µg/L, and contaminant B, with a LC50 of 50 µg/L, we could create a solution that had a concentration of 10 µg/L of contaminant A and 25 µg/L of contaminant B to represent one TU. This assumes that half of two LC50s equal one full LC50. This is the working definition of concentration additivity that we used throughout the study. When the TU is equal to one, the mixture is predicted to result in 50% organism mortality if the interaction is additive. If the toxic response deviates such that it is less than or greater than 50% (i.e., one TU), then it is not additive [15].

Assuming the null hypothesis of an additive interaction between the chemicals, the mixtures used in all experiments were achieved by adding the ratios of LC50 concentrations obtained in preliminary tests for each of the individual chemicals such that the sum of the fractions would result, theoretically, in 50% mortality, or one TU. Although the LC50 is the most commonly used metric to characterize toxicity, it clearly is an arbitrary choice along a dose–response curve that may take many forms. The general limitations of quantal metrics [31,34] should be kept in mind when using TUs, just as they are with any other single metric.

Toxicants

Dilution water for test solutions consisted of reconstituted water used in culturing. Reagent-grade copper, as cupric chloride, and zinc, as zinc chloride, were obtained from Sigma Chemical (St. Louis, MO, USA) and dried to constant weight before they were used to make stock solutions. Diazinon treatments were made using 99.5% pure diazinon from Chem Service (West Chester, PA, USA). Treatment solutions containing copper or zinc or combinations of the two metals were mixed every other day from type I water-based stock solutions and stored, along with stock solutions, in sealed containers at 4.4°C. To decrease the effect of chemical degradation, treatment solutions containing diazinon were mixed daily from solvent (analytical-grade acetone; Fisher Scientific, Fair Lawn, NJ, USA)-based stock solutions.

Treatments

Separate 7-d LC50 (one-TU) values were determined twice for copper and diazinon and four times for zinc through preliminary experimentation. Based on these, a total of 17 treatments of various mixtures were then prepared and tested simultaneously. These treatments are described in Table 1 as the theoretical percentage of the TU of each chemical in the mixture. Analytical-grade acetone was added to the solvent control and to all noncontrol treatments so that each treatment contained the same concentration of acetone that was added from the diazinon stock to the one-TU diazinon treatment. For the November 2000 test, the nominal 7-d LC50s for one TU each of copper, zinc, and diazinon (treatments 3–5), from which the test concentrations (treatments 6–17) were derived, were 21.8, 110, and 0.4 µg/L, respectively, and for the May 2002 test, these values were 78, 122, and 0.29 µg/L, respectively. These nominal LC50 values were adjusted between the November and May tests to account for deviations in toxicity that most likely resulted from differences in the S. capricornutum concentration [32]. Of particular interest were the copper LC50 values, which, consistent with metal ion adsorption onto algal surface or algal uptake, increased greatly when algae concentrations were increased [35]. With the exception of the May 2002 copper concentration, which was higher than typically
The effects of metal and organophosphate mixtures on *C. dubia* were studied in continuous-exposure, static-renewal toxicity tests. The temperature was maintained at 25\(^\circ\)C, and a 16:8-h light:dark photoperiod was provided, with temperatures recorded. Replicates of each treatment were used.

Table 1. Mixture test concentrations as percentages of one toxic unit (TU)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Copper (%)</th>
<th>Zinc (%)</th>
<th>Diazinon (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 Solvent control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3 1 TU Cu</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4 1 TU Zn</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>5 1 TU diazinon</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>6 Cu + Zn</td>
<td>25</td>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td>7 Cu + Zn</td>
<td>50</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>8 Cu + Zn</td>
<td>75</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>9 Cu + diazinon</td>
<td>25</td>
<td>0</td>
<td>75</td>
</tr>
<tr>
<td>10 Cu + diazinon</td>
<td>50</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>11 Cu + diazinon</td>
<td>75</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>12 Zn + diazinon</td>
<td>0</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>13 Zn + diazinon</td>
<td>0</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>14 Zn + diazinon</td>
<td>0</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>15 Cu + Zn + diazinon</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>16 Cu + Zn + diazinon</td>
<td>25</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>17 Cu + Zn + diazinon</td>
<td>50</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

For tests to be considered valid, total control survival per test must have remained at 80% or greater. Also, at least 60% of surviving adults in the controls must have had three broods, with an average total number of at least 15 neonates per surviving adult by test termination.

**Data verification**

For verification that actual metal concentrations approximated calculated concentrations, samples were taken from several representative treatments on a single test day, acidified with nitric acid to pH 2 according to standard methods and stored at 4.4\(^\circ\)C until analysis. Metal concentrations were then determined using the Perkin-Elmer Optima DV inductively coupled plasma atomic emission spectrometer (method 3120.B; Norwalk, CT, USA) [36]. Copper and zinc standard solutions, prepared from high-purity reference materials (High-Purity Standards, Charleston, SC, USA), were used during inductively coupled plasma atomic emission spectrometer analysis to ensure accuracy. Readings from each sample were replicated in triplicate, and blanks were used to ensure quality. All test samples remained at University of Vermont facilities in the custody of primary investigators throughout the course of the study. Actual diazinon treatment concentrations were not measured.

**Statistical analysis**

Survival and reproduction (neonates/female) were monitored daily. Survival was defined as the number of surviving adults per treatment at the end of the 7-d test period. The binomial parameter, with its associated 95% confidence intervals, was used to determine differences between the observed survival and the expected null of 50% survival for each mixture treatment [37].

To test the null of additive interaction of the chemicals on reproduction, the expected number of neonates produced in the mixture treatments was generated as weighted averages of the reproduction of the one-TU copper, one-TU zinc, and one-TU diazinon treatments. One-way \(t\) tests were then conducted to identify significant differences between the observed and expected number of young for each treatment [38]. The 95% confidence intervals associated with the one-way \(t\) test were generated [37]. Analysis of variance with Student-Newman-Keuls multiple comparisons was used to compare the relative toxicities of the treatments in terms of reproduction [38].

**RESULTS**

Temperature and dissolved oxygen concentration remained within satisfactory limits in all tests [30]. The toxicity of diazinon likely was not affected by hydrolysis or photolysis or its breakdown products, because diazinon is relatively stable. It has a half-life of 138 d under neutral aquatic conditions and of 77 d under alkaline aquatic conditions (pH 9); at approximately pH 8, little degradation would have occurred within our one-week experiments [4]. Actual copper test concentra-
tions, as measured by inductively coupled plasma atomic emission spectrometer analysis, were within 12.7% of their calculated target concentrations in greater than 95% of the analyzed samples. Actual zinc test concentrations were within 10.2% of their calculated target concentrations in greater than 95% of analyzed samples.

Survival

In the first mixture test (November 2000), control survival was 90% or greater. However, because 90% survival occurred in the one-TU copper treatment, all treatments containing copper were excluded from these results. For both the one-TU zinc and one-TU diazinon treatments, the observed survival was not significantly different from the expected 50% survival (Fig. 1). For all three zinc-and-diazinon mixture treatments, the observed survival was significantly greater than the expected survival.

In the second mixture test (May 2002), control survival was 100%. For all but one of the TU treatments, the observed survival was not significantly different from the expected 50% survival, but variability was high (Fig. 2). The observed survivals in the 75% zinc plus 25% diazinon and the 50% copper plus 25% zinc plus 25% diazinon treatments were not significantly different from those expected. For all other treatments, the observed survival was significantly greater than the expected survival. Significant mortality only occurred during treatments in which either copper or zinc represented 50% or more of the TU. In neither test was mortality associated with treatments containing 75% of one TU of diazinon.

Reproduction

For the November test, the average number of neonates produced in both control and solvent control treatments was slightly less, although not significantly so, than the 15 neonates per surviving adult necessary to constitute a valid test. Averaging 1.3 neonates per adult, with adults at 40% survival, the one-TU zinc treatment had significantly lower reproduction than the 8.2 neonates per adult produced by the one-TU diazinon treatment, which had 50% survival (Fig. 3). In all the three zinc-plus-diazinon mixture treatments, the observed reproduction was significantly greater than the expected reproduction. Both the expected and observed number of neonates reflected a pattern in which the greater the percentage of zinc in the mixture, the fewer average neonates produced. For survival, this pattern of increased effect was not observed as zinc was increased from 25 to 75% of the TU.

As in the November test, average neonate production in the May test was slightly greater, although not significantly so, for the control than for the solvent control. In both controls, the average neonate production per surviving adult was significantly greater than the U.S. EPA minimum. Again, with 6.7 neonates per adult and 40% survival, the one-TU zinc treatments had significantly fewer neonates than the one-TU diazinon treatment, with 20.6 neonates per adult and 50% adult survival (Fig. 4). The one-TU copper treatment, which had 15.8 average neonates and 60% adult survival, was not significantly different in reproduction from either the one-TU zinc or the one-TU diazinon treatments. In three of the mixture treatments—75% copper plus 25% zinc ($p = 0.1648$), 75% copper plus 25% diazinon ($p = 0.0594$), and 75% zinc plus...
Effects of metal and organophosphate mixtures on *C. dubia*

Fig. 3. Average number of observed and expected *Ceriodaphnia dubia* neonates per adult in the November 2000 toxicity test with 95% confidence intervals. Mixtures of zinc and diazinon are given as percentages of one toxic unit (TU). The asterisk (*) indicates a significant difference at $\alpha = 0.05$.

25% diazinon ($p = 0.0662$)—the observed number of neonates was not significantly different from the expected, although the last two mixtures were marginally different. In all other treatments, the observed number produced was significantly greater than the expected. A significant difference between observed and expected numbers of neonates was associated only with treatments in which copper or zinc were represented as 75% of the TU of the mixture and never associated with diazinon at greater than 25% TU.

In both tests, the expected and the observed zinc-plus-diazinon mixture treatments followed a general pattern: The greater the percentage of zinc that the mixture contained, the fewer average neonates produced. The observed number of neonates in the mixtures of copper plus diazinon also reflected the expected trend that the greater the percentage of copper in the mixture, the fewer average neonates produced.

For the mixtures of copper and zinc and those containing all three chemicals, the expected patterns of toxicity differed from those observed. For copper-plus-zinc mixtures, it was expected that as the TU percentage zinc increased and the TU percentage copper decreased, the number of neonates would decrease. However, in our experiments, as either of the metals increased to 75% TU of the mixture, the number of neonates decreased. For the mixtures of all three chemicals, it was expected that the treatment with the greatest percentage of zinc would have the lowest reproduction. In our experiments, the treatments comprised primarily of either copper or zinc had lower reproduction than the treatments composed primarily of diazinon.

**Relative reproduction toxicity**

One way to view reproductive impairment is through a three-dimensional grid, with one TU of each chemical extending along an axis from the origin to form a triangle (Fig. 5). Fewer average neonates were produced as one moves diagonally along a dimensional representation of the mixture treatments, from a large percentage diazinon to combinations with either a large percentage of copper and small percentage of zinc or a large percentage of zinc and small percentage of copper. In terms of the average number of neonates produced, the one-TU zinc treatment produced significantly fewer neonates than all other treatments. The 25% zinc plus 75% diazinon treatment had significantly more neonates than the 25% copper plus 75% zinc, 75% copper plus 25% zinc, 75% zinc plus 25% diazinon, or one-TU copper and one-TU diazinon treatment. Additionally, the 25% copper plus 75% diazinon, 50% zinc plus 50% diazinon, and 25% copper plus 25% zinc plus 50% diazinon treatments produced significantly more neonates per adult than the 75% copper plus 25% zinc, 75% zinc plus 25% diazinon, and one-TU copper treatments.

**DISCUSSION**

Additive responses, in which the combinations of chemicals were neither more nor less toxic than would be expected for one TU of the chemicals individually, were detected only in the May 2002 test. For survival, additive interactions were observed in the 75% zinc plus 25% diazinon and the 50%...
copper plus 25% zinc plus 25% diazinon treatments. For reproduction, additive responses were detected in 75% copper plus 25% zinc, 75% copper plus 25% diazinon, and 75% zinc plus 25% diazinon treatments. Generally, additive toxicity is thought to occur when the chemicals in combination have similar primary sites or modes of action within the exposed organism, so four of these five seemingly additive responses were unexpected. Less-than-additive interactions were found in all remaining mixture treatments for both tests. Less-than-additive interactions often occur when chemicals in mixture have dissimilar sites or modes of action [23,39].

Metals have several common modes of toxicity. Enzyme inhibition occurs when a metal displaces an essential metal cofactor of the enzyme or when the metal interacts with sulfhydryl groups on the enzyme [40]. At the cellular level, such enzyme inhibition can disrupt mitochondrial respiration and normal functions of the endoplasmic reticulum. Metals also may disrupt the structure and function of cells by metal accumulation in lysosomes and the formation of metal inclusions in the cell nucleus [40] or by disruption of calcium uptake [41] and interference with ion regulation, leading to suffocation [42]. The primary mode of action for diazinon is through inhibition of the enzyme acetylcholinesterase, resulting in disruption of the nervous system by inhibition of cholinesterase activity, which results in acetylcholine accumulation at nerve synapses, leading to the continuous firing of impulses. Death generally is attributed to respiratory failure [20]. Because diazinon causes toxicity through acetylcholinesterase inhibition and metals act through more diverse modes, this may explain the results in the majority of treatments.

Similarly, if the chemicals in the mixture are not interactive (i.e., the presence of one does not influence the other), then the toxic response may be expected to be additive. If the chemicals in the mixture are interactive, then factors such as uptake rates and availability or physiological processes such as chemical metabolism, transport, or excretion can result in a response that may be either greater or less than additive [43]. However, previous research has found that these general rules may not be sufficient to explain all mixture toxicity. For example, the planar chlorinated hydrocarbons 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and polychlorinated biphenyl congener 156 (PCB 156) appeared to share a similar primary site of action within the rainbow trout (Oncorhynchus mykiss), yet in terms of hepatic enzyme activity, equipotent mixtures of TCDD and PCB 156 produced a less-than-additive response [44]. Non-additive responses are thought to have resulted because the less toxic PCB 156 was an effective competitor for the binding sites that otherwise would be filled by the more toxic TCDD.

In the May experiment, different combinations of the same chemicals produced less-than-additive responses in some treatments and additive responses in others. It is possible that the degree of interaction between the chemicals differs, depending on the TU percentages of metals and organophosphates that make up the mixture treatment. If the dose–response curves of the individual chemicals are of different shapes, it is reasonable that as the TU percentages of the chemicals change, differing toxic responses would result. Other investigators have found inconsistent toxic responses at varying effect levels [29]. For example, at the 10% effective concentration level, mixtures of cadmium and zinc, which exhibit different shapes in their dose–response curves, acted on the reproduction of Folsomia candida in a less-than-concentration-additive manner, whereas at the 50% effective concentration level, a greater-than-concentration-additive effect was observed [45]. In this case, the additivity of the mixture depended on the effect level being examined. In our experiment, the mixture components are taken from different locations on what probably are differently shaped dose–response curves. For this reason, it is essential to evaluate multiple combinations of the same chemical mixture, because a single estimate of the type of toxic response resulting from the mixture may not give a complete picture concerning the type of interaction that occurs at varying composition levels.

Because most additivity occurred when metals comprised 75% of the TU, an additional explanation for both the additive and less-than-additive responses of mixtures containing the same chemicals is that for metals at 75% TU, the degree of associated toxicity may be similar enough to the toxicity associated with one TU that these two treatments cannot be differentiated. Therefore, instead of seeing additivity at the 75% TU metal plus 25% TU organophosphate treatments, we really may be seeing a less-than-additive response.

The 75% zinc plus 25% diazinon treatment that consistently showed additive toxicity for both survival and reproduction in the May test exhibited less-than-additive toxicity for both these endpoints in the November test. Such variability between test results appears to be common. Within a single treatment, the type of interaction exhibited in mixtures that contain only a few compounds is more variable than that of mixtures composed of many chemicals [28]. For example, four mixture tests by Hermens et al. [28] containing a hydrocarbon and a chloraniline exhibited toxicity ranging from partial additivity to additivity to more-than-additive toxicity. Even within a single treatment, the type of toxic interaction exhibited was not always consistent in both evaluated endpoints. In our May tests, of the four treatments that showed additivity, only one was additive for both survival and reproduction.

Three treatments in the May test looked at binary metal combinations of copper and zinc. The interaction of these metals was less than additive for survival and reproduction in all treatments but for the 75% copper plus 25% zinc for reproduction. Other binary and tertiary metal mixture studies have
found similar results. The effects of combinations of copper and zinc on zebra mussel (Dreissena polymorpha) filtration rates were less than concentration additive [12]. For growth of duckweed (Lemma minor) and Microtox bacteria (Vibrio fischeri), the interaction between copper and zinc most often also was less than additive [22]. Contrary to our results, combinations of copper and cadmium were more-than-concentration additive, and combinations of zinc and cadmium and of all three metals were concentration additive in terms of D. polymorpha filtration rate [12]. The genetically modified, luminescence-based microbial biosensor Escherichia coli showed significant greater-than-additive interactions when exposed to equitoxic mixtures of copper and zinc and of zinc and cadmium [21]. The microbial biosensor Pseudomonas fluorescens showed significant greater-than-additive interactions for copper and cadmium and showed additive interactions with exposure to copper-and-zinc mixtures [21]. These differences in the type of toxic interactions exhibited by E. coli, mussels, plants, and bacteria in response to zinc and copper mixture exposure likely are a result of basic differences in physiological and structural makeup of these organisms as well as the different metal concentration levels both between and within these studies.

Three mixture treatments in the November test and nine mixture treatments in the May test examined the responses associated with metal–organophosphate mixes. Only two similar studies evaluating metal–organophosphate mixtures were found in the literature. In one, copper and diazinon showed less-than-additive effects on mayfly larva survival [29]. This is consistent with our results, in which additivity was detected at high metal–low organophosphate concentrations. The other study showed that both mixtures of copper and malathion and of cadmium and malathion had strongly more-than-additive lethal effects for the marine microcrustacean T. bevicornis [30].

Other studies have shown that the patterns of toxic response to mixtures vary with the sensitivity of the endpoints evaluated, with a general trend that the type of response becomes less additive with the degree of endpoint sensitivity increases [23,27,46]. Overall, in this study, effects on survival were less than additive in 13 of 15 treatments, and effects on reproduction were less than additive in 12 of 15 treatments. Although this difference is slight, differences were observed in which endpoint was most sensitive for each chemical. For diazinon, survival was the most sensitive endpoint. For copper and zinc, reproduction was more sensitive, as previously shown [32]. Therefore, it would be expected that when metal combinations are tested alone, there would be more additive interactions in the reproduction endpoint than in the survival endpoint. This was the pattern in our data. Likewise, across the experiment, copper and zinc both played a greater role in reproductive toxicity than diazinon did. When tested individually and in combination, zinc and copper exposures have been shown to result in reduced daphnid reproduction [14,47]. Additionally, Daphnia magna population growth decreased as the concentration of a seven-metal mixture increased [16]. A possible explanation is that, at sublethal concentrations, electropositive metals, such as zinc and copper, which adsorb to suspended particulates, cause feeding inhibition when ingested by D. magna. This inhibition is suspected to be a mechanism impairing reproduction [48].

Metals and organophosphates commonly are found together in urban stormwater runoff and in urban surface waters at or above their effect concentrations. Our results indicate that such metal–organophosphate mixtures likely are not more toxic than would be expected from the chemicals individually. In fact, these mixtures often seem to be less than additive in combination. In natural systems, many other factors, such as the presence of additional chemicals or aquatic particulates, the total concentration of all the chemicals, and differences in general water quality and health of the exposed aquatic life, also will play a role in determining the toxicity of metal–organophosphate mixtures. Therefore, risk assessments should consider mixtures and their complex interactions to a greater extent if environmentally realistic projections are desired.

Acknowledgement—This research was funded by Green Mountain Power Corporation. We thank A. Pitt and A. Shambaug for technical assistance and S. Mahar and A. Howard for statistical advice. We also thank A. McIntosh, N. Hayden, and two anonymous reviewers for their helpful comments on the manuscript.

REFERENCES


