EFFECTS ON SURVIVAL, REPRODUCTION, AND GROWTH OF *Ceriodaphnia dubia* FOLLOWING SINGLE EPISODIC EXPOSURES TO COPPER OR CADMIUM

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Effects of episodic exposures have gained attention as the regulatory focus of the Clean Water Act has shifted away from continuous-flow effluents. Standardized laboratory toxicity tests require that exposure be held constant. However, this approach may not accurately predict organism responses in the field following episodic exposures such as those associated with rain-driven runoff events or accidental pollutant discharge. Using a modified version of the 7-day short-term chronic test recommended by the US Environmental Protection Agency, *Ceriodaphnia dubia* were exposed to copper or cadmium for durations ranging from 1 minute to 24 hours. In addition, adult reproductive recovery and effects on second generation individuals was assessed following select copper exposures. Finally, cadmium exposures were compared in reconstituted hard water (RHW) and municipal treated wastewater effluent (TWE). Following exposure, organisms were transferred to clean RHW or TWE and maintained for the remainder of the test. No- and lowest observed effect concentrations (NO- and LOECs) increased logarithmically with respect to logarithmic decreases in duration regardless of metal, endpoint or water type. Effective concentrations of cadmium however, were usually higher than those of copper, especially in TWE. LOECs for *C. dubia* survival following 24-hour and 5-minute exposures to copper were 116 and 417 µg/L, respectively. LOECs for fecundity were 58 and 374 µg/L, respectively. Neonate production of first generation adult *C. dubia* appeared to recover from pulsed copper

exposure upon examination of individual broods. Cumulative mean neonate production however, showed almost no signs of recovery at exposure durations ≥3 hours. Pulse exposure to copper also resulted in diminished fecundity of unexposed second generation individuals. Such effects were pronounced following parental exposure for 24 hours but lacking after parental exposures ≤3 hours. LOECs for *C. dubia* survival following 24-hour and 5-minute exposures to cadmium in RHW were 44 and 9000 µg/L, respectively. LOECs for fecundity were 16 and 5000 µg/L, respectively. In TWE, LOECs for *C. dubia* survival were 83 and >10,000 µg/L, respectively. LOECs for fecundity in TWE were 48 and 7000 µg/L, respectively. Runoff pollution is site and event specific, however, data presented herein may be useful as a predictive tool under various conditions.

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CHAPTER 1

EFFECTS ON SURVIVAL, REPRODUCTION AND GROWTH OF *Ceriodaphnia dubia* FOLLOWING SINGLE EPISODIC EXPOSURES TO COPPER

Introduction

Since its inception, the US Environmental Protection Agency (EPA) has expended considerable effort and resources developing means of assessing quality and controlling toxics in our nation's surface waters. Section 101(a)(3) of the Clean Water Act (CWA) states "it is the national policy that the discharge of toxic pollutants in toxic amounts be prohibited." As put forth in the regulation, 54 *FR* 23868, June 2, 1989, the EPA recommended an integrated approach to be used in water quality-based toxics control (EPA, 1991a). Implementation of at least part of the integrated approach contributes to development of water quality criteria (WQC) for receiving waters of point-source discharges.

The protective nature of WQC in relation to non-point source pollution such as rain-driven runoff events has been a matter of debate for many years. Numerous researchers have compared intermittent and continuous exposure to toxicants and have used such data to directly or implicitly challenge the reliability of WQC – which are based on continuous exposure data. Some have shown where intermittent exposure is more toxic (Abel, 1980; Abel and Garner, 1986; Ingersoll and Winner, 1982; Seim et al., 1984; Holdway and Dixon, 1986; Jarvinen et al., 1988a and 1988b) and criteria would be underprotective. Others have presented data suggesting that WQC may be overprotective (Bailey, 1985; Holdway and Dixon, 1985; Fisher et al., 1994; Holdway et

al., 1994; Handy, 1995; Novotny and Witte, 1997; Hosmer et al., 1998) in that intermittent or pulse exposures are less toxic.

The results of earlier research (mostly those prior to 1991) and others suggest WQC may be inadequate for protecting aquatic organisms against pulsed exposures. Such findings lead to research at EPA Duluth to develop the criterion maximum concentration (CMC) with exposure period assumption of 1 hour and the criterion continuous concentration (CCC) with an average period assumption of 4 days (Burton, 1999). Whether or not WQC for priority pollutants can be used for stormwater protection remains controversial in the U.S. and other countries. Consequently, stormwater abatement efforts are hampered by unavailability of wet-weather quality standards (Novotny and Witte, 1997). Regardless of whether criteria are over- or underprotective, intermittent exposure has been the subject of regulatory criticism. Many of those studies however, may not have posed realistic exposure scenarios. For example, daily pulses of a toxicant are not likely to occur unless some component of an effluent is not released continuously, but intermittently. Field application of pesticides, rain-driven runoff events, and accidental spills may be intermittent, but their regularity is generally seasonal and less predictable. In addition, toxicant exposure during such events is not likely the same each time. Environmental regulations do not address such scenarios, and the scientific literature has not addressed them in a regulatory framework.

It is important to note here that the CMC and CCC consider *average* concentrations over an *average* time period. It is also important to note that these scenarios are generally based on continuous exposure. A toxicant average concentration could be the result of many exposure scenarios – some of which having

more impact than others. For example, we often do not know whether a higher concentration of shorter duration is more or less affective than a lower concentration of longer duration when both have an equal *average* concentration. The average concentration over a 4-day period should not exceed the CCC. However, it is possible that the concentration on the first day is very high and effective, whereas concentrations of the other three days are much lower. It is also possible that higher concentrations on that first day render organisms more susceptible to successive lower concentrations. CMC and CCC averaging periods were derived with the understanding that in-stream toxicant concentrations often fluctuate (EPA, 1991a). The premise behind the averaging periods suggests that the concentration of fast-acting toxicants may exceed the CMC or CCC provided that the average concentration over that averaging period does not. In this, an attempt is made to reduce impacts of brief exposure to elevated concentrations. Unfortunately, extremes can not always be detected, and effective events will likely be missed.

Effects of wet weather discharges on the environment have gained more attention as the regulatory focus of the Clean Water Act has shifted from continuous-flow effluents to episodic discharges produced by storm events (Herricks and Milne, 1998). However, the difficulty of estimating effects from time-varying exposures from measurements obtained from continuous exposure tests is often an important source of uncertainty in ecological risk assessment (Reinert et al., 2002). For many years, researchers have been proposing incorporation of pulse-exposure test designs in risk assessment (Abel, 1980; Holdway and Dixon, 1986; Pascoe and Shazili, 1986; McCahon and Pascoe, 1990; Parsons and Surgeoner, 1991; Brent and Herricks, 1998;

Naddy et al., 2000; Naddy and Klaine, 2001). Such tests could be used to better evaluate effects that may occur under more natural exposure conditions while retaining the advantages of simplicity and repeatability associated with single-species laboratory tests (Peterson et al., 2001).

Whole effluent toxicity (WET) tests are single-species laboratory tests clearly designed for examination of point-source and continuous exposure scenarios. They are the most widely used and accepted standard aquatic toxicity tests in the United States. They have proven to be precise and reliable in most cases (EPA, 2001a), although this point remains controversial. Finally, WET test data has often agreed with observed instream impacts (EPA, 1991a; EPA, 2001a; e.g.s, Eagleson et al., 1990; Dickson et al., 1992; Diamond and Daley, 2000). Other single-species laboratory toxicity tests have also been shown to predict field effects when exposure regimes are similar (Borthwick et al., 1985; Clark et al., 1987). With modification, WET-type tests may be as useful in assessing non-point source and episodic exposure scenarios that have more complicated issues of time-scale toxicity.

Published research on effects of episodic exposure are dominated by studies of pesticides, however, several have investigated metals. Among these, many have examined effects from intermittent exposure (Lappivaara and Marttinen, 2005; Siddens et al., 1986; Seim et al., 1984; Hodson et al., 1983; Ingersoll and Winner, 1982). Several have also examined lethal (Brent and Herricks, 1998; Hutchinson et al., 1989; Abel and Garner, 1986; Pascoe and Shazili, 1986) and sublethal (McWilliams and Baird, 2002; Williams and Holdway, 2000; Handy, 1992; McCahon and Pascoe, 1991) effects of single-pulse exposures. Only two have investigated single pulse effects on

cladocerans. Brent and Herricks (1998) reported significant post-exposure immobility of *Ceriodaphnia dubia* at 2.5 and 0.37 mg/L cadmium following 30-minute and 2-hour exposures, respectively. 15-minute exposures did not result in significant post-exposure immobility at cadmium concentrations as high as 11.8 mg/L but did at 4.8 mg/L zinc. McWilliam and Baird (2002) examined feeding behavior in *Daphnia magna* exposed to heavy metals. Following 24-hour exposures, post-exposure feeding depression was significant at ≥0.2 µg/L cadmium, ≥14 µg/L copper and 100 µg/L zinc.

Most of the literature on sublethal effects of single pulse exposures have examined arbitrary, often environmentally realistic, exposure scenarios to determine whether or not they were problematic. Unfortunately, even many of environmentally realistic scenarios are site specific and may not be predictive of different sites or varying events. No other research has actually sought out specific effect levels for a wide range of exposure scenarios. The objective of this research was to identify post-exposure effect concentration-duration combinations of single episodic exposures of copper on survival, reproduction and growth of the cladoceran, *Ceriodaphnia dubia*. Results are expressed as traditional no- and lowest observed effect concentrations (NOEC, LOEC) as well as novel no- and lowest observed effect durations (NOET, LOET). Research presented here may have applications in ecological risk assessments beyond single sites or events.

Materials and Methods

Ceriodaphnia dubia *Cultures*

C. dubia were cultured in standard synthetic reconstituted hard water (RHW, EPA, 1991) without addition of sodium selenate. RHW also served as control water for all toxicity tests. RHW was prepared in 50-L batches following procedures outlined in Knight and Waller (1987) with the exceptions listed in Hemming et al. (2002). *C. dubia* were cultured as described by Knight and Waller (1992) also with modifications described in Hemming et al. (2002) except that 25 to 30 neonates were used to start each mass culture jar. *C. dubia* received the same feeding suspension in mass and individual cultures as well as during 7-day toxicity tests. The final feeding suspension consisted of a mixture of the unicellular green algae, *Pseudokirchneriella subcapitata* (formerly, *Selenastrum capricornutum*), and Cerophyl or cereal grass media (Wards Scientific) and was prepared as described by Knight and Waller (1992).

Pseudokerchneriella subcapitata *Cultures*

P. subcapitata were cultured in three steps: 1) 50-ml screw cap culture tubes, 2) 2-L Erlenmeyer flasks, and 3) 20-L polycarbonate carboys. Agar slant starter cultures were obtained from the University of Texas Culture Collection of Algae. Cells were dislodged from agar and resuspended in 2.5 to 3.0 ml sterile Gorhams media (American Type Culture Collection). 50-ml screw-cap culture tubes containing 15 ml sterile Gorhams media were then inoculated with 0.5 ml cell suspension. Tubes were capped with sterile foam plugs, placed on a wrist-action shaker (Burrel Corporation, Model 75) and shaken (240 rotations per minute) for 7 days. On the seventh day, 1 ml algae

suspension from each tube was used to inoculate new tubes to be stored for future use. The remaining 14 ml was used to inoculate 2-L flasks containing 1 L sterile Algal Assay Production (AAP) media as described by EPA (1994). These flasks were also incubated for 7 days with continuous gentle stirring. Algae grown in 2-L flasks were used to inoculate carboys containing 6 L sterile AAP media. Approximately 7 L additional AAP media was added two and four days after inoculation. On the sixth day, a vitamin suspension was added to each carboy. The vitamin suspension consisted of one multivitamin/multimineral tablet crushed in 100 ml deionized water. 25 ml of the suspension was added to each carboy – the equivalent of one-quarter tablet per carboy. Carboys were vigorously aerated throughout with air pumped through a granular activated carbon filter or through foam plugs similar to those used during media sterilization. On the seventh day, Carboys were capped with parafilm and stored in the dark at 4°C. Algae were retrieved by centrifugation. The contents of each carboy was centrifuged at 8,800 rpm. Algae pellets were rinsed with RHW, resuspended in 500 to 600 ml RHW, and stored in the dark at 4°C until needed. Concentrates generally ranged between 1.2 and 1.6×10^8 cells/ml. Within a 10-month to 1-year period, this three-step process was repeated as many times as needed (never more than four times) before a new agar slant was obtained. Culture temperatures ranged between 22 and 25°C. Lighting consisted of cool white or sunlight spectrum fluorescent lights. Light intensity ranged between 320 (tubes and flasks) and 540 (carboys) lux. Photoperiod was 16 light:8 dark with a 30-minute transition period at dawn and dusk.

Toxicity Tests

7-day short-term chronic toxicity tests with *C. dubia* were conducted following general procedures recommended by the US EPA (1994) except tests were ended after a maximum of 7 days or after all control organisms had released their third brood. Any fourth-brood neonates were not counted. Although blocking by known parentage is only a recent requirement of the EPA (2002), it was employed throughout this study. In addition, the yeast-cerophyl-trout chow feeding suspension was replaced by the algae-Cerophyl, or cereal grass media, diet described above.

Less than 24-hour-old (usually <12-hour-old) *C. dubia* neonates were exposed to copper (as copper nitrate) for durations ranging from 1 minute to 24 hours. 7-day continuous exposure tests were also performed. Nominal concentrations ranged from 1.0 to 1000 µg/L. Over eighty tests were performed, many with overlapping concentration-duration combinations. Exposure scenarios are given in Table 1.1. Food was provided during exposure regardless of duration. Following exposure, organisms were transferred to clean RHW for the remainder of the test period. Adult survival and fecundity (number of neonates per female) were assessed each day prior to renewals. Adult growth (as dry weight) was assessed after 7 days. Adults were rinsed with deionized water and placed in individual wells of porcelain well plates. Organisms were then dried at 60°C for 24 hours, allowed to cool to room temperature in a dessicator and weighed using a Cahn C-31 microbalance. Individual organisms were transferred to the balance using the tip of a #0 paint brush.

Table 1.1. Exposure concentration-duration combinations examined in this study.

Exposure Duration	Copper (µg/L)[1]
1 min	350, 400, 500, 600, 800, 1000
2 min	350, 400, 500, 600, 800, 1000
3 min	500, 600, 800, 1000
4 min	500, 600, 800, 1000
5 min	300, 350, 400, 410, 420, 430, 440, 450, 500
10 min	250, 300, 350, 400
15 min	225, 250, 300, 350, 400
20 min	250, 300, 350
25 min	250
30 min	200, 225, 250, 300
40 min	225
1 h	200, 225, 250, 300
3 h	150, 180, 190, 200, 210, 225, 250
6 h	110, 120, 130, 140,150, 175, 200, 225, 250
12 h	150, 175, 200, 225
24 h	20, 40, 60, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300
7 d	1.0, 3.0, 6.0, 12, 25, 50, 100, 120

[1] Nominal concentrations.

Statistical Analyses

Data were analyzed using Toxstat Version 3.4 following guidelines of the EPA (1994). Differences were accepted at $\alpha \leq 0.05$. Where assumptions of normality or homogeneity of variance were not met, Dunnett's test was performed in addition to non-parametric analyses in order to estimate percent minimum significant differences (PMSDs). It is important to note here that the EPA has set lower (13%) and upper (47%)

PMSD limits for the 7-day short-term chronic test with *Ceriodaphnia dubia* (EPA, 2001b and c).

Test Solutions and Analyses

Copper stock solutions were prepared in Milli-Q water using certified atomic absorption spectrometry standard solutions. Test solutions were prepared in RHW. Milli-Q stock solution concentrations and volumes used were chosen such that water quality parameters (conductivity, pH, hardness and alkalinity) of RHW were not altered. Samples of RHW test solutions were acidified to a pH ≤2 and analyzed by atomic absorption spectrometry (Varion SpectrAA 600 with graphite tube atomizer, GTA-100).

Results

Measured concentrations of copper were generally very close to nominal values (Table 1.2). The vast majority of measured concentrations were within 8% of nominal. In only one test (400-450 µg/L for 5 minutes) did the measured and nominal concentrations disagree with regards to the observed concentration response. In that test, the concentration response was confirmed by measured values.

Survival was adversely affected by increasing concentration-decreasing duration combinations (Table 1.3). Most mortality occurred within 24 hours post-exposure, however, some mortality was observed after 48 hours. Only periodically was mortality observed beyond 48 hours. Immobile organisms were transferred for at least two additional days, and recovery was never observed in that time. The NOEC and LOEC for 24-hour exposure were 92 and 112 µg/L, respectively. Surprisingly, these values

Table 1.2. Nominal versus measured copper concentrations (µg/L).

	Measured Concentration			
Nominal Conc.	Mean	1 SD	Range	n[1]
20.0	30.0		na	1
40.0	38.0		na	1
50.0	46.0		na	2
60.0	58.0		na	1
80.0	76.0		na	1
100	92.2	6.11	80-97	6
120	112	7.33	99-117	5
130	130	8.96	121-140	4
140	136	6.22	132-147	5
150	152	8.63	144-169	12
160	155	5.73	149-165	6
180	175	2.88	171-178	5
190	196		na	1
200	202	12.10	182-218	9
225	224	5.54	217-230	6
250	251	18.78	235-278	4
300	283	10.50	273-294	3
350	341		328-354	2
400	394		374-413	2
410	388		na	1
420	417		na	1
430	398		na	1
440	412		na	1
450	469		na	1
500	492		na	1
600	nd		na	1
800	nd		na	1
1000	nd		na	1

[1] n = number of toxicity tests where concentrations were measured. Atomic absorption analyses were performed with three replicates per sample, per test. na = not applicable, nd = not determined.

Table 1.3. No observed effect- and lowest observed effect durations for *C. dubia* survival, fecundity and growth at given copper concentrations (µg/L).

	Survival		Fecundity		Growth	
Conc.[1]	NOET	LOET	NOET	LOET	NOET	LOET
40.0			24 h		24 h	
60.0				24 h		24 h
80.0						
100	24 h					
120		24 h	6 h		6 h	
130				6 h		6 h
140						
150	6 h		3 h		3 h	
160		6 h		3 h		3 h
190	3 h					
200		3 h	2 h	2.5 h	2 h	2.5 h
225	30 min	40 min	<30 min[2]	30 min	30 min	30-40 min[2]
250	20 min	25 min	20 min	20-25 min[2]	15 min	20 min
300	15 min	20 min	10 min	15 min	10 min	15 min
350	15 min	20 min	10 min	15 min	15 min	15-20 min
400	5 min	10 min	1 min	5 min	1 min	5 min
412	5 min					
417		5 min				
500	2 min	3 min	1 min	2 min	1 min	2 min
600	1 min	2 min	<1 min[2]	1 min	<1 min[2]	1 min
800	<1 min[2]	1min				
1000		<1 min[3]				

[1] Nominal concentrations. 412 and 417 are measured values.
[2] Not determined. Listed effect time is assumed.
[3] 100% mortality was observed at 1 minute, before transfer to clean water.

were almost identical to the NOEC and LOEC for 7-day continuous exposure (94 and 121 µg/L, respectively) suggesting 24-hour exposures with food and up to a 72-hour post-exposure observation period may be predictive of 7-day continuous exposures.

Fecundity and growth of *C. dubia* were also adversely affected by increasing concentration-decreasing duration combinations (Table 1.3) – much in the same way as survival. Combinations affecting fecundity were the same as those affecting growth with three exceptions. At 225 and 350 µg/L, fecundity appeared slightly more sensitive than growth, and at 250 µg/L, growth appeared more sensitive. The LOEC for fecundity following a 24-hour exposure was 58 µg/L. Again, this value is close to that for 7-day continuous exposure (46 µg/L). NOECs for 7-day and 24-hour exposures were 24 and 38 µg/L, respectively.

LOECs in *C. dubia* 7-day short-term chronic tests are traditionally based on significant reduction in total number of neonates after three broods. Such is also the case here, however, reductions in specific broods were noted. Effective combinations nearly always resulted in reduced neonate production in all three broods. Third brood production however, was often more similar to controls suggesting some degree of recovery within the 7-day test period. Many ineffective combinations exhibited recovery following reductions in first or second broods. At lower effective concentrations, regardless of duration, reductions of any one brood were rarely enough to result in significant reductions for the total of all three broods. At higher effective concentrations, reductions in all three broods were more pronounced, especially at combinations nearest those causing significant mortality.

A narrow threshold between lethal and sublethal effects was observed. For example, LOECs for 24-hour exposures were 112 and 58 µg/L for survival and fecundity, respectively. Percent minimum significant differences reached as low as 7% and as high as 40%, however, they generally ranged between 15 and 25%. This

threshold appeared fairly consistent with increasing concentration and subsequent decrease in duration. For any given duration, a slight concentration-response was observed in fecundity and growth before mortality occurred. The concentration-response was also apparent after combinations affecting survival. Survivors of the LOEC-Ts for survival usually reproduced similar to those of lower concentrations and sometimes even similar to controls. Survivors of concentrations higher than LOECs for survival were much less fecund. Effect combinations where there were less than three survivors usually hardly reproduced at all.

Relationships between concentration and duration were very similar for survival, fecundity and growth. NOECs and LOECs for fecundity and growth also increased logarithmically with respect to logarithmic decreases in duration. Differences in concentration between effective durations were relatively small at lower concentration-longer duration combinations and greater at higher concentration-shorter duration combinations. LOECs for survival (Figure 1.1) were 200 and 112 µg/L for 3- and 24-hour exposures, respectively. LOECs for 40- and 1-minute exposures were 225 and 800 µg/L, respectively. LOECs for fecundity and growth (Figures 1.2 and 1.3) were 160 and 58 µg/L for 3- and 24-hour exposures, respectively. LOECs for 2.5-hour and 1-minute exposures were 200 and 600 µg/L, respectively.

Data generated from this type of research can also be used to illustrate relationships between an exposure concentration for numerous durations or an exposure duration for numerous concentrations (e.g., Figures 1.4 and 1.5). If percent reduction in neonate production is plotted against concentration or duration, a trend is realized regardless of combination. No effects result from neonate reduction of ≤10 to

15%. Effects on reproduction result from 10 to 40% reduction. Finally, effects on survival will be observed with neonate reductions of >40%.

Discussion

In an extensive review of the effects of intermittent exposures, Handy (1994) stated toxicants such as copper and cadmium are considerably less toxic with small reductions in exposure duration when peak concentrations are the same. This suggests that any small change in duration would correspond to large changes in toxicity. Although this study did not produce effect combinations of equal peak concentration, results obtained here agree with this at higher concentration-lower duration but not at lower concentration-longer duration combinations. Regardless of endpoint, at lower concentrations (<200 µg/L), large decreases in duration corresponded to small increases in effective concentration (i.e., duration appeared to be less important). On the other hand, at higher concentrations (>300 µg/L), slight decreases in duration corresponded to large increases in effective concentration (duration appeared to be more important).

Sublethal effects of copper on aquatic organisms vary considerably. Of particular interest to this study may be its affect on feeding behavior. Exposure to heavy metals has been reported to depress feeding behavior of daphnids even at concentrations as low as 10 µg/L (Taylor et al., 1998; McWilliam and Baird, 2002; Clément and Zaid, 2004). Impaired feeding has been linked to reduced egg production in Copepods (Stearns et al., 1989). In addition, Munger et al. (1999) reported highest accumulation of cadmium in the gut of *C. dubia* and suggested toxic effects continued at that location. In

this study, growth may have been affected similarly. At effective concentration-duration combinations, release of first brood neonates was not only reduced, it was often delayed by 12 to 24 hours especially at concentrations near the lethal threshold. Aborted eggs or deceased neonates were rarely observed suggesting no developmental effects. In addition, affected adults appeared much smaller and pale (an indication of lower lipid content and poor health) through release of their first brood. By release of their third brood however, affected adults were still visibly smaller, but neonate production was usually closer to and their typical amber color could not be visibly discerned from controls – clear evidence of recovery. Sublethal effects from even the shortest exposures likely resulted from either 1) higher accumulation rates due to much higher copper concentrations or 2) continued feeding depression well beyond exposure or a combination of both.

Some of the exposure scenarios presented by this research may not appear environmentally realistic. For example, it seems unlikely that a runoff event will last for 24 hours or that environmental concentrations of copper will reach 1000 µg/L. In fact, such scenarios may be possible if greater than base-flow concentrations linger in waterbodies or the scale of an event is unprecedented. The magnitude and duration of all events would have to be recorded to avoid any uncertainty.

The US EPA has stated that runoff pollution was a major water quality problem in our nation (EPA, 1994). EPA reported that 87% of 246 major waterbodies in the United States were affected by non-point source pollutants (NURP, 1993 in Walker et al., 1999). Metals, in particular, were considered to be the largest contributor to runoff pollution. Cole et al. (1984) reported that several metals were often found in runoff at

concentrations greater than their respective water quality criteria. Copper, lead and zinc were detected in 75% of Nationwide Urban Runoff Program (NURP) samples. Freshwater acute and chronic criteria for copper were exceeded 50 and 87% of the time, respectively. Heavy metal pollution in urban runoff is rivaled only by suspended sediment loads, biological and chemical oxygen demand, and coliforms (Cheebo et al., 2001; Scholes et al., 1998).

Several generalizations can be made regarding metals in runoff. First, lead, zinc, copper and cadmium are the heavy metals most frequently found. Concentrations of copper and zinc are usually the highest, with zinc often being orders of magnitude above the others. Concentrations reported in the literature are zinc (20 – 5000 µg/L) > copper ≈ lead (5 – 200 µg/L) > cadmium (<12 µg/L) (Davis et al., 2001). Walker et al. (1999) also reported a range of copper concentrations between 0.06 and 1410 µg/L. Second, heavy metal contamination in runoff is most often associated with suspended particulate matter. Significant positive correlation between suspended sediment and heavy metal transport has been reported during rain events (Yuan et al., 2001). However, suspended sediment-associated metals can become available during transport. Cheebo et al. (2001) noted changes in chemical form of metals during transport in combined sewer system overflows. In a study on the dispersion plume of a major municipal effluent, Gagnon and Saulnier (2003) observed highly variable partitioning of metals between dissolved and particulate phases. Third, landuse types most responsible as metal sources are industrial > commercial > residential > open space. Fourth, highways and roadways may be the single largest contributing source of heavy metal pollution in urban runoff. Legret and Pagotto (1999) examined runoff from a

major rural highway over a year-long study encompassing 50 rain events. Total copper concentrations in raw runoff waters ranged from 11 to 146 µg/L with a mean of 45 µg/L. Mean dissolved copper concentration in raw waters was 25 µg/L and ranged from 7.3 to 139 µg/L. Sediment copper concentrations ranged between 20 and 260 mg/kg. The authors calculated a surface loading of 0.29 kg/km (1 km of a dual carriageway including hard shoulder covering 1.165 ha). Automobile brake linings (142,000 mg Cu/kg) were determined to be the primary source for copper in highway runoff. Gromaire et al. (2000) reported that pollutant loads removed by street cleaning waters were comparable to one rain event for suspended sediments and organic matter but were five times lower for heavy metals. Median copper values were 0.12 and 0.71 mg/m^2 street for street washing waters and runoff waters, respectively. Respective particle-bound copper concentrations were 200 and 500 mg/kg. Barbosa and Hvitved-Jacobsen (1999) reported an average dissolved copper concentration of 10.7 µg/L from a highway with average daily traffic of 6000 vehicles. Dissolved copper concentrations ranged from <1 to 54 µg/L, and the average event mean concentration was 24.1 µg/L. Hares and Ward (1999) examined a motorway with average daily traffic of 140,000 vehicles and observed a total copper concentration of 274 µg/L. Finally, Sörme and Lagerkvist (2002) estimated that asphalt and brake linings contributed 11 to 17 and 280 kg Cu/yr, respectively to a waste water treatment plant through stormwater runoff. A fifth generalization is that runoff contamination is greater with more intense, less frequent rain events. Finally, since heavy metals can not be chemically altered or destroyed, they are not likely to disappear as runoff contaminants.

Numerous other sources of copper in runoff have also been investigated. Davis et al. (2001) listed building and roofing materials, vehicle brake emissions and atmospheric deposition as major sources for copper. Synthetic rainwater rinses of unpainted wood yielded the highest copper concentrations of building materials tested (4 to 320 µg/m^2), followed by brick (6 to 280 µg/m^2), painted wood (<1 to 280 µg/m^2) and concrete (<1 to 170 µg/m^2). Metal yielded the lowest copper concentrations (<1 to 4.6 µg/m^2). Roofing sample rinses resulted in mean copper concentrations of 7.5, 200, and 5000 µg/L for residential, commercial and industrial, respectively. Front brake rinses resulted in a mean copper concentration of 280 µg/L. Annual copper loadings were estimated to be 0.038 kg/ha-yr for residential and 0.243 kg/ha-yr for commercial land use types. Sörme and Lagerkvist (2002) estimated that copper roofs contributed an estimated 700-920 kg Cu/yr. Duke et al. (1998) detected zinc and copper in >80% runoff samples from metal plating facilities. Mean copper concentrations were reported to be as high as 390 µg/L. During investigations of abandoned copper and sulfate mines, Gray (1998) measured copper concentrations from mine surface runoff ranging from 1 to 383 mg/L. Calculated zinc+copper toxic units for the receiving river were 3.01 at the mine site and remained >1 10 km downstream. Gray (1998) estimated a copper loading of 6 tons (from all sources) for that year. Larm (2000) monitored material transport into and out of stormwater treatment facilities and estimated a total copper mass loading of 54 kg/yr from five separate subwatersheds. Finally, Ribolzi et al. (2002) reported an average particulate-associated copper concentration of 245 mg/L in runoff following treatment of a vineyard with a copper-based fungicide.

WQC are not applicable to episodic events such as those associated with rain-driven runoff events. However, with no other available benchmarks, predictions may be made using worst-case exposure scenarios as to whether or not they may be problematic. Reconstituted hard water used throughout this work had a hardness of 180 mg/L as $CaCO_3$. Given this, the CMC and CCC for copper would be approximately 24 and 15 µg/L (EPA, 1996), respectively and assumes no water effects ratio. If the CMC is not exceeded, it is clearly protective for combinations up to and including 3 minutes at 500 µg/L. For these, the average concentration over 1 hour is greater than 24 µg/L – effect levels exceed the CMC. At higher concentration-lower duration combinations, the average 1-hour concentration is less than 24 µg/L, however, significant mortality was observed within 48 hours. Combinations affecting reproduction and growth are deceptive. Although all effective combinations were ≤15 µg/L when averaged over 4 days, the CCC would not have been protective – effect levels were below the CCC. Given reported environmental concentrations above, effect concentrations observed in these studies are possible, and WQC may be exceeded.

There are still no actual regulations for intermittent or single pulse events, but only recommendations as to how to reduce their impact. Single chemical numeric criteria would be inappropriate as exposure to multiple toxicants likely occurs during storm events. It may in fact, be impossible to develop regulations for individual single events because each is site and event specific. We can however, study them as predictive tools. Such efforts may be the best way to study results of best management practices implemented to reduce the impact of runoff. In addition, most WQC have allowable exceedances in terms of flow. This can not apply to stormwater events

because these WQC presume no flows (including storms) during periods of drought (Novotny and Witte, 1997).

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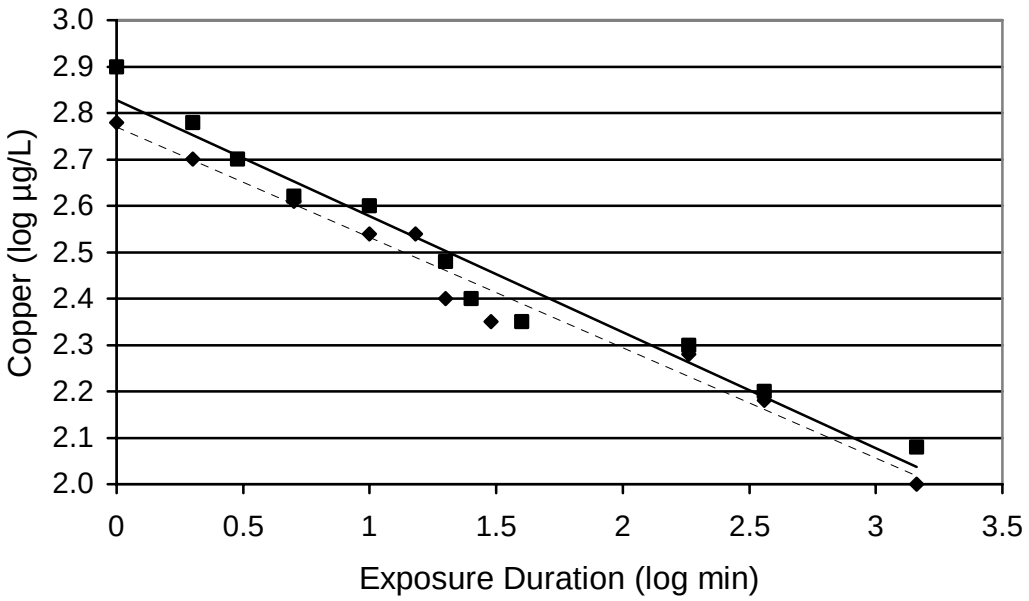

Figure 1.1. No- (diamonds) and lowest (squares) observed effect concentration-duration combinations for *C. dubia* survival. Trendlines are also shown as dashed and solid, respectively. NOEC-T: $R^2 = 0.9734$, log y = -0.2382 logx + 2.771; LOEC-T: $R^2 = 0.9633$, log y = -0.2502 logx + 2.8275.

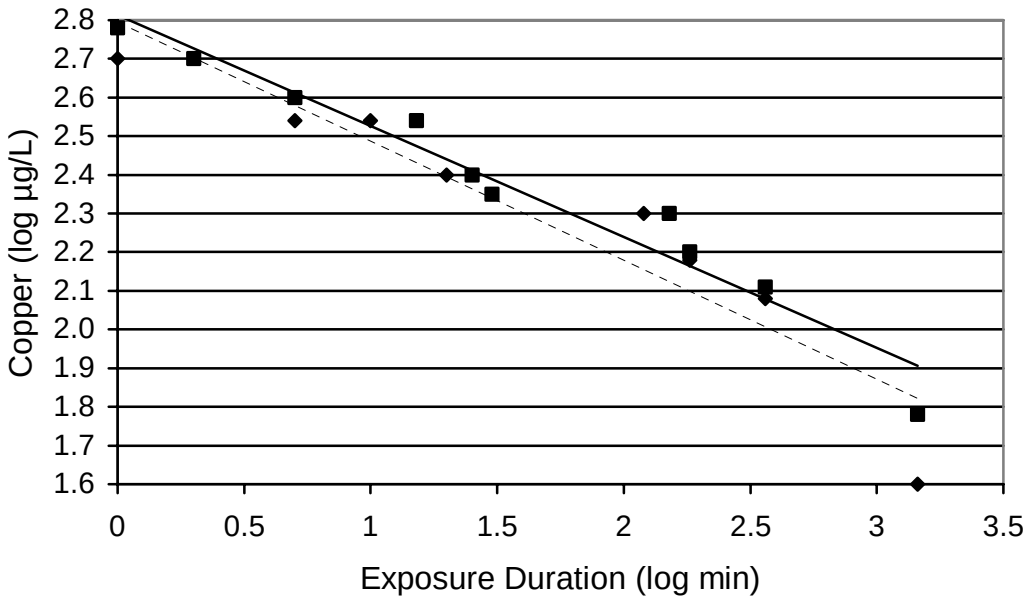

Figure 1.2. No- (diamonds) and lowest (squares) observed effect concentration-duration combinations for *C. dubia* fecundity. Trendlines are also shown as dashed and solid, respectively. NOEC-T: $R^2 = 0.8857$, log y = -0.3077 logx + 2.7949;
LOEC-T: $R^2 = 0.9522$, log y = -0.2870 logx + 2.8128.

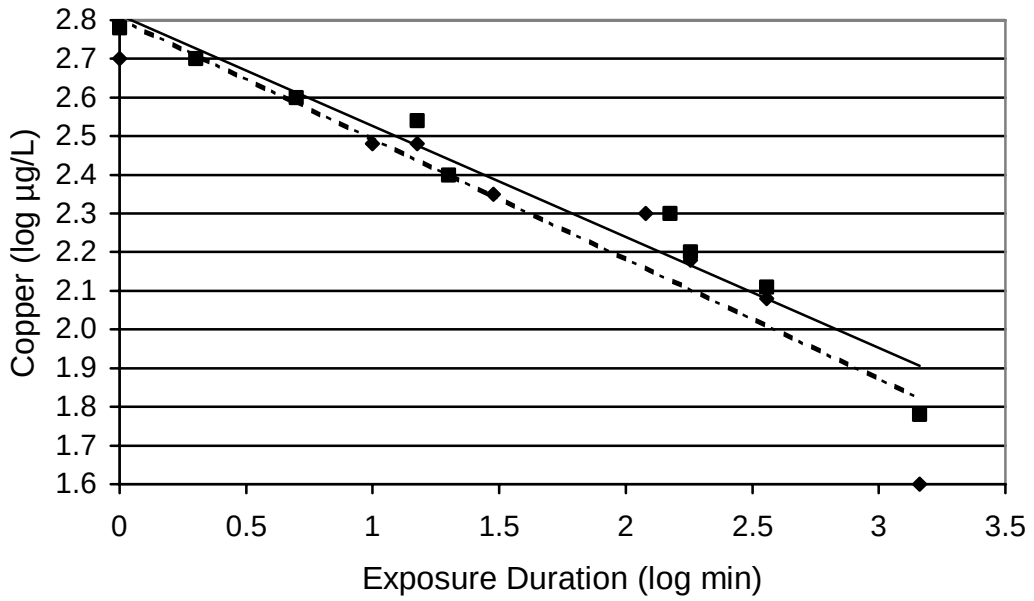

Figure 1.3. No- (diamonds) and lowest (squares) observed effect concentration-duration combinations for *C. dubia* growth. Trendlines are also shown as dashed and solid, respectively. NOEC-T: $R^2 = 0.8797$, log y = -0.3114 logx + 2.8046; LOEC-T: $R^2 = 0.9528$, log y = -0.2867 logx + 2.8129.

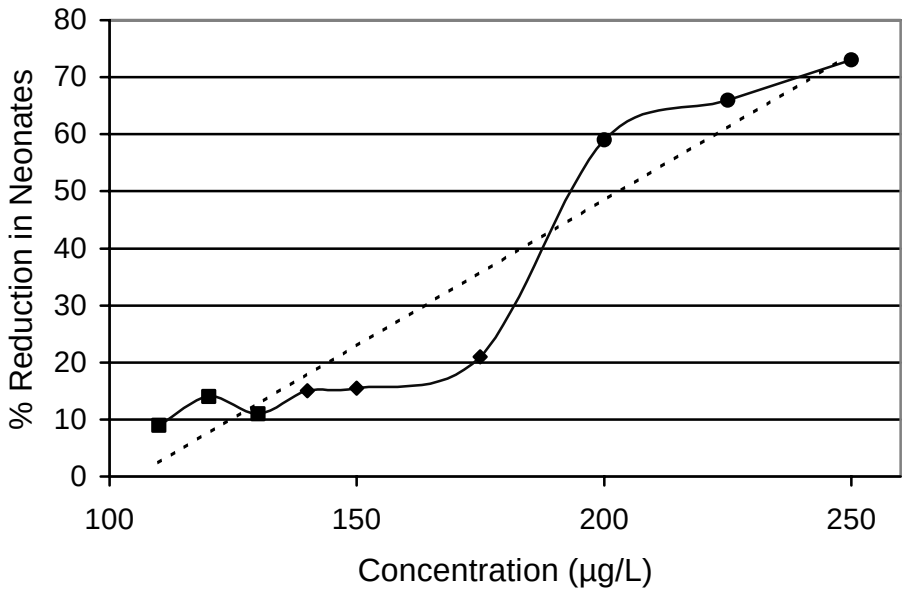

Figure 1.4. Relationship between concentration and percent reduction in neonate production with 6-hour exposures. No effect (squares), fecundity effect (diamonds) and survival effect concentrations (circles) are shown. $R^2 = 0.9098$, $y = 0.5117x – 53.781$.

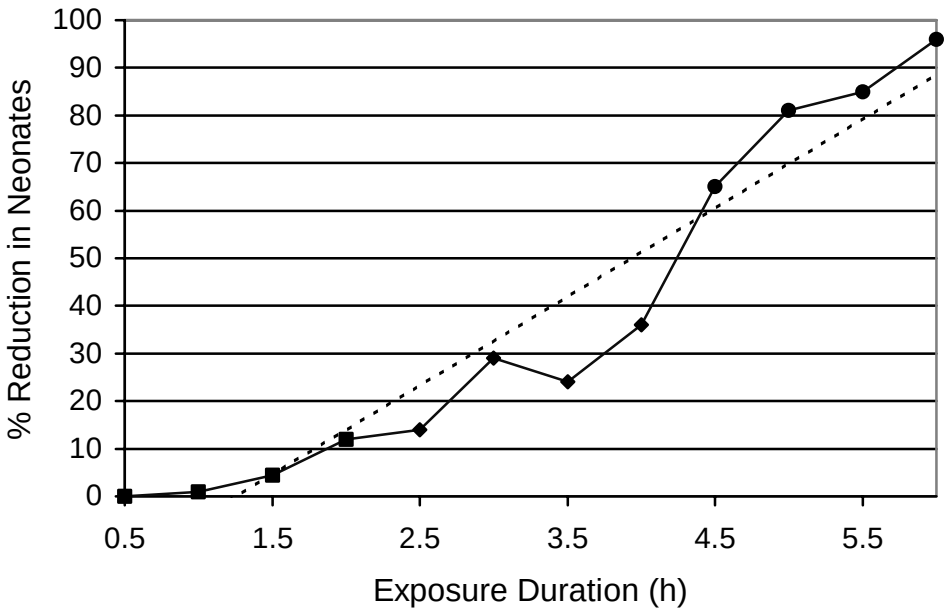

Figure 1.5. Relationship between duration and percent reduction in neonate production with exposure to 200 µg/L copper. No effect (squares), fecundity effect (diamonds) and survival effect concentrations (circles) are shown. $R^2 = 0.9179$, $y = 18.696x – 23.47$.

CHAPTER 2

EFFECTS OF SINGLE EPISODIC EXPOSURE TO COPPER ON *Ceriodaphnia dubia*: ADULT RECOVERY AND EFFECTS ON SECOND GENERATION INDIVIDUALS

Introduction

Traditional toxicity tests are generally based on assumptions of continuous exposure. However, exposures due to rain-driven runoff events, periodic pesticide application and accidental spills are not continuous. Exposures of this type occur episodically as intermittent (repeating) events or as a single pulse. It is important to understand the long-term toxicological impacts of episodic pollution incidents to protect aquatic organisms. Otherwise, regulatory agencies may fail to associate a decline in populations with a particular incident because of its brevity (Handy, 1994).

Episodic pollution may have no detectable effects, or it may lead to any observable array of sublethal effects to mortality (McCahon and Pascoe, 1990). Minor ecological perturbations or serious degradation can result from a single event. Because effects observed during an event are not different from continuous exposure (of given duration), most studies are interested in post-exposure effects. Seager and Maltby (1989) recommended that any study of episodic pollution include post-exposure periods. Post-exposure effects may include effects continued from initial exposure or effects not observed during initial exposure (latent effects). Recovery is also an important endpoint to consider in studies of episodic pollution. Although the use of recovery as an endpoint in toxicity testing was first recognized by Wright (1976), it is still generally overlooked in continuous-exposure designs. Ecological risk assessments of

episodic pollution would undoubtedly be enhanced with knowledge of population recovery.

Latent effects and recovery may be more important in the context of single-pulse events, however, they are also relevant to intermittent exposures provided enough time has elapsed between pulses. Latency can probably be assumed in all cases. It is the degree which must be explored (Reinert et al., 2002). Duration of time between pulses (sometimes called recovery interval) varies considerably depending on the exposure profile but is critical in determining whether natural populations will suffer. van der Hoeven and Gerristen (1997) reported that chlorpyrifos immobilized *Daphnia pulex* several days before actual death. Even when exposure was discontinued, immobilized daphnids died. Naddy and Klaine (2001) did not observe latent effects in *D. pulex* surviving initial chlorpyrifos exposure. They reported longer recovery intervals (≥72 h), and daphnids of the van der Hoeven and Gerristen experiments may have accumulated more toxicant. Hurd et al. (1996) observed latent effects of pesticide application on stream macroinvertebrates only after molting began – two to four weeks after exposure.

While many studies have been published examining organism recovery following runoff events (e.g., Schaefer et al., 1981; Liess and Schulz, 1999) accidental contaminant spills (e.g., Heimbach et al., 1996; Solà et al., 2004) and other episodic events (e.g., Parsons and Surgeoner, 1991; McHenery and Davies, 1996), fewer investigators have examined latent effects or recovery in aquatic invertebrates from pulse exposure to metals in single-species toxicity tests. McCahon and Pascoe (1991) reported reduced and delayed emergence of *Chironomus riparius* following single pulses of cadmium at 10% and 100% the 24-hour LC50s for first and fourth instar

larvae. Brent and Herricks (1998) observed delayed effects resulting in significantly increased immobility of *Hyalella azteca* and *Ceriodaphnia dubia* exposed to single pulses of zinc and cadmium. Zhao and Newman (2004) investigated latent mortality of *Hyalella azteca* exposed to copper. Post-exposure observation revealed 65 to 85% mortality after 48-hour exposures rather than the expected 50%. De Pirro et al. (2001) examined cardiac activity of Mediterranean limpets (*Patella rustica*, *P. caerulea* and *P. aspera*) following 6-hour exposures to copper. Even acardic individuals exhibited rapid recovery when returned to clean seawater. Gregory et al. (2002) noted increased filtration rates and recovery of gill filament histopathologies in the African rock mussel (*Perna perna*) following exposure to mercury.

Studies of contaminant effects on second generation individuals are numerous where they and first generation individuals were exposed (e.g., Hollister et al., 1980; Holdway and Dixon, 1986; Münzinger, 1990; Sánchez et al., 2000; Watts et al., 2001; Morris et al., 2003; Bossuyt and Janssen, 2004; and Muyssen and Janssen, 2004). Only two (to the best of the authors knowledge) however, investigated effects on unexposed second generation individuals following exposure of the first generation. Villarroel et al. (1999) examined feeding behavior of second generation *Daphnia magna* following parental exposure to the acaricide, tetradifon. Feeding rates of second generation individuals (first and third brood) were significantly reduced though to a lesser degree than that of parents. Barry et al. (1995) observed no significant effects on larvae growth of second generation Australian crimson-spotted rainbow fish (*Melanotaenia fluviatilis*) following parental pulse exposure to esfenvalerate. Lower survival, though not significant, was noted.

In the development of criterion maximum concentrations (CMCs) and criterion continuous concentrations (CCCs), the US Environmental Protection Agency (EPA) suggests an exceedance frequency of once every three years (EPA, 1991). This frequency is based in part on the potential of populations to recover following a pollution event, however, studies of recovery following single pulse events in nature are not likely to last three years without incidence of additional events. The risk of additional events also precludes practical study of unexposed second generation individuals in the field. Objectives of this research were to utilize a modified version of the 7-day short-term chronic toxicity test with *Ceriodaphnia dubia* to assess reproductive recovery of adult daphnids following single episodic exposures to copper and examine reproductive effects on unexposed second generation individuals. Research presented here may have applications in ecological risk assessments on single pulse heavy metal pollution events and subsequent population recovery.

Materials and Methods

A detailed description of *Ceriodaphnia dubia* and *Pseudokirchneriella subcapitata* cultures and performance of 7-day short-term chronic tests can be found in chapter 1. Exposure scenarios presented in this chapter are summarized in Table 2.1.

Adult Recovery and Effects on Second Generation Individuals

Reproductive recovery of adults was assessed simply by continuing clean water daily renewals for up to three weeks or ten broods. Second generation experiments were initiated just as the 7-day short-term chronic test without an exposure period.

Table 2.1. Exposure scenarios utilized in this research.

Exposure Duration	Copper (µg/L)[1]
1, 2, 3, 4 min	600
5 min	400, 410, 420, 430, 440, 450
3 h	180, 190, 200, 210
24 h	20, 40, 60, 80, 100, 120,
24 h	80, 100, 120, 140, 160, 180

[1] Nominal concentrations.

Briefly, individual neonates from the first, second, or third brood were transferred to cups containing RHW and algae suspension. Daily renewals were performed through production of third generation neonates. Blocking by known parentage was maintained where possible. Where an adult had died however, individual neonates from all remaining parents were pooled before assignment.

Statistical Analyses

Data were analyzed using Toxstat Version 3.4 (University of Wyoming, 1994) following guidelines of the EPA (2002). For comparison, fecundity data were also analyzed with surviving adults only where survival was not significantly affected. Differences were accepted at $\alpha \leq 0.05$. Where assumptions of normality or homogeneity of variance were not met, Dunnett's test was performed in addition to non-parametric analyses in order to estimate percent minimum significant differences (PMSDs).

Test Solutions and Analyses

Atomic adsorption analyses were performed as described in Chapter 1 using three replicates per sample, per concentration.

Results

Effects on First Generation: 7-day (baseline) Test

Measured concentrations of copper were generally very close to nominal values (Table 2.2). The vast majority of measured concentrations were within 8% of nominal. In only one test (400-450 µg/L for 5 minutes) did the measured and nominal concentrations disagree, however, the observed concentration response was confirmed by measured values.

Survival of first generation *C. dubia* were adversely affected by increasing concentration-decreasing duration combinations. Lowest observed effect concentrations (LOECs) for 24-hour and 1-minute exposure were 93 and 600 µg/L, respectively (Table 2.3). Most mortality occurred within 24 hours post-exposure, however, some mortality was observed after 48 hours. Immobile organisms were transferred for at least two additional days, and recovery was not observed in that time.

Fecundity of *C. dubia* was also adversely affected by increasing concentration-decreasing duration combinations – much in the same way as survival. LOECs for 24-hour and 1-minute exposures were 58 and 600 µg/L, respectively (Table 2.3). At effective (herein defined as significantly lower than controls) concentration-duration combinations, release of first brood neonates was not only reduced, it was often

Table 2.2. Nominal versus average measured copper concentrations (µg/L).

Nominal Conc.	Measured
20.0	30.0
40.0	38.0
60.0	58.0
80.0	77.0
100	93.0, 95.0
120	116
140	133
160	150
180	178, 171
190	196
200	218
210	233
400	374
410	388
420	417
430	398
440	412
450	469
600	nd

nd = not determined

delayed by 12 to 24 hours especially at concentrations near the lethal threshold. Reproduction was almost invariably delayed by one to three days at concentrations resulting in significantly reduced survival. The observed relationship between concentration and duration for survival and fecundity is illustrated in Figure 2.1.

Table 2.3. No- and lowest observed effect concentrations determined from 7-day short-term chronic tests with first generation *C. dubia*.

	Fecundity		Survival	
Exposure Duration	NOEC	LOEC	NOEC	LOEC
1 min[1]	<600	600	600	>600
2 min[1]			<600	600
5 min	<374	374	412	417
3 h	178	>178	196	218
24 h	38	58	93	116

[1] Nominal concentrations. All others were measured.

Adult Recovery

NOECs and LOECs of *C. dubia* 7-day short-term chronic tests are traditionally based on significant reduction in total number of neonates after three broods. Such is the case here, however, effects on individual broods were also determined. Effective combinations nearly always resulted in significantly reduced neonate production in all three broods. At lower effective concentrations, third brood production was often more similar to controls suggesting some degree of recovery within the 7-day test period. Some ineffective combinations exhibited recovery following significant reductions in first or second broods.

Two 24-hour exposures were performed, however, only one (20 to 120 µg/L) will be discussed here. See Appendix (Table A.1 and Figures A.1-A.3) for data from the 80 to 180 µg/L exposure. *C. dubia* fecundity was unaffected by <58 µg/L copper after ten broods. Table 2.4 shows mean number of neonates produced for each individual brood

and cumulative mean number of neonates produced per each subsequent brood. Concentrations >58 µg/L resulted in significantly reduced neonate production, also through ten broods. Mean neonate production for *individual broods* appeared to recover as they were generally not significantly different after the fifth brood. Mean neonate production at all concentrations steadily increased to the fifth or sixth brood before rapidly declining (Figure 2.2). The expected monotonic concentration-response also remained through five broods. After six broods however, neonate production from higher concentrations were often greater than that of lower concentrations.

Cumulative mean number of neonates per subsequent brood showed little sign of recovery. 58 µg/L was the only effective concentration where the total of ten broods was not significantly less than controls (Table 2.4). More time would not likely have made a difference as ten broods (3 weeks) approached the end of adult *C. dubia* expected life span, and mean individual brood production had diminished to between 2.1 and 3.6. Except at the lowest concentrations, a monotonic concentration response was observed throughout ten broods (Figure 2.3) – evidence against recovery of total neonate production.

EPA guidelines recommend not including concentrations where there is a significant effect on survival in analyses for reproduction. In the context of recovery however, a closer look at those groups may be warranted. 24-hour exposure to 114 µg/L copper resulted in 80% mortality of *C. dubia*, yet fecundity of the two surviving individuals was higher than all other concentrations. Cumulative mean neonate production was 104.4 and 97.0 for controls and 114 µg/L, respectively. Figure 2.4 offers

Table 2.4. Mean (1 SD) number of neonates produced for each individual brood and cumulative mean number per each subsequent brood following 24-hour exposure.

Brood	Concentration (µg/L)							
	RHW	MSD (PMSD)[1]	30	38	58	76	95	114[2]
1	3.6 (0.84)	0.9 (24.5)	3.7 (0.67)	3.7 (0.48)	3.4 (0.70)	1.7 (0.48)	1.6 (1.51)	0.6 (1.26)
2	8.8 (1.32)	1.5 (17.0)	9.1 (0.88)	9.0 (1.15)	7.2 (0.79)	5.2 (1.03)	4.3 (2.67)	1.4 (2.99)
1-2	12.4 (1.90)	2.6 (23.3)	12.8 (1.48)	12.7 (1.34)	10.6 (1.26)	6.9 (1.10)	5.9 (3.87)	2.0 (4.24)
3	12.6 (1.07)	2.3 (18.0)	11.9 (1.60)	12.2 (1.55)	11.0 (1.05)	9.1 (1.66)	7.3 (4.22)	2.1 (4.48)
1-3	25.0 (2.16)	3.6 (14.4)	24.7 (1.42)	24.9 (2.02)	21.6 (2.07)	15.8 (2.15)	13.2 (7.86)	4.1 (8.72)
4	13.4 (2.37)	2.6 (19.2)	13.3 (0.82)	13.2 (1.55)	11.3 (1.25)	10.8 (1.23)	9.4 (5.04)	2.6 (5.56)
1-4	38.4 (3.13)	6.0 (15.6)	38.0 (1.41)	38.1 (2.81)	32.9 (2.92)	26.4 (3.63)	22.6 (12.64)	6.7 (14.28)
5	15.5 (1.58)	2.9 (18.5)	15.1 (1.60)	15.5 (1.58)	14.1 (1.66)	12.4 (2.01)	10.4 (5.66)	2.5 (5.32)
1-5	53.9 (3.93)	8.4 (15.6)	53.1 (2.42)	53.6 (3.53)	47.0 (3.56)	38.8 (5.07)	33.0 (18.03)	9.2 (19.60)
6	15.6 (1.65)	3.8 (24.3)	14.4 (3.17)	13.2 (4.71)	14.3 (1.83)	14.3 (2.26)	10.7 (6.13)	2.9 (6.12)
1-6	69.5 (3.37)	11.1 (16.0)	67.5 (4.62)	66.8 (6.84)	61.3 (4.95)	53.1 (4.31)	43.7 (24.00)	12.1 (25.69)
7	12.7 (4.97)	5.4 (42.9)	9.4 (6.26)	12.2 (5.09)	10.6 (4.97)	11.7 (4.37)	7.9 (5.74)	2.1 (4.43)
1-7	82.2 (6.49)	14.3 (17.3)	76.9 (9.36)	79.0 (11.11)	71.9 (5.40)	64.8 (5.29)	51.6 (28.90)	14.2 (30.12)
8	12.3 (4.35)	4.9 (39.8)	8.6 (5.13)	7.1 (6.03)	6.7 (5.17)	9.1 (3.21)	3.5 (3.78)	1.9 (4.07)
1-8	94.5 (9.95)	16.8 (17.7)	85.5 (11.81)	86.1 (13.8)	78.6 (9.98)	73.9 (6.87)	55.1 (31.31)	16.1 (34.18)
9	6.3 (5.03)	4.7 (73.8)	4.5 (4.20)	2.9 (3.90)	3.8 (5.05)	4.2 (3.65)	3.3 (4.55)	1.8 (5.35)
1-9	100.8 (13.30)	19.2 (19.1)	89.0 (13.52)	89.0 (14.92)	82.2 (13.93)	78.1 (9.52)	58.4 (33.54)	17.9 (38.53)
10	3.6 (2.84)	3.9 (97.7)	3.1 (2.73)	2.1 (2.02)	4.3 (5.44)	2.8 (3.58)	2.8 (3.74)	1.5 (3.57)
1-10	104.4 (14.82)	22.0 (20.6)	92.1 (15.99)	91.1 (15.91)	86.5 (17.75)	80.9 (12.03)	61.2 (35.52)	19.4 (41.97)

Values highlighted were significantly less than controls.
Thick bold line after broods 1-3 denotes results at the end of the traditional 7-day test.
[1] MSD = minimum significant difference. PMSD = percent MSD.
[2] LOEC for survival (20%). Not included in analyses for reproduction.

a comparison between cumulative mean neonate production including and excluding deaths.

Trends in individual brood and cumulative neonate production recovery were generally the same regardless of exposure durations ≥5 minutes. Neonate production for individual broods appeared to recover by the fifth brood. Durations shorter than 3 hours however, revealed faster recovery of cumulative mean neonate production as duration decreased. Cumulative mean neonate production from the 3-hour exposure did not recover after five broods (Table 2.5, Appendix Figures A.4-A.6). Although not significantly reduced at 178 and 196 µg/L, survival was 70 and 80%, respectively. This resulted in considerable variability relative to controls. The 3-hour exposure experiment was therefore terminated after five broods with the assumption that recovery would not occur. After five broods, brood size was expected to decrease and variability was expected to increase (a trend also observed from 24-hour exposure). In addition, no changes in significance resulted when data were analyzed with survivors only.

Cumulative mean neonate production following 5-minute exposure to concentrations <412 µg/L were not significantly different from controls after five broods (Table 2.6, Appendix Figures A.7-A.9.) Lack of differences at 388 and 398 µg/L may be explained by the variability associated with at least two mortalities in every treatment except 374 µg/L. Again, analyses with survivors only revealed no changes in significantly effective concentrations.

Table 2.5. Mean (1 SD) number of neonates produced for each individual brood and cumulative mean number per each subsequent brood following 3-hour exposure.

	Concentration (µg/L)					
Brood	RHW	MSD (PMSD)[1]	178	196	218[2]	233[2]
1	3.9 (0.57)	1.1 (28.7)	1.9 (1.66)	1.7 (1.25)	1.4 (1.35)	0.4 (0.84)
2	10.2 (0.92)	2.6 (25.4)	4.3 (3.50)	4.6 (3.44)	2.4 (3.13)	0.6 (1.90)
1-2	14.1 (1.37)	3.6 (25.7)	6.6 (4.99)	6.3 (4.67)	3.8 (4.37)	0.6 (1.90)
3	14.4 (1.58)	4.5 (31.1)	8.3 (5.98)	8.3 (6.00)	0.0 (na)	0.9 (2.85)
1-3	28.5 (2.84)	8.1 (28.2)	14.9 (10.94)	14.6 (10.62)	3.8 (4.37)	1.9 (5.34)
4	13.7 (0.94)	4.8 (35.2)	9.1 (6.57)	9.3 (6.52)	8.4 (7.31)	2.6 (5.50)
1-4	47.2 (2.78)	12.7 (30.0)	24.0 (17.33)	23.9 (16.97)	20.1 (17.50)	7.0 (15.03)
5	12.9 (1.45)	5.3 (41.4)	10.3 (7.30)	9.9 (7.09)	9.0 (7.82)	3.1 (6.54)
1-5	56.1 (3.99)	17.9 (31.9)	34.3 (24.55)	33.8 (23.84)	29.1 (25.16)	10.1 (21.51)

Values highlighted were significantly less than controls.
Thick bold line after broods 1-3 denotes results at the end of the traditional 7-day test.
[1] MSD = minimum significant difference. PMSD = percent MSD.
[2] Significant effect on survival (40 and 20%, respectively). Not included in analyses for reproduction.

600 µg/L exposure had a significant effect on *C. dubia* fecundity following 1-minute exposure and survival after 2-minutes. *C. dubia* exposed to 600 µg/L for 3 and 4 minutes died within 48 hours. Like other effective combinations, individual brood neonate production for 1-minute exposure appeared to recover by the fifth brood (Table 2.7, Appendix Figures A.10-A.12). Unlike other effective combinations however, cumulative mean neonate production after five broods recovered at the highest effective combination not affecting survival. This held true through eight broods.

Table 2.6. Mean (1 SD) number of neonates produced for each individual brood and cumulative mean number per each subsequent brood following 5-minute exposure.

Brood	Concentration (µg/L)							
	RHW	MSD (PMSD)[1]	374	388	398	412	417[2]	469[2]
1	4.5 (0.85)	1.6 (35.6)	3.8 (1.03)	3.5 (1.90)	2.9 (1.66)	2.6 (2.17)	2.9 (2.60)	1.8 (2.04)
2	9.8 (1.14)	3.1 (32.1)	8.2 (1.40)	7.2 (3.88)	7.0 (3.86)	5.3 (4.06)	5.4 (4.74)	4.3 (4.64)
1-2	14.3 (1.64)	4.6 (32.0)	12.0 (2.11)	10.7 (5.72)	9.9 (5.42)	7.9 (5.99)	8.3 (7.20)	6.1 (6.66)
3	13.4 (1.35)	4.4 (32.8)	11.1 (2.23)	10.3 (5.50)	9.5 (5.19)	8.2 (5.75)	8.1 (7.05)	5.9 (6.24)
1-3	27.7 (2.03)	8.8 (31.7)	23.1 (3.98)	21.0 (11.13)	19.4 (10.54)	16.1 (11.49)	16.4 (14.20)	12.0 (12.84)
4	14.8 (1.03)	5.0 (33.7)	13.3 (1.83)	10.9 (5.78)	12.3 (6.58)	9.5 (6.64)	8.2 (7.63)	7.6 (8.03)
1-4	41.5 (4.25)	13.7 (32.9)	36.4 (5.12)	31.9 (16.86)	31.7 (16.93)	25.6 (17.95)	24.6 (21.58)	19.6 (20.81)
5	14.2 (1.23)	5.4 (37.7)	13.1 (3.76)	11.2 (6.07)	11.4 (6.28)	10.3 (7.23)	7.4 (7.81)	7.1 (7.59)
1-5	55.8 (4.32)	18.6 (33.3)	49.5 (6.52)	43.1 (22.79)	43.1 (23.06)	35.9 (25.08)	32.0 (28.85)	26.7 (28.37)

Values highlighted were significantly less than controls.
Thick bold line after broods 1-3 denotes results at the end of the traditional 7-day test.
[1] MSD = minimum significant difference. PMSD = percent MSD.
[2] Significant effect on survival (60 and 50%, respectively). Not included in analyses for reproduction.

Trends in neonate production of concentrations significantly affecting survival were similar regardless of duration. Although such populations would not be expected to recover, cumulative mean neonate production of survivors was usually very similar to controls. Cumulative mean fecundity of survivors from 3-hour exposure to 218 and 233 µg/L was 48.5 (3.21) and 50.5 (9.19), respectively after five broods. Cumulative mean fecundity of survivors from 5-minute exposure to 417 and 469 µg/L was 53.3 (11.52) and 53.4 (5.36), respectively after five broods. Finally, cumulative mean fecundity of

Table 2.7. Mean (1 SD) number of neonates produced for each individual brood and cumulative mean number per each subsequent brood following 600 µg/L exposures.

	Duration (600 ug/L)			
Brood	RHW	MSD (PMSD)[1]	1 min	2 min[2]
1	3.8 (0.79)	0.7 (17.4)	3.4 (0.84)	1.1 (1.10)
2	8.4 (1.07)	0.7 (8.2)	8.2 (0.79)	3.4 (3.17)
1-2	12.2 (1.55)	1.0 (8.2)	11.6 (1.43)	4.5 (4.25)
3	14.4 (1.43)	1.2 (8.1)	12.6 (2.07)	5.9 (5.45)
1-3	26.6 (2.95)	1.8 (6.8)	24.2 (3.08)	10.4 (9.62)
4	16.5 (0.71)	1.3 (8.0)	15.6 (2.95)	8.8 (7.61)
1-4	43.1 (2.86)	2.4 (5.5)	39.8 (4.44)	19.2 (16.97)
5	15.2 (2.04)	1.4 (9.1)	16.6 (1.96)	9.8 (8.79)
1-5	58.3 (2.10)	2.3 (4.0)	56.4 (3.24)	29.0 (25.61)
6	15.4 (2.46)	1.7 (11.0)	16.0 (2.40)	9.7 (8.52)
1-6	73.7 (2.62)	2.9 (4.0)	72.4 (4.60)	38.7 (34.09)
7	9.3 (3.16)	3.1 (31.9)	10.9 (4.23)	5.9 (6.49)
1-7	83.0 (5.03)	4.2 (5.0)	83.3 (4.57)	44.6 (38.83)
8	6.5 (2.59)	2.6 (40.6)	4.6 (4.25)	4.5 (5.23)
1-8	89.5 (7.34)	5.9 (6.6)	87.9 (5.52)	49.1 (42.73)

Values highlighted were significantly less than controls.
Thick bold line after broods 1-3 denotes results at the end of the traditional 7-day test.
[1] MSD = minimum significant difference. PMSD = percent MSD.
[2] Significant effect on survival (60%). Not included in analyses for reproduction.

survivors from 2-minute exposure to 600 µg/L was 81.8 (8.47) after eight broods. Figure 2.4 offers a comparison between cumulative mean neonate production including and excluding deaths for 24-hour exposure. See Appendix (Figures A.3, A.6, A.9 and A.12) for similar comparisons from the other exposure scenarios.

Effects on Second Generation Individuals

Reproductive effects were observed in second generation (F_1) individuals in every exposure scenario tested. Degree of effects however, decreased dramatically with decreasing exposure duration. 24-hour exposure resulted in significantly reduced fecundity of F_1 individuals at concentrations ≥30 µg/L (Table 2.8, Table A.2). This was surprising because 30 and 38 µg/L did not result in significantly reduced fecundity of first generation individuals (parents). Nearly every F_1 brood from parental first and second broods were significantly lower than controls. F_1 third brood showed signs of recovery as fewer broods and fewer concentrations were significantly lower than controls.

No effects on F_1 fecundity was observed from the 3-hour exposure experiment at any concentration tested (Table 2.9). No significant differences resulted even from concentrations previously affecting survival of parents. Parental first brood neonates were however, released two to three days later than in other concentrations. F_1 individuals from parental second and third broods were not examined with the assumption that effects would not be observed. F_1 individuals from 5-minute exposures exhibited significantly lower fecundity at 417 µg/L, the LOEC for survival effects on parents (Table 2.10). Slightly lower fecundity and increased variability were observed at 374, 398 and 469 µg/L due to one mortality in each of these concentrations. Finally, 1

Table 2.8. Mean (1 SD) number of neonates produced from second generation individuals following 24-hour exposure.

Brood	Concentration (µg/L)							
	RHW	MSD (PMSD)[1]	30	38	58	76	95	114[2]
Second Generation from Parent First Brood								
1	4.2 (0.63)	1.3 (31.3)	3.8 (1.40)	2.6 (1.17)	2.8 (1.23)	2.9 (1.52)	2.3 (1.34)	3.8 (1.17)
2	9.5 (1.08)	2.4 (25.3)	7.2 (2.62)	7.6 (1.51)	5.8 (2.20)	5.6 (2.55)	5.7 (3.40)	7.7 (1.03)
1-2	13.6 (1.17)	3.5 (26.0)	11.0 (3.97)	10.2 (2.49)	8.6 (3.30)	8.5 (3.89)	8.0 (4.62)	11.5 (1.87)
3	14.7 (1.16)	3.7 (25.1)	10.9 (4.20)	12.0 (1.41)	9.9 (3.81)	9.2 (3.97)	9.2 (4.96)	11.7 (2.42)
1-3	28.4 (1.35)	7.0 (24.5)	21.9 (8.02)	22.2 (3.64)	18.5 (7.00)	17.7 (7.72)	17.2 (9.31)	23.2 (3.76)
Second Generation from Parent Second Brood								
1	4.5 (1.08)	0.9 (20.9)	3.9 (0.57)	3.9 (0.32)	3.1 (0.88)	3.4 (1.08)	3.6 (1.17)	4.4 (0.84)
2	10.6 (1.17)	1.35 (12.7)	9.4 (1.43)	8.8 (1.03)	7.4 (1.35)	8.3 (0.82)	8.0 (1.49)	7.9 (1.52)
1-2	15.1 (2.02)	2.0 (13.0)	13.3 (1.77)	12.7 (1.06)	10.5 (1.90)	11.7 (1.42)	11.6 (2.32)	12.3 (2.21)
3	15.8 (1.55)	1.9 (12.1)	13.1 (1.97)	12.8 (1.55)	11.8 (1.87)	11.9 (1.52)	11.4 (1.65)	13.3 (2.41)
1-3	30.9 (3.44)	3.3 (10.8)	26.4 (2.84)	25.5 (2.37)	22.3 (3.47)	23.5 (2.51)	23.0 (2.94)	25.6 (4.30)
Second Generation from Parent Third Brood								
1	4.1 (0.32)	1.0 (23.5)	3.9 (0.74)	3.4 (1.08)	3.6 (1.65)	3.9 (0.57)	4.4 (0.52)	4.1 (0.88)
2	8.9 (0.74)	1.3 (15.1)	9.6 (1.08)	7.7 (1.64)	8.3 (1.64)	8.3 (1.42)	9.0 (1.05)	8.8 (1.14)
1-2	13.0 (0.82)	1.8 (13.6)	13.5 (1.65)	11.1 (2.33)	11.9 (2.18)	12.2 (1.62)	13.4 (1.35)	12.9 (1.29)
3	13.2 (1.69)	1.8 (13.3)	12.3 (2.06)	10.6 (2.32)	11.1 (1.60)	11.0 (1.76)	12.6 (0.70)	12.1 (0.99)
1-3	26.2 (1.48)	2.6 (9.8)	25.9 (3.25)	21.7 (2.75)	23.0 (3.12)	23.2 (2.57)	26.0 (1.33)	25.1 (1.66)

Values highlighted were significantly less than respective controls.
See Table 2.4 for results of first generation exposure.
[1] MSD = minimum significant difference. PMSD = percent MSD.
[2] For second generation from parent first brood, n = 10 for all concentrations except 114 µg/L where n = 6. MSDs (PMSDs) for those were 1.5 (36.2), 2.8 (29.2), 4.1 (30.0), 4.2 (28.9) and 8.0 (28.3), respectively. n = 10 in all other tests.

Table 2.9. Mean (1 SD) number of neonates produced from second generation individuals following 3-hour exposure.

Brood	Concentration (µg/L)					
	RHW	MSD (PMSD)[1]	178	196	218[2]	233[2]
Second Generation from Parent First Brood						
1	3.9 (0.31)	0.8 (19.9)	4.7 (0.68)	4.2 (0.79)	4.7 (1.06)	4.5 (0.85)
2	9.9 (1.73)	1.3 (13.5)	10.0 (0.94)	9.4 (1.26)	9.5 (1.34)	10.1 (1.29)
1-2	13.8 (1.55)	1.4 (9.9)	14.7 (1.16)	13.6 (1.51)	14.2 (1.48)	14.6 (1.08)
3	16.2 (1.62)	1.9 (11.8)	17.0 (1.25)	15.2 (2.15)	15.4 (2.12)	15.5 (2.22)
1-3	30.0 (3.05)	2.7 (9.2)	31.7 (3.09)	28.8 (2.62)	29.6 (2.63)	30.1 (1.90)

Values highlighted were significantly less than controls. n = 10 for all tests.
See Table 2.5 for results of first generation exposure.
[1] MSD = minimum significant difference. PMSD = percent MSD.
[2] Initiated on day 7 of the first generation study due to scant and unpredictable availability of second generation neonates. Parental broods were mixed and composed mostly of first and second broods.

Table 2.10. Mean (1 SD) number of neonates produced from second generation individuals following 5-minute exposure.

Brood	Concentration (µg/L)							
	RHW	MSD (PMSD)[1]	374	388	398	412	417	469
Second Generation from Parent First Brood								
1	4.3 (0.48)	1.0 (23.3)	3.4 (1.43)	4.1 (0.74)	4.1 (0.57)	4.1 (0.57)	3.6 (0.97)	3.8 (1.40)
2	8.3 (1.16)	2.2 (26.6)	7.7 (2.79)	8.2 (1.03)	7.6 (2.99)	8.0 (1.33)	7.0 (1.33)	7.6 (2.82)
1-2	12.6 (1.35)	3.0 (23.8)	11.1 (4.07)	12.3 (1.49)	11.7 (3.40)	12.1 (1.66)	10.6 (2.07)	11.4 (4.22)
3	13.4 (0.84)	3.1 (23.0)	12.0 (4.27)	13.3 (0.82)	11.9 (4.28)	12.7 (1.16)	12.3 (1.95)	11.3 (4.11)
1-3	26.0 (2.00)	6.0 (23.0)	23.1 (8.31)	25.6 (2.12)	23.6 (7.59)	24.8 (2.78)	22.9 (3.90)	22.7 (8.30)

Values highlighted were significantly less than controls. n = 10 for all tests.
See Table 2.6 for results of first generation exposure.
[1] MSD = minimum significant difference. PMSD = percent MSD.

Table 2.11. Mean (1 SD) number of neonates produced following 600 µg/L exposures.

	Duration (600 ug/L)			
Brood	RHW	MSD (PMSD)[1]	1 min	2 min[2]
Second Generation from Parent First Brood				
1	4.8 (0.45)	1.1 (23.8)	4.2 (1.30)	5.6 (0.55)
2	11.0 (0.71)	1.9 (16.9)	10.6 (1.95)	11.0 (1.22)
1-2	15.8 (1.10)	2.3 (14.8)	14.8 (2.59)	16.6 (1.14)
3	16.2 (0.84)	2.6 (16.0)	15.4 (2.70)	16.4 (1.82)
1-3	32.2 (1.92)	4.6 (14.4)	30.2 (5.12)	33.0 (2.55)

No significant effects were observed. n = 10 for all tests.
See Table 2.7 for results of first generation exposure.
[1] MSD = minimum significant difference. PMSD = percent MSD.
[2] Neonates from parent first brood were not released until day 6 of the original 7-d test.

and 2-minute exposure to 600 µg/L also did not result in any significant differences in F_1 fecundity from parental first brood individuals (Table 2.11). PMSDs for all F_1 analyses were generally in the low to mid 20s, but ranged between 10 and 38%.

Examination of Percent Minimum Significant Differences (PMSDs)

EPA has set lower (13%) and upper (47%) PMSD limits for the 7-day short-term chronic test with *Ceriodaphnia dubia* (EPA, 2001a and b). Differences observed outside these limits may not be considered realistic – either the power of the test was too high

or too low. The lack of significant differences, and apparent recovery, of individual later broods from the 24-hour exposure (Table 2.4) may therefore be explained by the increasing variability with time. PMSDs increased dramatically after the sixth brood (24 to 98%). This was not seen with cumulative mean neonate production as PMSDs remained fairly consistent over time (14 to 23%).

PMSDs from the 3-hour exposure (Table 2.5) were all between 25 and 42%. A slight but steady increase was observed for individual broods. PMSDs from the 5-minute exposure (Table 2.6) were very consistent, ranging between 31 and 38%. Again, individual broods exhibited slight increases over time. It is expected that these exposures would have followed similar trends observed from the 24-hour exposure had they been carried beyond five broods. PMSDs from the 600 µg/L exposure (Table 2.7) however, were nearly all below 9%. The few reproductive differences observed from this exposure may not be real.

PMSDs obtained from second generation studies were generally lower than those from first generation (baseline) tests. PMSDs from the 24-hour exposure (Table 2.8) ranged between 25 and 31% for F_1 from parent first brood. F_1 from parent second and third brood however, exhibited many PMSDs below 13%. More than half of the differences observed from F_1 from parent second brood are questionable, based on EPA's limits imposed on minimum significant differences.

Discussion

In a review of the effects of intermittent exposures, Handy (1994) stated toxicants such as copper are considerably less toxic with small reductions in exposure duration

when peak concentrations are the same. This suggests that any small change in duration would correspond to large changes in toxicity. Although this study did not produce effect combinations of equal peak concentration, results obtained here for first generation exposures agree with this at higher concentration-lower duration but not at lower concentration-longer duration combinations. Regardless of endpoint, at lower concentrations (<300 µg/L), large decreases in duration corresponded to small increases in effective concentration. On the other hand, at higher concentrations (>300 µg/L), slight decreases in duration corresponded to large increases in effective concentration.

Toxicant mode of action may offer insight as to whether latent effects are likely to occur (Reinert et al., 2002). Organisms have a greater chance of recovery and lesser latent effects when exposed to pollutants with a reversible mode of action. The opposite would be true for chemicals exhibiting an irreversible mode of action (such as copper). Handy (1994) stated that if a contaminant has a rapid and reversible toxic mechanism, then mortality would be proportional to the exposure duration. However, several explanations are possible when large increases in exposure duration produce small changes in toxicity: 1) a critical exposure duration is required to initiate the toxic mechanism, but has not been reached, or 2) the toxic action is much slower than the duration of the experiment, thus mortalities are not observed (Handy, 1994). Alternatively, if small changes in exposure duration produce large increases in mortality, then it can be assumed that the “critical exposure duration” has been reached and/or the toxic mechanism is rapid but not reversible (Handy, 1994).

According to statements above, results obtained from this study seem to suggest more than one mode of action for copper depending on concentration-duration combination. The two explanations offered by Handy (1994) for large increases in exposure duration-small changes in toxicity do not apply here where lower concentration-longer duration combinations resulted in just that—small changes in toxicity. On the other hand, the above model of rapid but not reversible toxic mechanism was observed with higher concentration-shorter duration exposure combinations—large changes in toxicity. Clearly, a critical exposure duration was reached, and the toxic action was actually much faster than the duration of the experiment in both scenarios. It is therefore suggested that mode of action is the same for different exposure combinations, but rate of accumulation is considerably faster at higher concentrations.

Reduced fecundity observed in this study may be explained by the effects of heavy metals on feeding behavior. Exposure to heavy metals has been reported to depress feeding behavior of daphnids even at concentrations as low as 10 µg/L (Taylor et al., 1998; McWilliam and Baird, 2002; Clément and Zaid, 2004). Impaired feeding has also been linked to reduced egg production in Copepods (Stearns et al., 1989). In addition, Munger et al. (1999) reported highest accumulation of cadmium in the gut of *C. dubia* and suggested toxic effects continued at that location. The same approximate reproductive effect and concentration-response occurred with each exposure scenario. Effects observed from longer exposures (lower effective concentrations) likely resulted in prolonged feeding depression in addition to accumulation whereas effects observed from shorter exposures (higher effective concentrations) were probably more the result of higher accumulation rates.

Reduced fecundity in second generation individuals may have resulted from maternal transfer of toxicant. Guan and Wang (2004) suggested transfer of metals from mother to offspring attributed to rapid elimination of selenium and zinc in *Daphnia magna*. Reproductive effects were notable following 24-hour exposure of parents. With these, more time was allowed for copper to be passed on to developing eggs. In shorter exposures however, effects were lacking. Reduced fecundity was only observed in second generation first brood individuals where parent survival had been significantly reduced by 5-minute exposure. Although reproductive effects were pronounced in first generation individuals, exposures ≤3 hours did not provide adequate time for transfer of toxicant to eggs.

24-hour pulse exposures from a runoff event or concentrations as high as 600 µg/L may not appear environmentally realistic. In fact, such scenarios may be possible if greater than base-flow concentrations linger in waterbodies, an organism is transported in elevated concentrations for some time, or the scale of an event is unprecedented. The magnitude and duration of all events would have to be recorded to avoid any uncertainty.

The US EPA has stated that runoff pollution was a major water quality problem in our nation (EPA, 1994). EPA reported that 87% of 246 major waterbodies in the United States were affected by non-point source pollutants (NURP, 1993 in Walker et al., 1999). Metals, in particular, were considered to be the largest contributor to runoff pollution. Cole et al. (1984) reported that several metals were often found in runoff at concentrations greater than their respective water quality criteria (WQC).

Next to zinc, copper is one of the most frequently found heavy metals in runoff. Concentrations reported in the literature are zinc (20 – 5000 µg/L) > copper ≈ lead (5 – 200 µg/L) > cadmium (<12 µg/L) (Davis et al., 2001). Walker et al. (1999) also reported a range of copper concentrations between 0.06 and 1410 µg/L. Heavy metal contamination in runoff is most often associated with suspended particulate matter, however, suspended sediment-associated metals can become available during transport (Cheebo et al., 2001; Gagnon and Saulnier, 2003). Highways and roadways may be the single largest contributing source of copper pollution in urban runoff. Legret and Pagotto (1999) examined runoff from a major rural highway and calculated a surface loading of 0.29 kg/km (1 km of a dual carriageway including hard shoulder covering 1.165 ha). Automobile brake linings (142,000 mg Cu/kg) were determined to be the primary source for copper in highway runoff. Gromaire et al. (2000) reported median copper values of 0.12 and 0.71 mg/m^2 street for street washing waters and runoff waters, respectively. Barbosa and Hvitved-Jacobsen (1999) reported an average dissolved copper concentration of 10.7 µg/L from a highway with average daily traffic of 6000 vehicles. Dissolved copper concentrations ranged from <1 to 54 µg/L, and the average event mean concentration was 24.1 µg/L. Hares and Ward (1999) examined a motorway with average daily traffic of 140,000 vehicles and observed a total copper concentration of 274 µg/L. Finally, Sörme and Lagerkvist (2002) estimated that asphalt and brake linings contributed 11 to 17 and 280 kg Cu/yr, respectively to a waste water treatment plant through stormwater runoff.

Numerous other sources of copper in runoff have also been investigated. Davis et al. (2001) listed building and roofing materials, vehicle brake emissions and

atmospheric deposition as major sources for copper. Annual copper loadings were estimated to be 0.038 kg/ha-yr for residential and 0.243 kg/ha-yr for commercial land use types. Sörme and Lagerkvist (2002) estimated that copper roofs contributed an estimated 700-920 kg Cu/yr. Duke et al. (1998) detected copper in >80% runoff samples from metal plating facilities with mean concentrations as high as 390 µg/L. Gray (1998) measured copper concentrations from copper mine surface runoff ranging from 1000 to 383,000 µg/L. Larm (2000) monitored material transport into and out of stormwater treatment facilities and estimated a total copper mass loading of 54 kg/yr from five separate subwatersheds. Finally, Ribolzi et al. (2002) reported an average particulate-associated copper concentration of 245 mg/kg in runoff following treatment of a vineyard with a copper-based fungicide.

The protective nature of WQC applied to episodic events is controversial and uncertain. Reconstituted hard water used throughout this work had a hardness of 180 mg/L as $CaCO_3$. Given this, the CMC and CCC for copper would be approximately 30 and 19 µg/L, respectively and assumes no water effects ratio. The CMC is clearly underprotective for combinations up to and including 5 minutes at 417 µg/L in that the average concentration over 1 hour is greater than 30 µg/L. The 1-hour average concentration of the 2-minute exposure to 600 µg/L is 20 µg/L, however, significant mortality was observed within 48 hours. Although all combinations affecting reproduction were below 19 µg/L when averaged over 4 days, the CCC also would not have been protective. An exceedance frequency of one year presumes organisms have the potential to recover following an event equal to or less than the CMC or CCC. Although neonate production of adult *C. dubia* appeared to recover by the fifth or sixth

individual brood, total neonate production remained significantly lower in almost all combinations except those with lowest effect concentrations. Recovery at concentrations above the lethal threshold, where survivor neonate production often exceeded controls, would be extremely slow and would depend on no additional environmental perturbations or pressures. Observed effects on second generation individuals from longer exposures compound the reduced potential for population recovery. Exposure scenarios presented here clearly show the importance of a single event in that concentrations averaging below recommended criteria can have deleterious effects and low potential for recovery.

Our understanding of the effects and sources of runoff-related pollution have increased over the years. Provisions of Section 319 of Clean Water Act have certainly improved upon the way non-point source pollution issues are handled. However, runoff-related toxicity is still a major problem. The US EPA (2000) still cites non-point source pollution as a serious water quality concern. Urban runoff has been reported as the second most frequent cause of surface water pollution in the United States (Walker et al., 1999). Studies of latent effects and population recovery will further our understanding of the real impacts of non-point pollution events.

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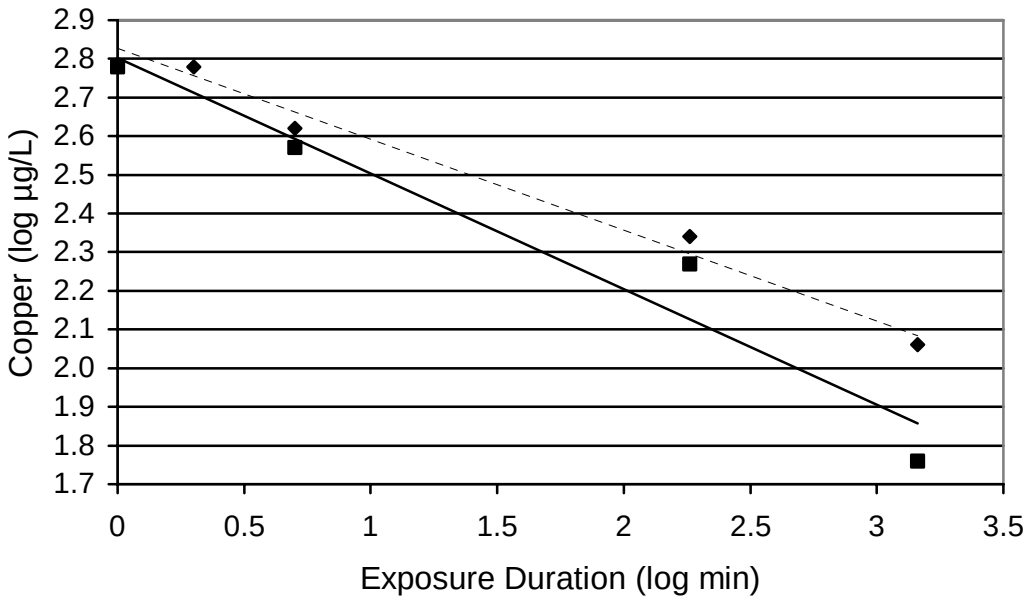

Figure 2.1. LOECs for survival (diamonds) and fecundity (squares). For illustrative purposes, the LOEC for fecundity from the 3-hour exposure (187 µg/L) was estimated as the average of the fecundity LOEC (>178 µg/L) and survival NOEC (196 µg/L). Trend lines for survival (dashed), R2 = 0.9838; log y = -0.2352 logx + 2.8276 and fecundity (solid), R^2 = 0.9471; log y = -0.2992 logx + 2.8027 are shown.

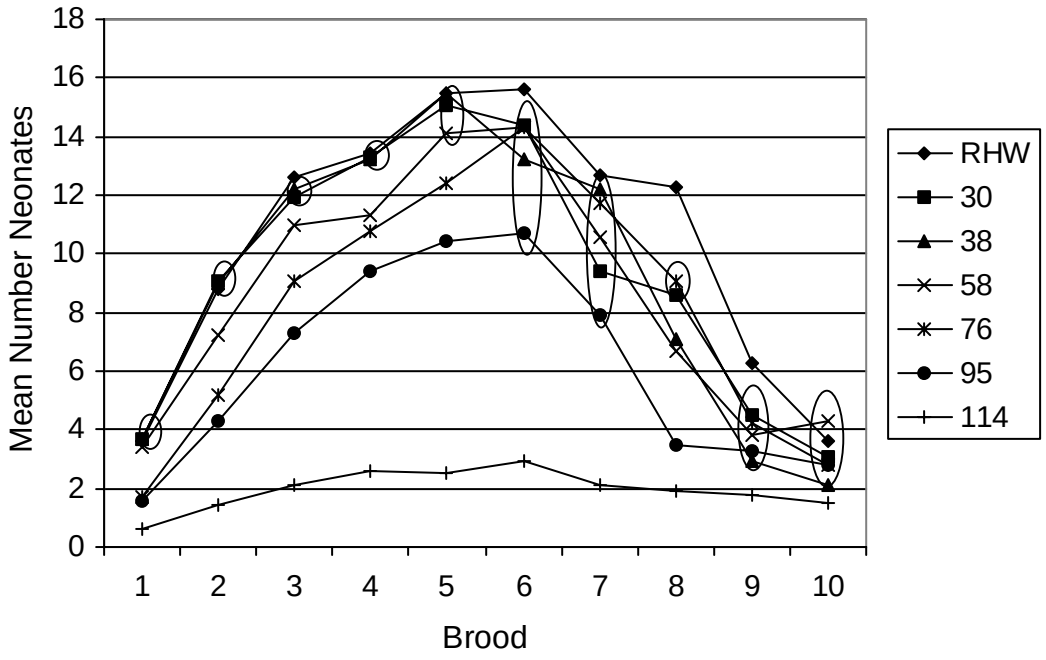

Figure 2.2. Mean number of neonates produced per adult female for each individual brood: 24-hour exposure. Error bars not shown for clarity. Ovals contain groups found not to be significantly less than controls.

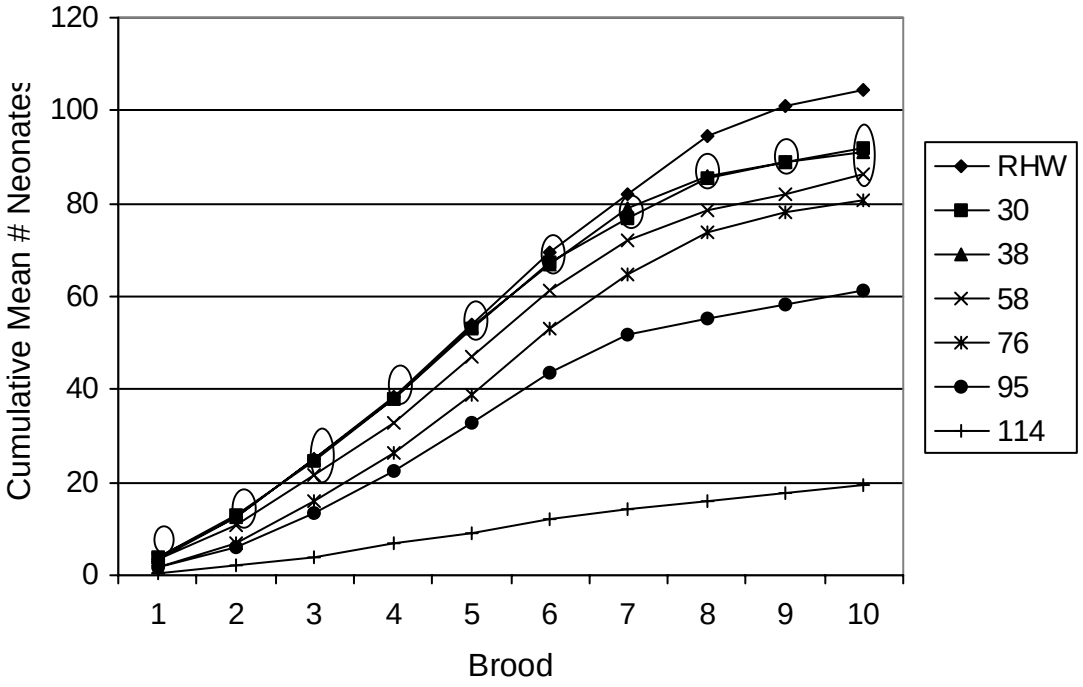

Figure 2.3. Cumulative mean number of neonates produced per adult a female following each subsequent brood: 24-hour exposure. Error bars not shown for clarity. Ovals contain groups found not to be significantly less than controls.

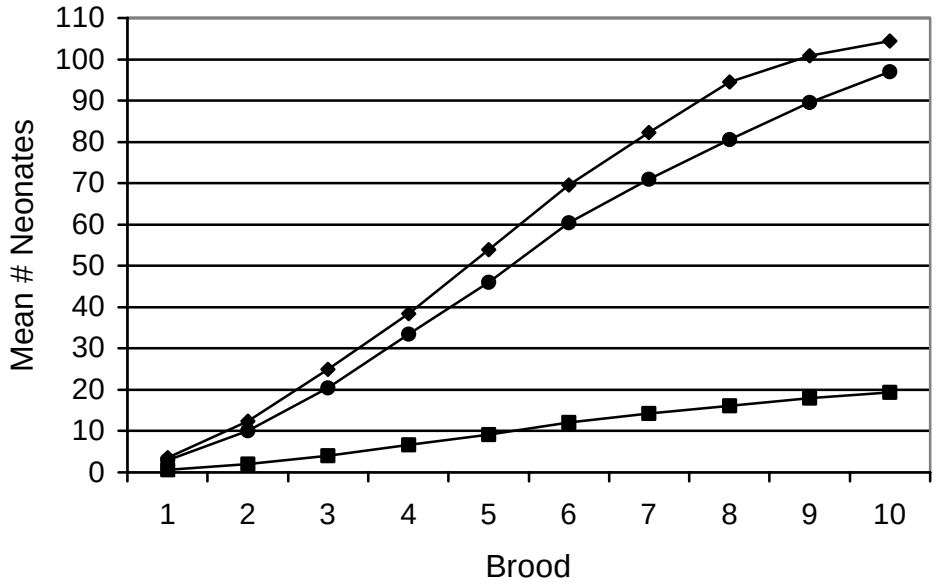

Figure 2.4. Cumulative mean number of neonates produced at 114 µg/L when deceased adults were counted as zero (n=10) neonates produced (squares) and when neonates of survivors only (n=2) were counted (circles). RHW reference (diamond): 24-hour exposure.

CHAPTER 3

EFFECTS ON SURVIVAL AND REPRODUCTION OF *Ceriodaphnia dubia* FOLLOWING SINGLE EPISODIC EXPOSURES TO CADMIUM: A LABORATORY TO FIELD COMPARISON

Introduction

Runoff related toxicity is a major problem in the United States and elsewhere. The US Environmental Protection Agency (EPA, 2000) cites non-point source pollution as a serious water quality concern. Urban runoff has been reported as the second most frequent cause of surface water pollution in the US (Walker et al., 1999). Storm-driven runoff toxicity may be especially problematic in the southwest where stream flow is often intermittent. Rain events in Texas and Oklahoma can last 7 to 9 hours on average (Novotny and Witte, 1997). Consequently, many countries require abatement of pollution by wet weather discharges, yet there is no yardstick included in stormwater regulations by which this problem can be quantified and the effect of abatement assessed. Abatement efforts are greatly hampered by unavailability of wet-weather water quality criteria (WQC) or standards (Novotny and Witte, 1997).

There is considerable controversy in the US and many other countries as to whether the water quality criteria for priority pollutants can be used for protection against stormwater toxicity or other episodic exposures. In addition, most water quality criteria have allowable exceedances in terms of low flow. This can not apply to stormwater events because these criteria presume no flows (including storms) during periods of drought (Novotny and Witte, 1997). It may in fact, be impossible to develop

regulations for individual single events because each is site and event specific. We can however, study them as predictive tools. Such efforts may be the best way to study results of best management practices implemented to reduce the impact of runoff.

Impacts of wet weather discharges on the environment have gained more attention as the regulatory focus of the Clean Water Act has shifted from continuous-flow effluents to episodic pollution produced by storm events (Herricks and Milne, 1998). Consequently, pulse-exposure tests have been advocated for use in risk assessment in order to more accurately evaluate effects that may occur under natural exposure conditions while retaining the advantages of simplicity and repeatability associated with single-species laboratory tests (Peterson et al., 2001). Numerous authors have proposed incorporation of pulse-exposure test designs in risk assessment (e.g.s, Abel, 1980; Pascoe and Shazili, 1986; McCahon and Pascoe, 1990; Brent and Herricks, 1998; Naddy and Klaine, 2001). However, such designs are still underemphasized and underutilized (Naddy et al., 2000).

The difficulty of estimating effects from time-varying exposures from measurements obtained from continuous exposure tests is often an important source of uncertainty in ecological risk assessment (Reinert et al., 2002). Many approaches for investigating episodic pollution have been developed from conventional toxicity testing methods (McCahon and Pascoe, 1990). Including Whole Effluent Toxicity (WET) tests, conventional toxicity tests are simply modified to accommodate issues of time-varying exposure. For example, exposure durations may be altered or post-exposure observation periods included in the study design. WET tests are clearly designed for examination of point-source and continuous exposure scenarios. They are the most

widely used and accepted standard tests in the United States. They have proven to be precise and reliable in most cases (EPA, 2001a), although this point remains controversial. Finally, WET tests often agree with observed instream impacts (e.g.s, Diamond and Daley, 2000; Kosmala et al., 1999; Dickson et al., 1992; Eagleson et al., 1990). Other single-species laboratory toxicity tests have also been shown to predict effects in the field when exposure regimes are similar (Clark et al., 1987). With modification, WET-type tests may be as useful in assessing non-point source exposure scenarios that have more complicated issues of time-scale toxicity.

Studies of pesticides dominate the peer-reviewed literature on episodic exposures. Several however, have investigated effects of metals. Among these, many have examined effects from intermittent exposure (Lappivaara and Marttinen, 2005; Siddens et al., 1986; Seim et al., 1984; Hodson et al., 1983; Ingersoll and Winner, 1982). Numerous investigators have also examined lethal (Brent and Herricks, 1998; Hutchinson et al., 1989; Abel and Garner, 1986; Pascoe and Shazili, 1986) and sublethal (McWilliams and Baird, 2002; Williams and Holdway, 2000; Handy, 1992; McCahon and Pascoe, 1991) effects of single-pulse exposures. Only two have investigated single pulse effects on cladocerans. Brent and Herricks (1998) reported significant post-exposure immobility of *Ceriodaphnia dubia* following single exposures to cadmium or zinc. 30-minute and 2-hour exposure to 2.5 and 0.37 mg/L cadmium, respectively resulted in significant immobility. 15-minute exposures however, did not result in significant post-exposure immobility at cadmium concentrations as high as 11.8 mg/L but did at 4.8 mg/L zinc. McWilliam and Baird (2002) examined feeding behavior in *Daphnia magna* exposed to heavy metals. Post-exposure feeding depression was

significantly lower at ≥0.2 µg/L cadmium, ≥14 µg/L copper and 100 µg/L zinc following 24-hour exposures.

Most of the literature on sublethal effects of single pulse exposures have examined site or event specific exposure scenarios to determine whether or not they were problematic. Unfortunately, these may not be predictive of different sites or varying events. No other research has actually sought to identify specific effect levels for a wide range of exposure scenarios. Moreover, very few have compared potential effects of different water types. The objective of this research was to define post-exposure effect concentration-duration combinations of single episodic exposures of cadmium on survival and reproduction of the cladoceran, *Ceriodaphnia dubia* in either standard laboratory reconstituted hard water (RHW) or a municipal treated wastewater effluent TWE). Results are expressed as traditional no- and lowest observed effect concentrations (NOEC, LOEC) as well as novel no- and lowest observed effect durations (NOET, LOET). Research presented here may have applications in ecological risk assessments beyond single sites or events and may be useful in introducing an exposure duration component to the biotic ligand model.

Materials and Methods

A detailed description of *Ceriodaphnia dubia* and *Pseudokirchneriella subcapitata* cultures can be found in Chapter 1.

Toxicity Tests

7-day short-term chronic toxicity tests with *C. dubia* were conducted following procedures recommended by the US EPA (1994) except tests were ended after a maximum of 7 days or after all control organisms had released their third brood. Any fourth-brood neonates were not counted. Although blocking by known parentage is only a recent requirement of the EPA (2002), it was employed throughout this study. In addition, the yeast-cerophyl-trout chow feeding suspension was replaced by the algae-Cerophyl, or cereal grass media, diet described in Chapter 1.

Short-term chronic toxicity tests were conducted in two series. Brooks et al. (2004) found that *C. dubia* survival was significantly reduced after 4 days when exposed to artificial stream water contaminated with 80 µg/L cadmium. Artificial streams were supplied by TWE and flowed continuously, however, laboratory *C. dubia* toxicity tests were static-renewal. 20 µg/L cadmium-contaminated stream water affected neither *C. dubia* survival nor fecundity after 7 days of exposure. The first series of experiments was performed to determine minimum pulse exposure duration of 80 µg/L stream water required to significantly lower *C. dubia* survival and fecundity. After exposure, organisms were transferred to control stream water (TWE flowing through streams without addition of cadmium) for the remainder of the 7-day test. The second series of experiments were performed to determine effective (herein defined as significantly less than controls) concentration-duration combinations for cadmium in different water types. Less than 24-hour-old (usually <12-hour-old) *C. dubia* neonates were exposed to cadmium in either RHW (EPA, 1991) or TWE that had not flowed through streams. Exposure durations ranged from 5 minutes to 24 hours. 7-day and a 48-hour

continuous-exposure tests were also performed. Nominal concentrations ranged from 3.0 to 10,000 µg/L. Exposure scenarios are given in Table 3.1. Food was provided during exposure regardless exposure water or duration. Following exposure, organisms were transferred to clean RHW or TWE for the remainder of the test period. Adult survival and fecundity (number of neonates per female) were assessed each day prior to renewals.

Table 3.1. Exposure concentration-duration combinations used in single episodic event exposures to cadmium.

Exposure Duration	Cadmium in RHW	Cadmium in TWE[1,2]
5 min	400 – 10,000	400 – 10,000
1.5 h	80 – 600	80, 200 – 800
3 h	80 – 500	80, 100 – 800
7 h	80	80
24 h	20 – 200	80, 10 – 100
48 h	3.0 – 100[3]	80
7 d	3.0 – 50	5 – 250[4]

All concentrations in this table are nominal as cadmium (µg/L).
[1] TWE = Final treated wastewater effluent.
[2] Each independent listing of 80 µg/L refers to tests performed directly from the 80 µg/L stream mesocosm.
[3] 48-h acute test, not a 48-h episode.
[4] All from stream mesocosms.

Stream Mesocosms

The University of North Texas Stream Research Facility and municipal effluent supplying stream mesocosms are described in Brooks et al. (2004). Water quality measurements were taken three times a day during the study (Table 3.2).

Table 3.2. Water quality parameters (1 SD) of final treated wastewater effluent supplying stream mesocosms.

Parameter	6 am	2 pm	10 pm
pH	6.9 (0.10)	6.9 (0.09)	6.9 (0.09)
Temperature (°C)	29.3 (0.18)	31.3 (0.12)	30.8 (0.10)
Dissolved Oxygen (mg/L)	4.5 (0.29)	7.3 (0.43)	4.2 (0.23)
Alkalinity (mg/L as $CaCO_3$)	64 (8.2)	69 (8.9)	69 (12.4)
Hardness (mg/L as $CaCO_3$)	146 (11.5)	140 (5.7)	142 (9.2)
Total Organic Content (mg/L)	7.6 (1.05)	7.8 (2.05)	7.3 (2.20)
Total Dissolved Solids (mg/L)	0.57 (0.006)	0.58 (0.002)	0.58 (0.002)
Specific Conductance (µs/cm)	898 (9.7)	903 (2.8)	901 (3.9)
Turbidity (NTU)	9.2 (3.20)	12.2 (5.45)	9.4 (3.86)
Total Copper (µg/L)	17.5 (1.3)	19.3 (3.1)	17.7 (2.6)
Dissolved Copper (µg/L)	16.5 (1.0)	17.3 (3.3)	16.7 (0.6)

Data taken directly from Brooks et al. (2004).

Statistical Analyses

Data were analyzed using Toxstat Version 3.4 (University of Wyoming, 1994) following guidelines of the EPA (1994). Differences were accepted at α=0.05. Where assumptions of normality or homogeneity of variance were not met, Dunnett's test was performed in addition to non-parametric analyses in order to estimate percent minimum significant differences (PMSDs). It is important to note here that the EPA has set lower

(13%) and upper (47%) PMSD limits for the 7-day short-term chronic test with *Ceriodaphnia dubia* (EPA, 2001b and c).

Test Solutions and Analyses

Cadmium stock solutions were prepared in Milli-Q water using certified atomic absorption spectrometry standard solutions. Test solutions were prepared in RHW or TWE. Milli-Q stock solution volumes were chosen such that water quality parameters (conductivity, pH, hardness and alkalinity) were not altered. Samples of test solutions were acidified to a pH ≤2 and analyzed by atomic absorption spectrometry (Varion SpectrAA 600 with graphite tube atomizer, GTA-100).

Results

Measured concentrations of cadmium were 60 to 120% of nominal values (Table 3.3). The vast majority of measured concentrations were within 80 to 100% of nominal. In two experiments (1.5- and 3-hour exposure to 400-600 µg/L in RHW), most measured concentrations were considerably higher than nominal. This did not affect the observed concentration response.

Exposures to 80 µg/L Stream Water

Continuous exposure of 80 µg/L stream water resulted in significantly reduced survival of *C. dubia* in 3 to 7 days. A 96-hour pulse was required to significantly reduce survival after transfer to non-dosed TWE (Figure 3.1). A 48-hour pulse did not affect survival. *C. dubia* fecundity was significantly reduced following exposure durations >3-

Table 3.3. Nominal versus measured cadmium concentrations (µg/L).

	Measured Concentration			
Nominal Conc.	Mean	SD	Range	n[1]
10.0	6.0	na	na	1
20.0	15.5	na	15-17	2
40.0	30.5	na	27-34	2
60.0	44.5	na	41-48	2
70.0	54.0	na	na	1
80.0	71.4	10.3	60-84	5
100	83.4	1.82	81-86	5
120	92.7	9.87	86-104	3
140	114	21.2	97-138	3
160	135	na	na	1
180	163	17.9	142-174	3
200	199	na	na	1
220	200	na	171-229	2
240	236	na	na	1
260	229	19.6	218-252	3
280	241	na	241	2
300	252	5.2	246-255	3
400	375	18.8	363-397	3
425	410	na	na	1
450	474	na	na	1
475	492	na	na	1
500	535	na	513-557	2
525	573	na	na	1
550	528	na	na	1
575	655	na	na	1
800	656	63.5	584-704	3
1000	865	na	800-930	2
2000	1530	na	1380-1680	2
>2000	nd			

[1] n = number of toxicity tests where concentrations were measured. Atomic absorption analyses were performed with three replicates per sample. Concentrations >2000 µg/L were not measured.

hours. The relationship between percent neonate reduction and exposure duration is shown in Figure 3.2.

Effective Concentration-duration Combinations in RHW or TWE

The City of Denton, TX Waste Water Reclamation Facility is obligated to measure for priority pollutants and other constituents in their final treated effluent on a regular basis. Few organic pollutants exceeded regulated limits in TWE. On the other hand, several metals regularly exceeded regulated limits; including Zn, Cu, Fe, Mg, Ca, Mn and K. Mean total cadmium concentrations were 0.002 mg/L, and measured values ranged between -0.010 and 0.005 mg/L. Total cadmium and dissolved cadmium were not significantly different. Results of water quality measurements taken during these experiments are summarized in Table 3.4.

Survival was adversely affected by increasing concentration-decreasing duration combinations (Table 3.5). Most mortality occurred within 24 hours post-exposure, however, some mortality was observed after 48 hours. Seldom was mortality observed beyond 48 hours. Immobile organisms were transferred for at least two additional days, and recovery was never observed in that time. The NOEC and LOEC for 24-hour exposure in RHW were 34 and 41 µg/L, respectively. These values are considerably higher than those for 7-day continuous exposure (3.4 and 12 µg/L, respectively). The NOEC and LOEC for 24-hour exposure in TWE were 61 and 83 µg/L, respectively – much higher than those of RHW.

Fecundity of *C. dubia* was also adversely affected by increasing concentration-decreasing duration combinations (Table 3.5) – much in the same way as survival. The LOEC for fecundity following a 24-hour exposure in RHW was 17 µg/L. Again, this value

Table 3.4. Measured water quality constituents in TWE.

Constituent [1,2]	Mean	SD	Range
Aluminum	0.061	0.0417	0.001-0.138
Bicarbonate	142.7	25.90	108.6-183.3
C-BOD	1.9	0.81	0.7-6.2
Chloride	81.6	10.95	61.9-95.8
Chlorine-Residual	1.25	0.240	1.01-1.97
Chlorine-DE	0.05	0.022	0.01-0.08
Dissolved Oxygen	7.9	0.46	6.8-8.7
Fecal Coliforms (CFU/100 ml)	0.46	1.091	0-8
Fluoride	0.681	0.060	0.592-0.766
pH (S.U.)	7.3	0.10	7.0-7.7
Total Phosphates	1.71	0.385	1.05-2.20
Sodium	79.2	6.95	70.3-92.8
Sulfates	92.9	13.39	77.4-112.5
Total Alkalinity	117.0	21.23	89.0-150.2
Total Ammonia	0.54	0.601	0.05-2.88
Total Hardness	157	28.1	98-196
Total Solids	464	47.1	388-536
Total Suspended Solids	1.6	2.45	0.3-22.2
Turbidity (N.T.U.)	1.31	0.653	0.50-3.34
Specific Conductance (µmhos)	675	69.8	574-799
Nitrate	0.98	na[3]	na
Total Metals (mg/L)[4]			
Chromium	0.001	0.0128	-0.028-0.029
Zinc	0.045	0.0293	0.015-0.208
Copper	0.046	0.0233	0.002-0.111
Lead	0.009	0.0042	-0.07-0.15
Iron	0.143	0.2561	0.07-1.84
Cadmium	0.002	0.0026	-0.010-0.005
Nickel	0.022	0.0277	-0.022-0.115
Magnesium	6.68	1.000	3.95-8.69
Calcium	51.7	7.37	41.4-69.7
Manganese	0.036	0.0482	0.006-0.319
Silver	0.001	0.0030	-0.033-0.188
Potassium	34.6	36.69	10.08-9.94
Arsenic	0.003	0.0051	-0.010-0.020
Molybdenum	0.026	0.0562	-0.065-0.274

Table 3.4. continued.

Constituent	Mean	SD	Range
Dissolved Metals (mg/L)[5]			
Chromium	0.002	0.0013	-0.040-0.039
Zinc	0.047	0.0325	0.026-0.243
Copper	0.047	0.0276	0.015-0.139
Lead	0.003	0.0300	-0.07-0.09
Iron	0.078	0.0431	-0.04-0.21
Cadmium	0.003	0.0055	-0.012-0.008
Nickel	0.020	0.0328	-0.048-0.130
Magnesium	6.70	1.021	3.78-9.15
Calcium	51.5	7.92	35.9-68.9
Manganese	0.037	0.0494	0.007-0.322
Silver	-0.002	0.0132	-0.030-0.061
Potassium	30.2	33.49	10.19-9.86
Arsenic	0.006	0.0144	-0.007-0.090
Molybdenum	0.011	0.0273	-0.072-0.055
Other Pollutants (µg/L)			
bis-(2-Ethylhexyl)phthalate[6]	55.1	42.03	14-98
Methylene Chloride[6]	254	417	1.5-736
T. Bromodichloromethane	0.540	na	na
Total Chloroform	1.42	na	na

[1] mg/L unless otherwise noted.
[2] n = 91 for these constituents except; n = 90 for pH, chlorine-residual, chlorine-DE, and dissolved oxygen; n = 86 for fecal coliforms; n = 49 for turbidity; n = 303 for ammonia.
[3] na = not applicable due to less than three measurements.
[4] n = 47
[5] n = 46
[6] n = 3

Table 3.5. No- and lowest observed effect durations for *C. dubia* survival and fecundity in RHW or TWE at given cadmium concentrations (µg/L).

	Survival				Fecundity			
	RHW		TWE[1]		RHW		TWE	
Conc.[2]	NOET	LOET	NOET	LOET	NOET	LOET	NOET	LOET
20					<24 h	24 h		
40	24 h							
50					<7 h	7 h	24 h	
60		24 h						24 h
80			72 h[3]	96 h[3]				
90			24 h					
100				24 h	3 h			
120					1.5 h	3 h		
140						1.5 h		
160							3 h	
180								3 h
200							1.5 h	
220								1.5 h
450	3 h							
475		3 h						
500	1.5 h		3 h					
525		1.5 h		3 h				
550			1.5 h					
575								
600				1.5 h				
4800					5 min			
5000						5 min		
6000							5 min	
7000								5 min
8000	5 min							
9000		5 min						
10000			5 min	>5 min				

[1] TWE = final treated wastewater effluent.
[2] Nominal concentrations (µg/L).
[3] Values associated with 80 µg/L stream mesocosm static-renewal exposure.

is considerably higher than that for 7-day continuous exposure (3.4 µg/L). NOECs for 7-day and 24-hour exposures were 1.1 and <17 µg/L, respectively. The NOEC and LOEC for 24-hour exposure in TWE were 34 and 48 µg/L, respectively. Again, these values were higher than those for 24-hour exposures in RHW.

A narrow threshold between lethal and sublethal effects was observed regardless of water type. For example, LOECs for 24-hour exposures in RHW were 41 and 17 µg/L for survival and fecundity, respectively and 83 and 48 µg/L, respectively in TWE. Percent minimum significant differences reached as low as 10% and as high as 42%, however, they generally ranged between 15 and 25%. This threshold appeared fairly consistent with increasing concentration and subsequent decrease in duration. For any given duration, a slight concentration-response was observed in fecundity before mortality occurred. The concentration-response was also apparent beyond combinations affecting survival. Survivors of the LOEC-Ts for survival usually reproduced similar to those of lower concentrations and sometimes even similar to controls. Effective combinations where there were less than three survivors usually hardly reproduced at all.

Relationships between concentration and duration were very similar for survival and fecundity. NOECs and LOECs increased logarithmically with respect to duration. Differences in concentration between effective durations were relatively small at lower concentration-longer duration combinations and greater at higher concentration-shorter duration combinations. LOECs for survival (Figure 3.3) were 8000 and 41 µg/L for 5-minute and 24-hour exposures in RHW, respectively. In TWE, LOECs for survival were >10,000 and 61 µg/L for 5-minute and 24-hour exposure, respectively. LOECs for

fecundity followed nearly identical trends although concentrations were proportionally lower. The relationship between concentration and duration are illustrated in Figures 3.3 (survival) and 3.4 (fecundity).

Discussion

This study showed that as exposure duration decreased, concentrations required to cause an effect dramatically increased. This trend held true regardless of water type although effective concentrations in TWE were considerably higher than in RHW. At lower concentrations (<600 µg/L), large decreases in duration corresponded to small increases in effective concentration. On the other hand, at higher concentrations (>600 µg/L), slight decreases in duration corresponded to large increases in effective concentration. Handy (1994) stated toxicants such as copper and cadmium are considerably less toxic with small reductions in exposure duration when peak concentrations are the same. This suggests that any small change in duration would correspond to large changes in toxicity. Although this study did not produce effect combinations of equal peak concentration, results obtained here agree with this at higher concentration-shorter durations but not at lower concentration-longer durations.

Effects of cadmium are numerous, however, reduced fecundity observed in this study may be explained by the effects of cadmium on feeding behavior. Exposure to heavy metals has been reported to depress feeding behavior of cladocerans even at concentrations as low as 1.4 µg/L (Taylor et al., 1998; McWilliam and Baird, 2002; Orchard et al., 2002; Clément and Zaid, 2004). Impaired feeding has also been linked to reduced egg production in Copepods (Stearns et al., 1989). Munger et al. (1999)

reported highest accumulation of cadmium in the gut of *C. dubia* and suggested toxic effects continued at that location. In addition, Griffiths (1980) found that exposure to cadmium for just 2 hours resulted in paralysis and shrinking of gut diverticula in *Daphnia magna*. The same approximate reproductive effect and concentration-response occurred with each exposure scenario. Effects observed from longer exposures may have resulted in prolonged feeding depression and accumulation of cadmium, whereas effects observed from shorter exposures were probably more the result of higher accumulation rates.

The US EPA has stated that runoff pollution is a major water quality problem in our nation (EPA, 2000). EPA reported that 87% of 246 major waterbodies in the United States were affected by non-point source pollutants (NURP, 1993 in Walker et al., 1999). Metals, in particular, were considered to be the largest contributor to runoff pollution. Cole et al. (1984) reported that several metals were often found in runoff at concentrations greater than their respective water quality criteria. Cadmium was detected in 55% of Nationwide Urban Runoff Program (NURP) samples with concentrations ranging from 0.1 to 14 µg/L. Freshwater acute and chronic criteria for cadmium were exceeded 9 and 55% of the time, respectively. Heavy metal pollution in urban runoff is rivaled only by suspended sediment loads, biological and chemical oxygen demand, and coliforms (Cheebo et al., 2001; Scholes et al., 1998).

Lead, zinc, copper and cadmium are the heavy metals most frequently found in runoff. Concentrations of copper and zinc are usually the highest, with zinc often being orders of magnitude above the others. Concentrations reported in the literature are zinc (20 – 5000 µg/L) > copper ≈ lead (5 – 200 µg/L) > cadmium (<12 µg/L) (Davis et al.,

2001). Makepeace et al. (1995) also reported cadmium concentrations as high as 13.7 µg/L. Heavy metal contamination in runoff is most often associated with suspended particulate matter. Significant positive correlation between suspended sediment and heavy metal transport has been reported during rain events (Yuan et al., 2001). However, particle-bound metals can become available during transport (Cheebo et al., 2001; Gagnon and Saulnier, 2003). Highways and roadways may be the single largest contributing source of heavy metal pollution in urban runoff. Legret and Pagotto (1999) examined runoff from a major rural highway over a year-long study encompassing 50 rain events. Total cadmium concentrations in raw runoff waters ranged from 0.2 to 4.2 µg/L with a mean of 1.0 µg/L. Mean dissolved cadmium concentration in raw waters was 0.53 µg/L and ranged from 0.11 to 3.6 µg/L. Sediment cadmium concentrations ranged between 0.25 and 1.7 mg/kg. The authors calculated a surface loading of 0.0094 kg/km (1 km of a dual carriageway including hard shoulder covering 1.165 ha). Automobile brake linings and tire wear (2.7 and 2.6 mg Cd/kg) were determined to be the primary source for cadmium in highway runoff. Gromaire et al. (2000) reported median cadmium values of 0.65 and 5.50 mg/m^2 street for street washing waters and runoff waters, respectively. Respective particle-bound concentrations were 2.0 and 0.5 mg/kg. Hares and Ward (1999) examined a motorway with average daily traffic of 140,000 vehicles and observed a total cadmium concentration of 14.1 µg/L. Finally, Sörme and Lagerkvist (2002) estimated that tires and brake linings contributed 0.23 and 0.08 to 0.12 kg Cd/yr, respectively to a waste water treatment plant through stormwater runoff. Pekey et al. (2004) attributed 8 µg/L concentrations in a polluted stream partly to road traffic and the paint industry. Finally, Sansalone et al. (1996) noted that metals are

found mostly in dissolved form in pavement runoff. Dissolved to particulate metal element mass ratios for cadmium were as high as 3.6 mg/mg.

Numerous other sources of cadmium in runoff have also been investigated. Davis et al. (2001) listed building and roofing materials, vehicle brake emissions and oil as major sources for cadmium. Synthetic rainwater rinses of painted wood yielded the highest concentrations of building materials tested (<0.2 to 15 µg/m^2), followed by brick (<0.2 to 6.8 µg/m^2). Roofing sample rinses resulted in mean cadmium concentrations of 0.12, 1.3 and 0.6 µg/L for residential, commercial and industrial, respectively. Front brake rinses resulted in a mean cadmium concentration of 1.9 µg/L and used oil yielded concentrations as high as 100 µg/L. Annual cadmium loadings were estimated to be 0.0012 kg/ha-yr for residential and 0.0022 kg/ha-yr for commercial land use types. Sörme and Lagerkvist (2002) estimated that galvanized building materials contributed an estimated 0.25 kg Cu/yr. Duke et al. (1998) detected cadmium in >25% runoff samples from metal plating facilities. Mean cadmium concentrations were reported to be as high as 120 µg/L. During investigations of abandoned copper and sulfate mines, Gray (1998) measured cadmium concentrations from mine surface runoff ranging from 0 to 880 mg/L. Gray (1998) estimated a cadmium loading of 0.3 tons for that year.

Some exposure scenarios presented in this research may not seem environmentally realistic. For example, one may not expect a single event to last 24 hours or 5-minute exposures to reach cadmium concentrations as high as 10,000 µg/L. In fact, such scenarios may be possible if greater than base-flow concentrations linger in waterbodies or the scale of an event is unprecedented. The magnitude and duration of all events would have to be recorded to avoid any uncertainty. Review of

environmental concentrations above suggests a low probability that the shorter effective exposures of much higher concentrations observed here are likely to occur.

The protective nature of WQC in relation to non-point source pollution has been a matter of debate for many years. Numerous researchers have compared intermittent and continuous exposure to toxicants and have used such data to directly or implicitly challenge the reliability of WQC – which are based on continuous exposure data. Some have shown where intermittent exposure is more toxic (Abel, 1980; Abel and Garner, 1986; Ingersoll and Winner, 1982; Seim et al., 1984; Holdway and Dixon, 1986; Jarvinen et al., 1988a and 1988b). Others have presented data suggesting intermittent or pulsed exposure may be more toxic (Bailey, 1985; Holdway and Dixon, 1985; Fisher et al., 1994; Holdway et al., 1994; Handy, 1995; Novotny and Witte, 1997; Hosmer et al., 1998). Regardless of whether criteria are over- or underprotective, protection against intermittent exposure has been the subject of regulatory criticism.

WQC are clearly not applicable to pollution associated with runoff events. However, in the absence of other available benchmarks, they may be used as predictive tools. For example, if WQC are not exceeded, *C. dubia* exposed to single pulses of copper were usually protected by the criterion maximum concentration (CMC), but rarely protected by the criterion continuous concentration (CCC) (see Chapter 1). RHW used throughout this work had a hardness of 180 mg/L as $CaCO_3$. Given this, the CMC and CCC for cadmium would be approximately 3.77 and 0.39 µg/L, respectively and assumes no water effects ratio (EPA, 2001d). If criteria are not exceeded, the CMC for cadmium should be protective for most exposure combinations regardless of water type. 1-hour average concentrations for all exposures were well above the CMC – effective

concentrations exceed the CMC. The CCC for cadmium also appeared protective. All combinations affecting reproduction in RHW or TWE were above 0.39 µg/L when averaged over 4 days – effective concentrations exceed the CCC. Given environmental concentrations above, effective concentrations observed in these studies are not likely to occur, and WQC may not be exceeded.

Higher concentrations were required to achieve the same effect at the same duration in TWE. Protective effects of TWE are likely due to it having much higher values for total organic content (~8 mg/L) compared to that of RHW (<1 mg/L). Despite a water effects ratio ranging between 1.1 and 3.0 (depending on endpoint and duration) and lower hardness, the CMC and CCC for TWE are only slightly higher (4.95 and 0.55 µg/L, respectively) than those for RHW. These criteria are clearly overprotective for single pulse exposures to cadmium in more natural waters.

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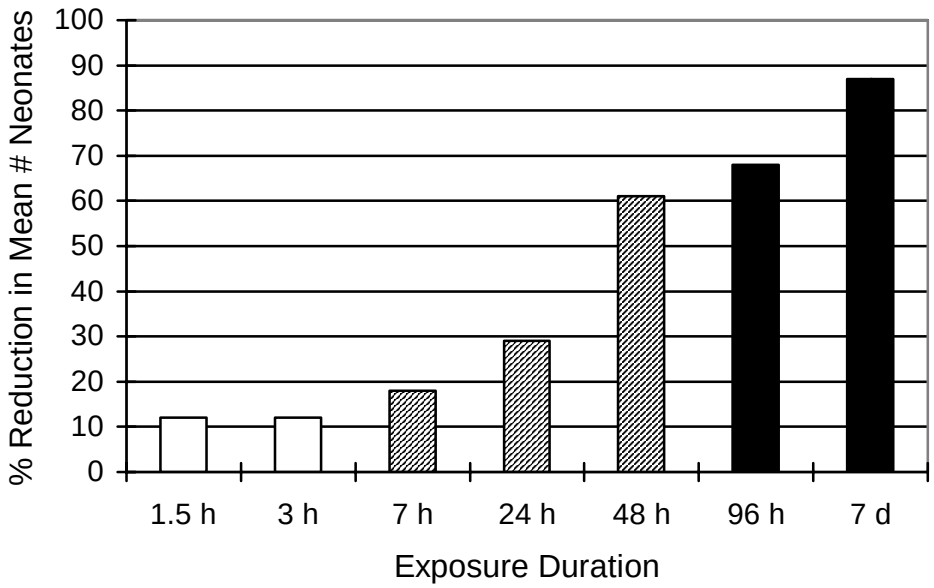

Figure 3.1. Percent reduction in neonate production from various durations in 80 µg/L cadmium contaminated stream water. Striped bars indicate significantly lower fecundity than control stream water. Solid bars also indicates significantly lower survival.

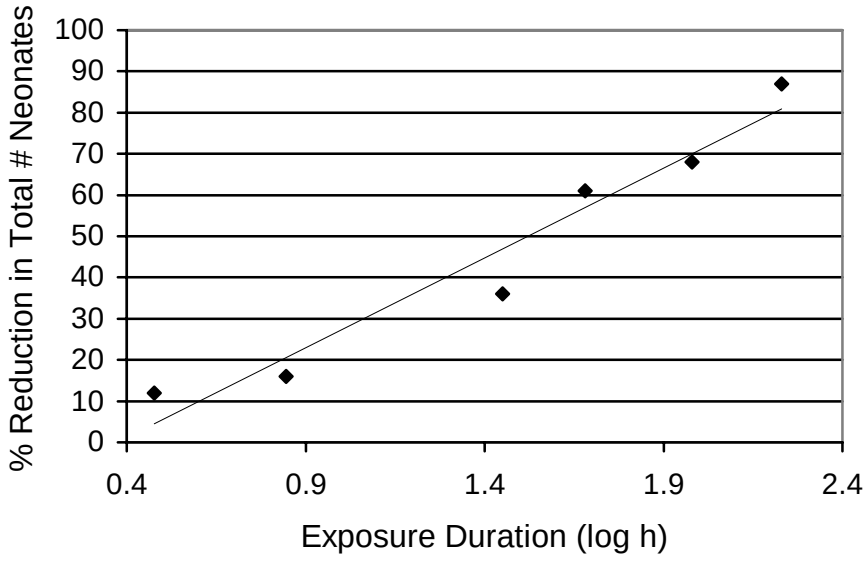

Figure 3.2. Percent reduction in neonate production following single pulse exposure to 80 µg/L cadmium contaminated stream water. The first point (log h 0.477) represents the longest duration having no significant affect on reproduction. The last two points (log h 1.98 and 2.23) also indicate significantly reduced survival. $R^2 = 0.9442$; $y = 43.619 \log x - 16.304$.

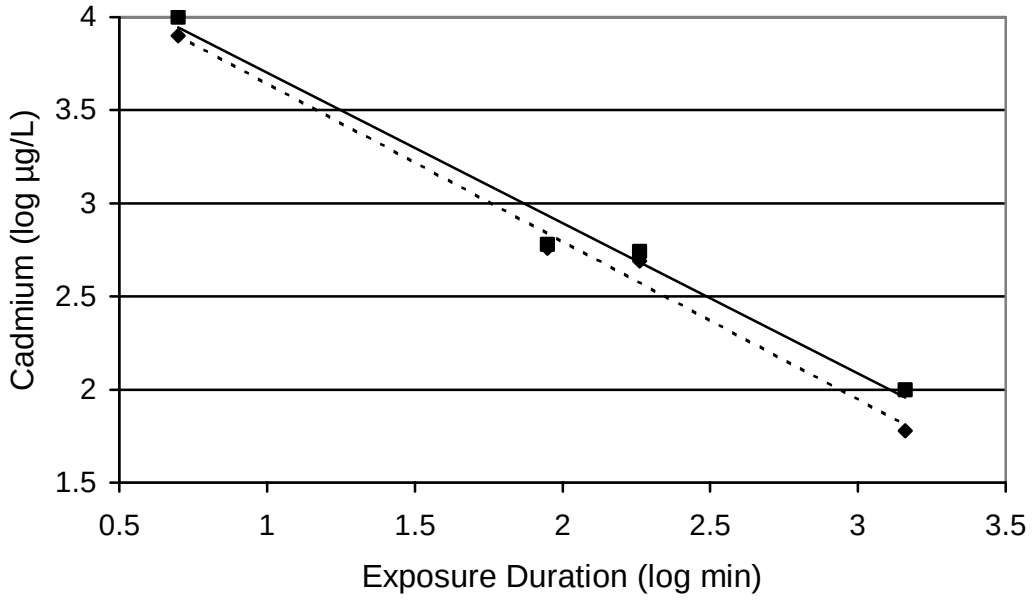

Figure 3.3. Lowest observed effect concentration-duration combinations for *C. dubia* survival in RHW (♦) or TWE (■). Trendlines are also shown as dashed or solid, respectively. RHW: $R^2 = 0.991$; log y = -0.8503 logx + 4.4973. TWE: $R^2 = 0.9845$; log y = -0.8076 logx + 4.5091.

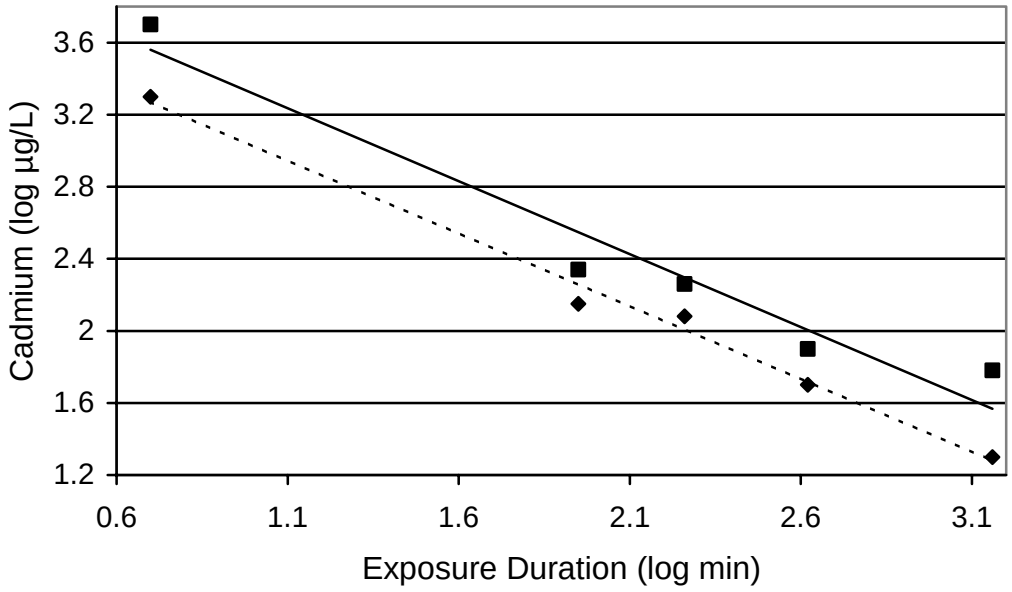

Figure 3.4. Lowest observed effect concentration-duration combinations for *C. dubia* fecundity in RHW (♦) or TWE (■). Trendlines are also shown as dashed or solid, respectively. RHW: $R^2 = 0.9917$; log y = -0.8091 logx + 3.8356. TWE: $R^2 = 0.9489$; log y = -0.8097 logx + 4.127.

CHAPTER 4

SUMMARY AND CONCLUSION

Introduction

Traditional toxicity tests are generally based on assumptions of continuous exposure. However, exposures due to rain-driven runoff events, periodic pesticide application and accidental spills are not continuous. Exposures of this type occur episodically as intermittent (repeating) events or as a single pulse. It is important to understand the long-term toxicological impacts of episodic pollution incidents to protect aquatic organisms. Otherwise, regulatory agencies may fail to associate a decline in populations with a particular incident because of its brevity (Handy, 1994).

Episodic pollution may have no detectable effects, or it may lead to any observable array of sublethal effects to mortality (McCahon and Pascoe, 1990). Minor ecological perturbations or serious degradation can result from a single event. Because effects observed during an event are not different from continuous exposure (of given duration), most studies are interested in post-exposure effects. Seager and Maltby (1989) recommended that any study of episodic pollution include post-exposure periods.

Effects of wet weather discharges on the environment have gained more attention as the regulatory focus of the Clean Water Act has shifted from continuous-flow effluents to episodic discharges produced by storm events (Herricks and Milne, 1998). However, the difficulty of estimating effects from time-varying exposures from measurements obtained from continuous exposure tests is often an important source of

uncertainty in ecological risk assessment (Reinert et al., 2002). For many years, researchers have been proposing incorporation of pulse-exposure test designs in risk assessment (Abel, 1980; Holdway and Dixon, 1986; Pascoe and Shazili, 1986; McCahon and Pascoe, 1990; Parsons and Surgeoner, 1991; Brent and Herricks, 1998; Naddy et al., 2000; Naddy and Klaine, 2001). Such tests could be used to better evaluate effects that may occur under more natural exposure conditions while retaining the advantages of simplicity and repeatability associated with single-species laboratory tests (Peterson et al., 2001).

Many approaches for investigating episodic pollution have been developed from conventional toxicity testing methods (McCahon and Pascoe, 1990). Including Whole Effluent Toxicity (WET) tests, conventional toxicity tests are simply modified to accommodate issues of time-varying exposure. For example, exposure durations may be altered or post-exposure observation periods included in the study design. WET tests are clearly designed for examination of point-source and continuous exposure scenarios, however, with modification, WET-type tests may be as useful in assessing non-point source exposure scenarios that have more complicated issues of time-scale toxicity.

Whether or not water quality criteria (WQC) for priority pollutants can be used for stormwater protection remains controversial in the U.S. and other countries. Consequently, stormwater abatement efforts are hampered by unavailability of wet-weather quality standards (Novotny and Witte, 1997). Field application of pesticides, rain-driven runoff events, and accidental spills may be intermittent, but their regularity is generally seasonal and less predictable. In addition, toxicant exposure during such

events is not likely the same each time. Environmental regulations do not address such scenarios, and the scientific literature has not addressed them in a regulatory framework.

Published research on effects of episodic exposure are dominated by studies of pesticides, however, several have investigated metals. Among these, many have examined effects from intermittent exposure (Lappivaara and Marttinen, 2005; Siddens et al., 1986; Seim et al., 1984; Hodson et al., 1983; Ingersoll and Winner, 1982). Several have also examined lethal (Brent and Herricks, 1998; Hutchinson et al., 1989; Abel and Garner, 1986; Pascoe and Shazili, 1986) and sublethal (McWilliams and Baird, 2002; Williams and Holdway, 2000; Handy, 1992; McCahon and Pascoe, 1991) effects of single-pulse exposures. Only two have investigated single pulse effects on cladocerans (McWilliam and Baird, 2002; Brent and Herricks, 1998).

Summary of Results

Effects of Single Pulse Exposures of Copper on C. dubia

Survival, fecundity and growth of *C.dubia* were adversely affected by increasing concentration-decreasing duration combinations (Table 1.3). Most mortality occurred within 24 hours post-exposure, however, some mortality was observed after 48 hours. The No- and Lowest observed effect concentrations (NOEC, LOEC) for 24-hour exposure were 92 and 112 µg/L, respectively. With few exceptions, combinations affecting fecundity were the same as those affecting growth. LOECs for fecundity and growth following a 24-hour exposure were both 58 µg/L.

A narrow threshold between lethal and sublethal effects was observed. For example, LOECs for 24-hour exposures were 112 and 58 µg/L for survival and fecundity, respectively. This threshold appeared fairly consistent with increasing concentration and subsequent decrease in duration. For any given duration, a slight concentration-response was observed in fecundity and growth before mortality occurred.

Relationships between concentration and duration were very similar for survival, fecundity and growth (Figures 1.1-1.3). NOECs and LOECs increased with respect to logarithmic decreases in duration. Differences in concentration between effective (significantly lower than controls) durations were relatively small at lower concentration-longer duration combinations and greater at higher concentration-shorter duration combinations. For example, LOECs for survival were 200 and 112 µg/L for 3- and 24-hour exposures, respectively. LOECs for 40- and 1-minute exposures were 225 and 800 µg/L, respectively.

Recovery of first generation adult *C. dubia* following pulsed exposure to copper.

Four different experiments from first generation studies were continued beyond the traditional 7-day test period. This was done to monitor reproductive recovery of adult *C. dubia* following 24- and 3-hour, and 1 to 5-minute exposures. LOECs for 24-hour and 1-minute exposures were 58 and 600 µg/L, respectively for fecundity (Table 2.3). At effective concentration-duration combinations, release of first brood neonates was often delayed by 12 to 24 hours especially at concentrations near the lethal threshold. Reproduction was almost invariably delayed by one to three days at concentrations resulting in significantly reduced survival. Effective combinations nearly always resulted

in significantly reduced neonate production in all three broods. At lower effective concentrations, third brood production was often more similar to controls suggesting some degree of recovery within the 7-day test period.

C. dubia fecundity was significantly reduced by 24-hour exposure to >58 µg/L copper after ten broods. Table 2.4 shows mean number of neonates produced for each individual brood and cumulative mean number of neonates produced per each subsequent brood. Mean neonate production for *individual broods* appeared to recover as they were generally not significantly different from controls after the fifth brood. Mean neonate production at all concentrations steadily increased to the fifth or sixth brood before rapidly declining (Figure 2.2). The expected monotonic concentration-response also remained only through five broods. Cumulative mean number of neonates per subsequent brood showed little sign of recovery. 58 µg/L was the only effective concentration where the total of ten broods was not significantly less than controls (Table 2.4). In addition, the observed monotonic concentration-response was observed throughout ten broods (Figure 2.3) – evidence against recovery of total neonate production.

Trends in individual brood and cumulative neonate production recovery were generally the same regardless of exposure durations ≥5 minutes. Durations shorter than 3 hours however, revealed faster recovery of cumulative mean neonate production as duration decreased. Cumulative mean neonate production from the 3-hour exposure did not recover after five broods (Table 2.5, Appendix Figures A.4-A.6). On the other hand, cumulative mean neonate production following 5-minute exposure to concentrations <412 µg/L were not significantly different from controls after five broods (Table 2.6,

Appendix Figures A.7-A.9.). Finally, individual brood neonate production for 1-minute exposure to 600 µg/L appeared to recover by the fifth brood (Table 2.7, Appendix Figures A.10-A.12).

Reproductive recovery of individuals from combinations affecting survival were also monitored. 24-hour exposure to 114 µg/L copper resulted in 80% mortality of *C. dubia*, yet fecundity of the two surviving individuals was higher than all other concentrations. Figure 2.4 offers a comparison between cumulative mean neonate production including and excluding deaths. Trends in neonate production of concentrations significantly affecting survival were similar regardless of duration. Although such populations would not be expected to recover, cumulative mean neonate production of survivors was usually very similar to controls. Cumulative mean fecundity of survivors from 2-minute exposure to 600 µg/L was 81.8 (8.47) after eight broods. See Appendix (Figures A.3, A.6, A.9 and A.12) for similar comparisons from the other exposure scenarios.

Effects on second generation individuals following parental exposure to copper.

Reproductive effects were observed in second generation (F_1) individuals in every exposure scenario tested. Degree of effects however, decreased dramatically with decreasing exposure duration. 24-hour exposure resulted in significantly reduced fecundity of F_1 individuals at concentrations ≥30 µg/L (Table 2.8, Table A.2). This was surprising because 30 and 38 µg/L did not result in significantly reduced fecundity of first generation individuals (parents). Nearly every F_1 brood from parental first and second broods were significantly lower than controls. F_1 third brood showed signs of

recovery as fewer broods and fewer concentrations were significantly lower than controls.

No effects on F_1 fecundity was observed from the 3-hour exposure experiment at any concentration tested (Table 2.9), even those previously affecting survival of parents. Parental first brood neonates were however, released two to three days later than in other concentrations. F_1 individuals from 5-minute exposures exhibited significantly lower fecundity at 417 µg/L, the LOEC for survival on parents (Table 2.10). Finally, 1 and 2-minute exposure to 600 µg/L also did not result in any significant differences in F_1 fecundity from parental first brood individuals (Table 2.11).

Effects of Single Pulse Exposures of Cadmium on C. dubia

Continuous and pulse exposure to 80 µg/L cadmium.

Continuous exposure of 80 µg/L cadmium-contaminated stream mesocosm water (see Brooks et al., 2004) resulted in significantly reduced survival of *C. dubia* in 3 to 7 days. A 96-hour pulse was required to significantly reduce survival after transfer to control stream water (Figure 3.1). *C. dubia* fecundity was significantly reduced following exposure durations >3-hours. The relationship between percent neonate reduction and exposure duration is shown in Figure 3.2.

Effective concentration-duration combinations in RHW or TWE.

Survival was adversely affected by increasing cadmium concentration-decreasing duration combinations (Table 3.5). The LOEC for 24-hour exposure in reconstituted hard water (RHW) was 41 µg/L, whereas the LOEC for 24-hour exposure in treated wastewater effluent (TWE) was 83 µg/L – much higher than that of RHW.

Fecundity *C. dubia* was also adversely affected by increasing concentration-decreasing duration combinations (Table 3.5) – much in the same way as survival. LOECs for fecundity following a 24-hour exposure were 17 and 48 µg/L in RHW and TWE, respectively.

As with copper, a narrow threshold between lethal and sublethal effects was observed. This was observed regardless of water type. For example, LOECs for 24-hour exposures in RHW were 41 and 17 µg/L for survival and fecundity, respectively and 48 and 83 µg/L, respectively in TWE. This threshold appeared fairly consistent with increasing concentration and subsequent decrease in duration.

Relationships between concentration and duration were very similar for survival and fecundity. NOECs and LOECs increased logarithmically with respect to logarithmic decreases in duration (also regardless of water type). This relationship was very similar to that observed with copper, however, effective concentrations of cadmium were much higher at comparable shorter durations. Differences in cadmium concentration between effective durations were relatively small at lower concentration-longer duration combinations and greater at higher concentration-shorter duration combinations. For example, LOECs for survival (Figure 3.3) were 8000 and 41 µg/L for 5-minute and 24-hour exposures in RHW, respectively. LOECs for fecundity followed nearly identical trends although concentrations were proportionally lower. The relationship between concentration and duration are illustrated in Figures 3.3 (survival) and 3.4 (fecundity).

Application of Results

Over 100 tests were performed to determine no- and lowest observed effect concentration-duration combinations for copper and over 40 for cadmium. From these data, relationships were established that may be useful in predicting lethal and sublethal effects from single-pulse exposure to these metals. Runoff events are highly event and site specific, however, research presented herein may be used as a predictive tool under various conditions.

Effective concentration-duration relationships were determined for *C. dubia* survival (Figure 4.1) and fecundity (Figure 4.2) following single-pulse exposures to copper. The relationship for growth was also established but is not shown here as it is nearly identical to that for fecundity. Effective concentration-duration relationships were also determined for *C. dubia* survival (Figure 4.3) and fecundity (Figure 4.4) following single-pulse exposures to cadmium in RHW or TWE. Most experiments were conducted in RHW, however, relationships in TWE were very similar though effective concentrations were somewhat higher for given durations. Therefore, relationships presented here should be applicable to more natural waters.

It is the authors' intention that data and relationships presented herein may be used by anyone studying or monitoring runoff event or accidental spill toxicity of cadmium or copper. For example, watershed monitoring programs may apply this information to known or suspected metal concentrations measured during storm events. A measured value of copper from a stream or runoff sample, can be applied to the relationship established in Figures 4.1 or 4.2 to predict the exposure duration required to cause effects. Conversely, if the exposure duration of an event is known, then the

concentration required to cause effects can be estimated. Relationships shown in Figures 4.3 and 4.4 can be utilized if the toxicant of interest is cadmium.

This research focused on single toxicants, however, information on toxicity of mixtures, along with professional judgment, may enhance application of concentration-duration relationships presented herein. In addition, knowledge of water effects ratios for specific sites may also enhance use of these relationships. Such are areas for further research.

Conclusions

Survival, growth and fecundity of *Ceriodaphnia dubia* were adversely affected by single pulse exposures to copper. Survival and fecundity are also adversely affected by single pulses of cadmium. Regardless of endpoint, similar effects (NOECs, LOECs) result from low concentration-longer duration exposure combinations and high concentration-shorter duration combinations. This relationship is logarithmic with respect to duration and concentration. At lower concentrations, large decreases in duration corresponded to small increases in effective concentration. On the other hand, at higher concentrations, slight decreases in duration corresponded to large increases in effective concentration. This held true regardless of endpoint or metal but was more pronounced with cadmium. Finally, a narrow threshold between lethal and sublethal effects was observed. This also appeared to be independent of endpoint or toxicant.

Neonate production of first generation adult *C. dubia* appeared to recover from pulsed copper exposure upon examination of individual broods. Mean neonate production was generally not significantly less than controls by the fifth or sixth brood,

however, this is likely a consequence of increased variability and reduced statistical power. Cumulative mean neonate production however, showed almost no signs of recovery at exposure durations ≥3 hours. Cumulative mean neonate production did appear to recover following ≤5-minute exposures except at concentrations nearest and above the lethal threshold. Pulsed exposure to copper also resulted in diminished fecundity of unexposed second generation individuals. Effects on second generation individuals were pronounced following parental exposure for 24 hours but lacking after parental exposures ≤3 hours.

C. dubia survival and fecundity were also adversely affected by single-pulse exposures to cadmium. Effective concentration-duration combinations were determined for laboratory RHW and TWE. Concentrations significantly affecting survival were slightly higher in TWE regardless of duration. As shown in Figure 3.3 or 4.3, the slope of the relationship between survival effects in RHW or TWE are very similar and begin to approach each other at the highest concentration-shortest duration. Concentrations significantly affecting fecundity were considerably higher in TWE compared to RHW. The slope of the relationship between affects on fecundity in RHW or TWE were nearly identical although RHW had a slightly higher R^2 value (Figure 3.4 or 4.4). Higher effect concentrations in TWE are likely due to much higher measured values for total organic content (~8 mg/L) as compared to RHW (<1 mg/L). Hardness and alkalinity were generally lower in TWE.

Effect trends of single-pulse exposures to cadmium were very similar to those of copper regardless of water type. Briefly, effective concentrations increased logarithmically with respect to decreasing duration. Combinations significantly affecting

survival almost always exerted their effect within 24 hours. A sharp threshold between effects on fecundity and survival was observed, and only a slight concentration-response was observed between the two.

Although not a direct part of previous studies, comparisons of copper and cadmium pulse exposures in RHW offered some interesting results. A much greater range of effective cadmium concentrations was observed for the same exposure durations (5 minutes to 24 hours). Cadmium concentrations significantly affecting *C. dubia* fecundity and survival were lower for exposure durations >2 and >8 hours, respectively (Figures 4.5 and 4.6). On the other hand, effective copper concentrations were lower for durations <2 and <8 hours, respectively. These results are interesting though not terribly surprising. Our laboratory has shown that cadmium is less acutely toxic to *C. dubia* but more sublethally toxic than copper – the longer the exposure, the more toxic cadmium becomes relative to copper. 48-hour LC50s average ~40 to 50 µg/L and ~20 to 30 µg/L for cadmium and copper, respectively. 7-day LOECs for *C. dubia* fecundity have been 3.4 and 46 µg/L, respectively. Results of single-pulse exposures agree with these findings that cadmium in more toxic than copper with longer exposure durations.

A review of environmental concentrations and sources of cadmium and copper in runoff was presented in previous chapters. Lead, zinc, copper and cadmium are the heavy metals most frequently found. Concentrations of copper and zinc are usually the highest, with zinc often being orders of magnitude above the others. Concentrations reported in the literature are zinc (20 – 5000 µg/L) > copper ≈ lead (5 – 200 µg/L) > cadmium (<12 µg/L) (Davis et al., 2001). Walker et al. (1999) also reported a range of

copper concentrations between 0.06 and 1410 µg/L. Makepeace et al. (1995) also reported a cadmium concentrations as high as 13.7 µg/L. Highways, roadways (vehicle emissions) and certain building materials are the principal sources of cadmium and copper in runoff.

It is clear that WQC are not applicable to single-pulse exposures such as those associated with rain-driven runoff events. However, with no other available benchmarks, predictions may be made using worst-case exposure scenarios as to whether or not they may be problematic. The criterion maximum concentration (CMC) and criterion continuous concentration (CCC) for total recoverable copper in RHW are 24 and 15 µg/L, respectively (US Environmental Protection Agency, (EPA) 1996). A 24-hour pulse exposure to 120 µg/L (LOEC for survival) or 60 µg/L (LOEC for fecundity) represent worst-case scenarios presented in these studies for copper. Based on reported environmental concentrations, such exposures *are possible* and the respective WQC may be exceeded. The CMC and CCC for total recoverable cadmium in RHW are 3.9 and 0.42 µg/L, respectively (EPA, 2001). A 24-hour pulse exposure to 60 µg/L (LOEC for survival) or 20 µg/L (LOEC for fecundity) represent worst-case scenarios for cadmium in RHW. Given reported environmental concentrations above, 60 or 20 µg/L cadmium are *not likely* to occur, and effect levels definitely exceed WQC.

Data from the City of Denton Watershed Protection Program (April 2001 to present) support the above findings (Banks and Wood, 2001; Banks, 2002; and subsequent Monthly Reports). Mean measured total cadmium and copper concentrations have been reported as 1.6 and 22.0 µg/L, respectively from Cooper Creek stormwater samples (Table 4.1). Among the major watersheds under City of

Denton jurisdiction, Cooper Creek was the only one to show regular acute toxicity to *C. dubia* following storm events (unpublished data). Toxicity due to metals however, is unclear. Stormwater samples were acutely toxic two and six times when cadmium and copper, respectively were detected. Samples were not toxic twice when neither metal was detected. Toxic samples were observed when cadmium was not detected and when concentrations reached 11 µg/L. Toxic samples were also observed when copper was not detected to when concentrations reached as high as 37 µg/L. Toxicity was observed only twice when both metals were detected. Frequency of stormwater toxicity has decreased considerably in the last two years while metal concentrations have not. This may be explained, in part, by reductions in concentrations of diazinon (Banks et al., In press) and chlorpyrifos (Banks et al., 2005) since the Federally mandated ban of December, 2001.

Runoff related toxicity is a major problem in the United States and elsewhere. The EPA (2000) cites non-point source pollution as a serious water quality concern. Urban runoff has been reported as the second most frequent cause of surface water pollution in the US (Walker et al., 1999). EPA reported that 87% of 246 major waterbodies in the United States were affected by non-point source pollutants (NURP, 1993 in Walker et al., 1999). Metals, in particular, were considered to be the largest contributor to runoff pollution. Cole et al. (1984) reported that several metals were often found in runoff at concentrations greater than their respective WQC. Many countries require abatement of pollution by wet weather, yet there is no yardstick by which to regulate wet-weather pollution. Abatement efforts are greatly hampered by

Table 4.1. Measured total metal concentrations (µg/L) from Cooper Creek, Denton, Texas when above detection limits.

	Measured Concentration			
Metal	Mean	1 SD	Range	n[1]
Cadmium	1.6	3.30	0.2-11.0	10
Copper	22.0	12.08	4.7-55.2	25

[1] Numbers of samples where metal was detected. 36 samples reported.

unavailability of wet-weather WCQ or standards (Novotny and Witte, 1997). Because episodic pollution is highly site and event specific, it is the hope of the author that research presented herein will have applications in ecological risk assessment of episodic metal pollution beyond single sites or events and subsequent population recovery. Research presented herein may also have applications towards the development of a biotic ligand model that incorporates duration of exposure and sublethal effects.

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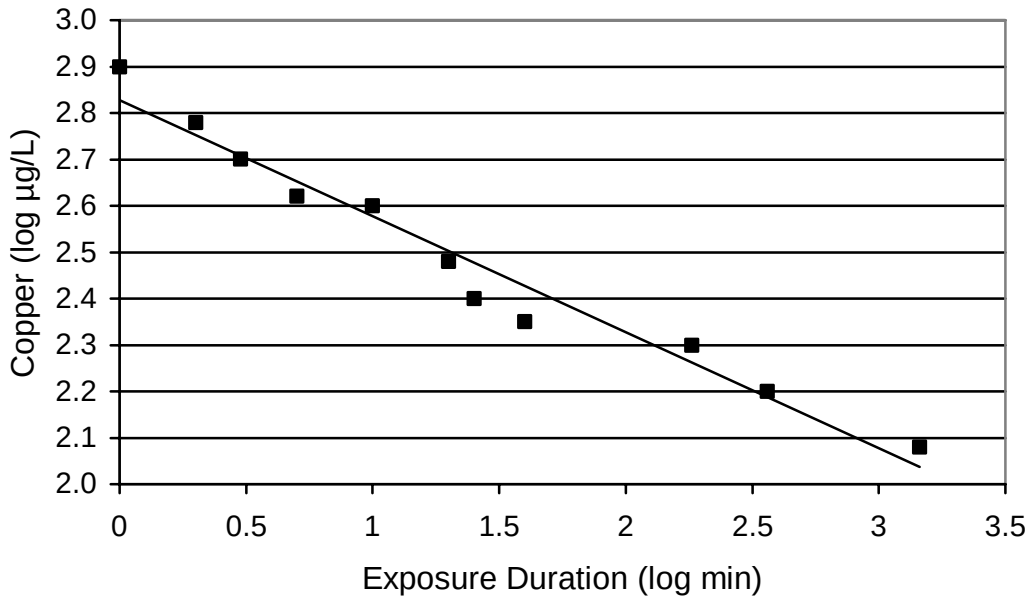

Figure 4.1. Lowest observed effect concentration-duration combinations for *C. dubia* survival following exposure to copper. LOEC-Ts: $R^2 = 0.9633$, log y = -0.2502 logx + 2.8275.

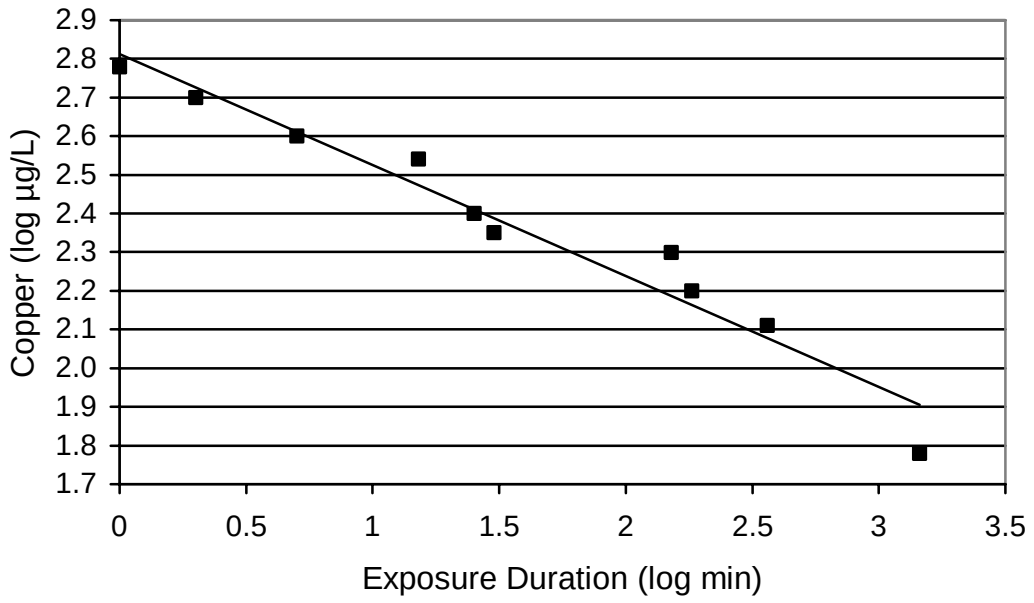

Figure 4.2. Lowest observed effect concentration-duration combinations for *C. dubia* fecundity following exposure to copper. LOEC-Ts: $R^2 = 0.9522$, log y = -0.2870 logx + 2.8128.

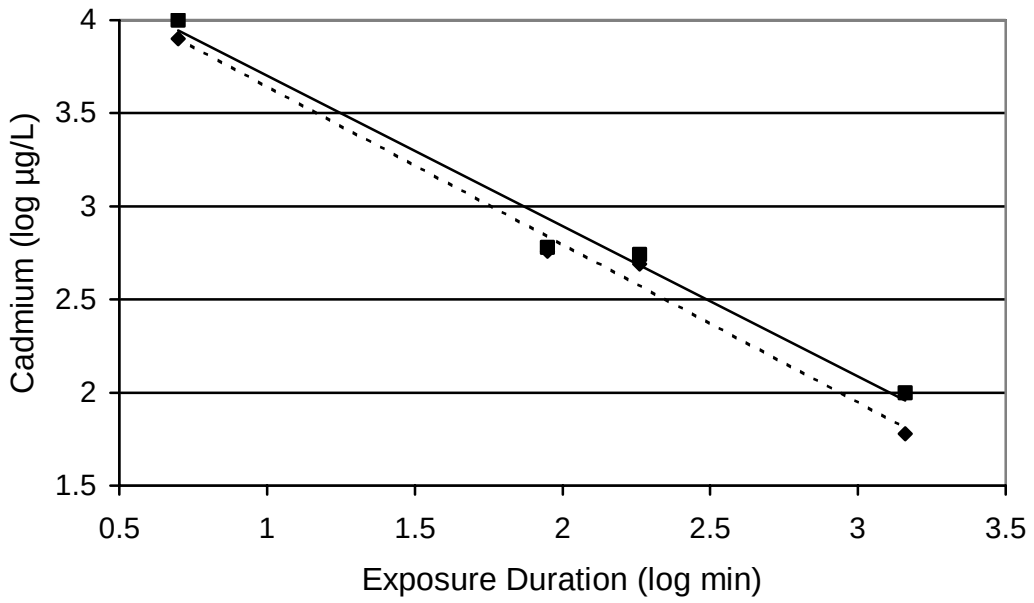

Figure 4.3. Lowest observed effect concentration-duration combinations for *C. dubia* survival following exposure to cadmium in RHW (diamonds) or TWE (squares). Trendlines are also shown as dashed or solid, respectively. RHW: $R^2 = 0.991$; log y = -0.8503 logx + 4.4973. TWE: $R^2 = 0.9845$; log y = -0.8076 logx + 4.5091.

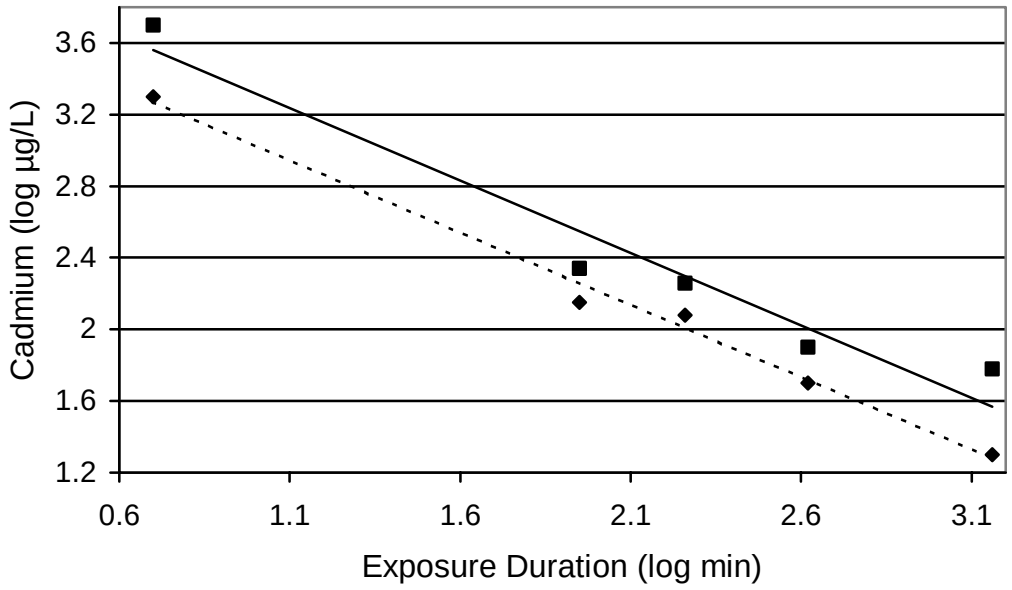

Figure 4.4. Lowest observed effect concentration-duration combinations for *C. dubia* fecundity following exposure to cadmium in RHW (diamonds) or TWE (squares). Trendlines are also shown as dashed or solid, respectively. RHW: $R^2 = 0.9917$; log y = -0.8091 logx + 3.8356. TWE: $R^2 = 0.9489$; log y = -0.8097 logx + 4.127.

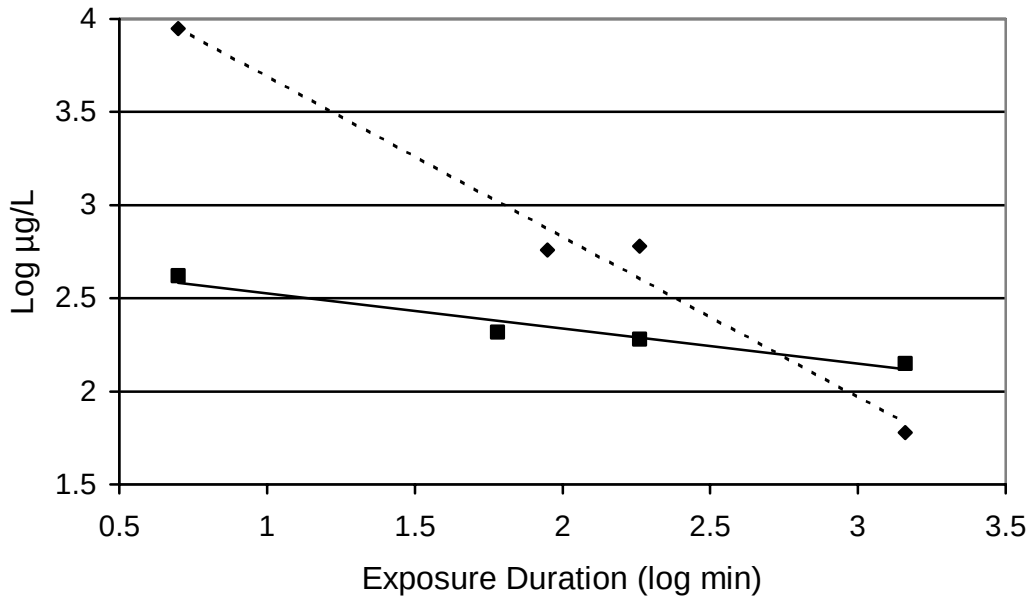

Figure 4.5. Lowest observed effect combinations for *C. dubia* survival exposed to either cadmium (diamonds) or copper (squares) in RHW. Trendlines are also shown as dashed or solid, respectively. Cadmium: $R^2 = 0.9807$; log y = -0.8637 logx + 4.5599. Copper: $R^2 = 0.9501$; log y = -0.489 logx + 2.7157.

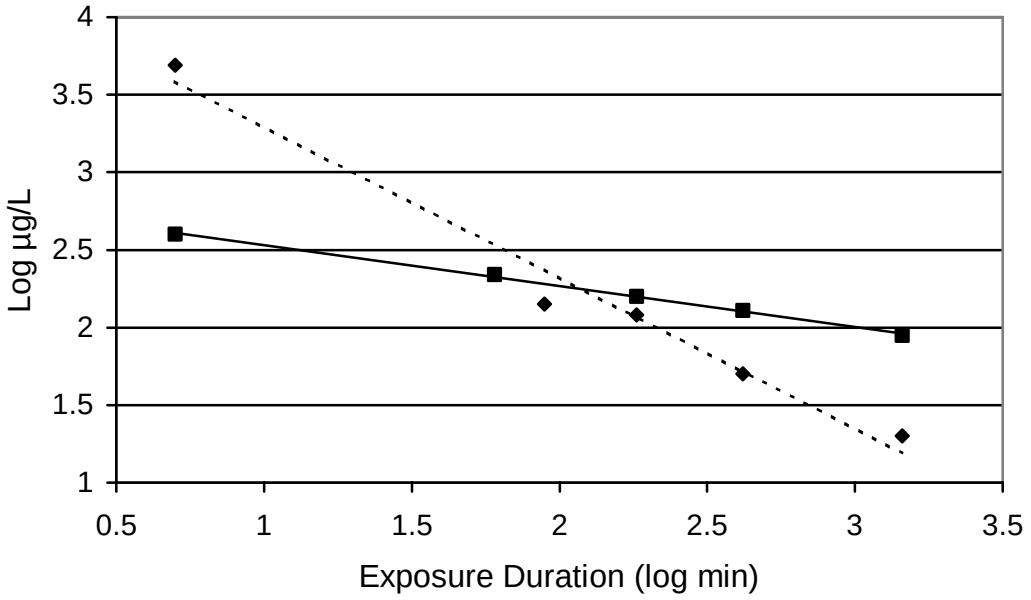

Figure 4.6. Lowest observed effect combinations for *C. dubia* fecundity exposed to either cadmium (diamonds) or copper (squares) in RHW. Trendlines are also shown as dashed or solid, respectively. Cadmium: $R^2 = 0.9785$; log y = -0.9742 logx + 4.2667. Copper: $R^2 = 0.9980$; log y = -0.2634 logx + 2.7941.

APPENDIX:

SUPPLEMENTAL DATA AND FIGURES TO CHAPTER 2

Table A.1. Mean (1 SD) number of neonates produced following 24-hour exposure: second experiment.

Brood	Concentration (µg/L)							
	RHW	MSD (PMSD)[1]	77	93	116	133	150[2]	171[2]
1	4.5 (0.85)	0.9 (20.8)	3.2 (0.92)	3.3 (0.82)	1.8 (1.03)	1.0 (1.05)	1.2 (1.40)	0.8 (1.32)
2	9.8 (1.40)	1.9 (19.2)	8.3 (0.95)	7.2 (1.03)	5.1 (2.69)	2.8 (2.57)	3.0 (2.83)	2.0 (2.71)
1-2	14.3 (1.89)	2.6 (18.1)	11.5 (1.18)	10.5 (1.35)	6.9 (3.54)	3.8 (3.43)	4.2 (4.13)	2.8 (3.94)
3	14.6 (0.97)	2.9 (19.8)	12.9 (0.99)	11.3 (1.42)	9.3 (3.77)	6.5 (4.90)	5.6 (5.12)	2.9 (4.01)
1-3	29.9 (4.04)	5.1 (17.6)	24.4 (1.90)	21.8 (2.53)	16.2 (6.94)	10.3 (8.14)	9.8 (9.10)	5.7 (7.38)
4	15.7 (1.42)	3.5 (22.2)	15.8 (1.46)	13.3 (2.00)	11.6 (4.43)	8.1 (5.76)	6.7 (5.91)	4.7 (6.13)
1-4	44.7 (3.40)	8.2 (18.3)	40.2 (2.62)	35.1 (4.36)	26.8 (10.69)	18.4 (13.56)	16.5 (14.76)	10.4 (13.43)
5	13.4 (0.97)	3.5 (26.3)	14.3 (1.06)	10.8 (3.88)	11.1 (3.98)	7.8 (5.43)	7.2 (6.32)	4.1 (6.61)
1-5	58.1 (3.90)	10.9 (18.7)	54.5 (2.64)	45.9 (4.51)	37.9 (14.20)	26.2 (18.75)	23.7 (20.08)	14.5 (19.22)
6	13.6 (1.78)	4.9 (36.3)	15.0 (2.11)	11.5 (6.19)	12.9 (4.84)	10.3 (7.27)	8.2 (7.15)	4.7 (7.60)
1-6	71.7 (4.50)	14.7 (20.6)	69.5 (2.68)	57.4 (7.71)	50.8 (18.68)	36.5 (25.61)	31.9 (27.76)	19.2 (26.35)
7	11.5 (2.59)	5.3 (46.1)	14.6 (4.20)	11.2 (6.10)	11.0 (4.35)	10.1 (7.64)	9.3 (8.15)	2.7 (5.70)
1-7	83.2 (5.05)	19.0 (22.9)	84.1 (5.38)	68.6 (12.95)	60.6 (23.09)	46.6 (32.66)	41.2 (35.79)	21.9 (30.88)
8	10.6 (3.98)	5.7 (53.4)	10.9 (4.53)	7.4 (6.13)	9.2 (5.51)	10.4 (7.52)	8.1 (7.16)	3.6 (5.85)
1-8	93.8 (7.63)	23.2 (24.8)	95.0 (8.12)	76.0 (16.91)	69.8 (26.79)	57.0 (39.78)	49.3 (42.65)	25.5 (36.58)

Values highlighted were significantly less than controls.
Thick bold line after broods 1-3 denotes results at the end of the traditional 7-day test.
[1] MSD = minimum significant difference. PMSD = percent MSD.
[2] Concentrations resulting in significantly reduced survival and were not included in analyses for reproduction.

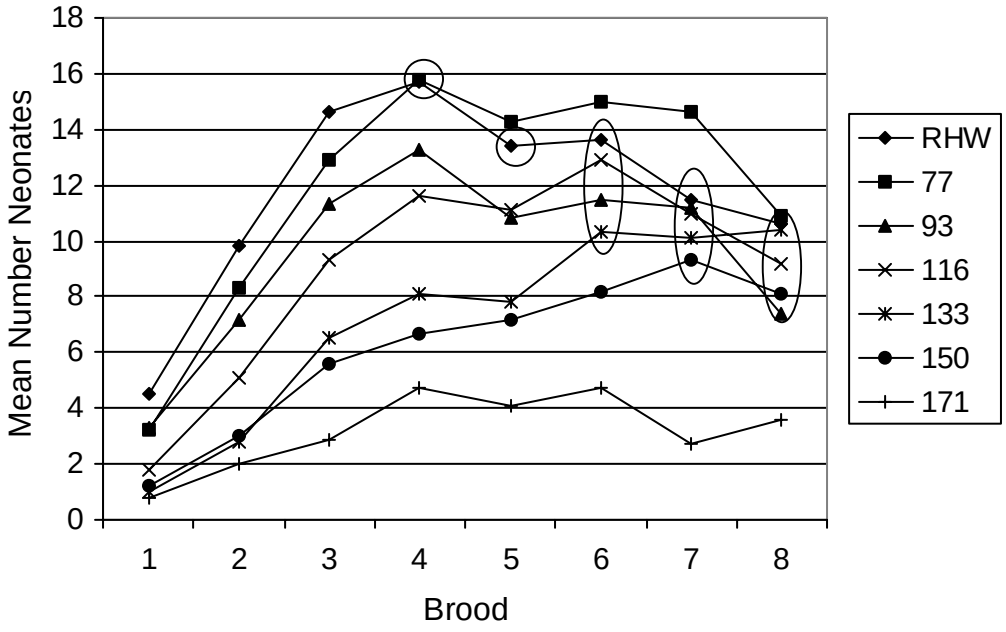

Figure A.1. Mean number of neonates produced per female for each individual brood (24-hour exposure, second experiment). Error bars not shown for clarity. Ovals contain concentrations found not to be significantly less than controls.

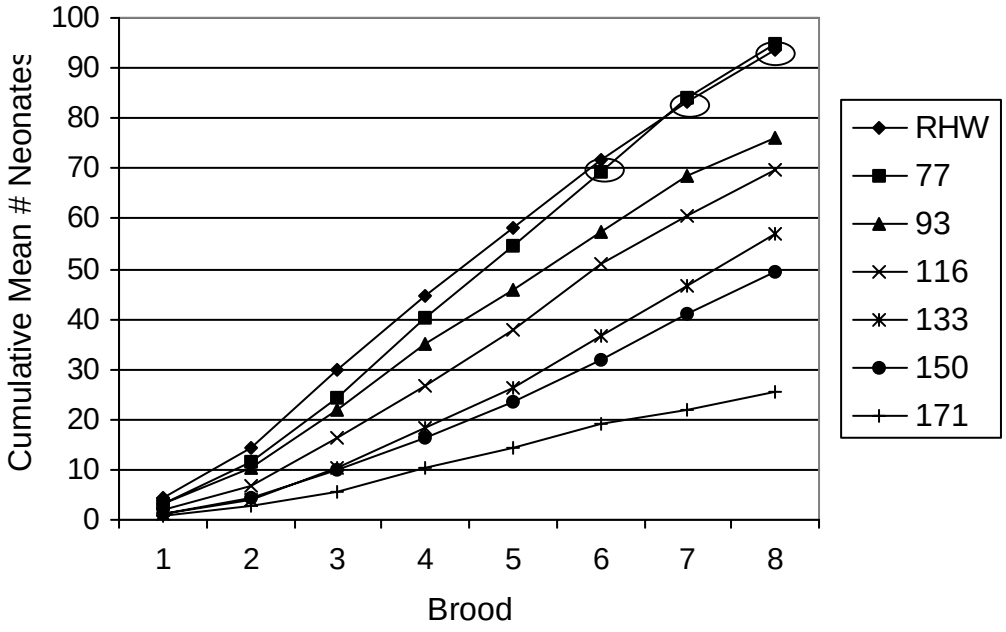

Figure A.2. Cumulative mean number of neonates produced per female following each subsequent brood (24-hour exposure, second experiment). Error bars not shown for clarity. Ovals contain groups found not to be significantly less than controls.

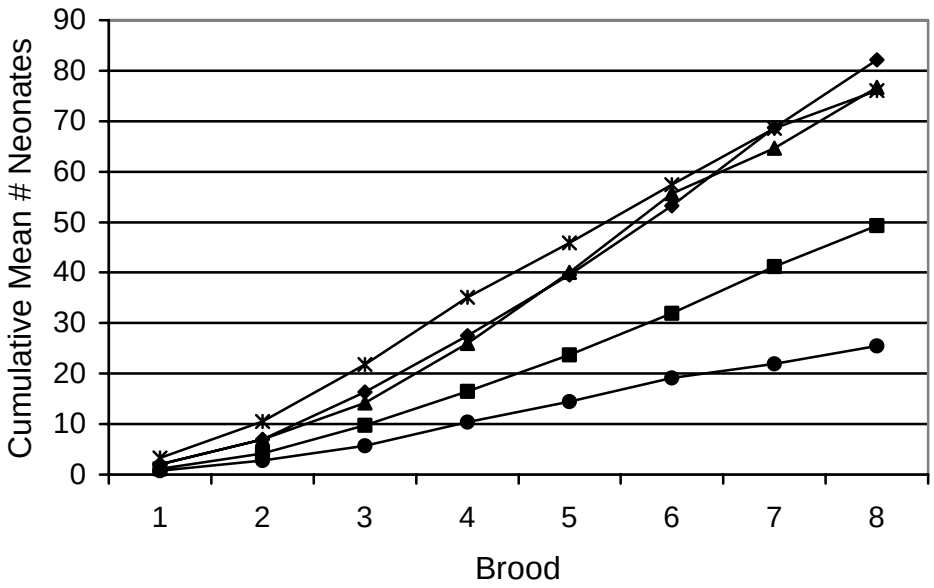

Figure A.3. Cumulative mean number of neonates produced (24-hour exposure, second experiment) at 150 and 171 µg/L when deceased adults were counted as zero (n=10) neonates produced (squares and circles, respectively) and when neonates of survivors only (n=6 and 4, respectively) were counted (diamonds and triangles, respectively). RHW reference (star).

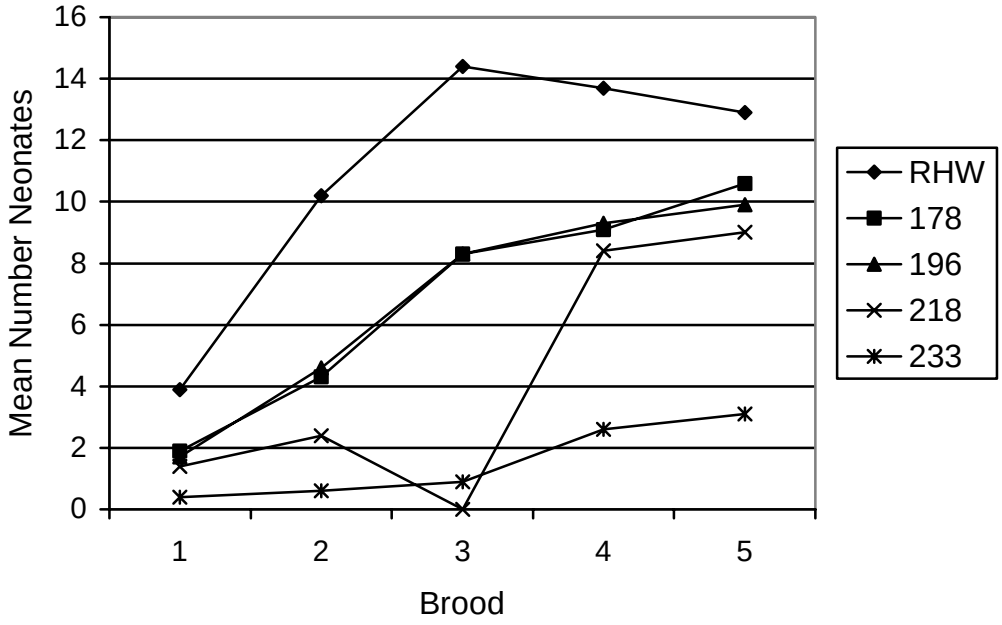

Figure A.4. Mean number of neonates produced per adult female for each individual brood: 3-hour exposure. Error bars not shown for clarity. Neonate production was not significantly less than controls only from broods four and five at 178 and 196 µg/L.

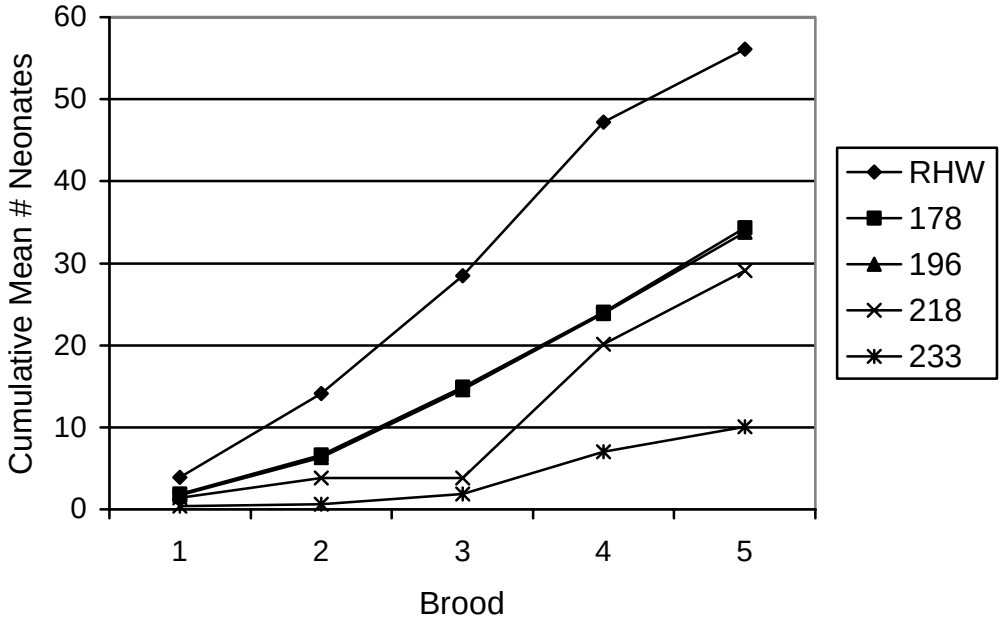

Figure A.5. Cumulative mean number of neonates produced per adult a female following each subsequent brood: 3-hour exposure. Error bars not shown for clarity. Neonate production was significantly lower than controls for at all concentrations and broods.

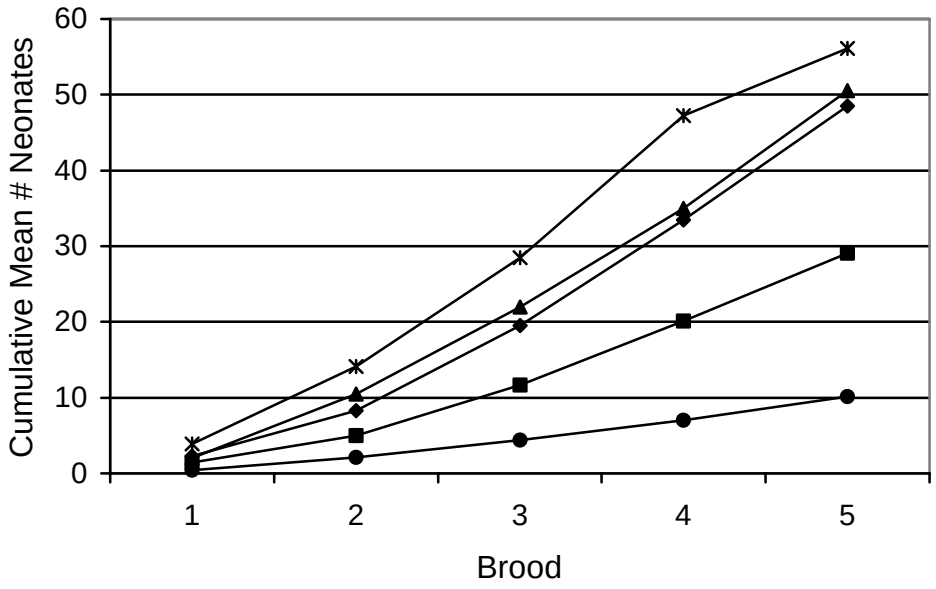

Figure A.6. Cumulative mean number of neonates produced at 218 and 233 µg/L when deceased adults were counted as zero (n=10) neonates produced (squares and circles, respectively) and when neonates of survivors only (n=6 and 2, respectively) were counted (diamonds and triangles, respectively): 3-hour exposure. RHW reference (star).

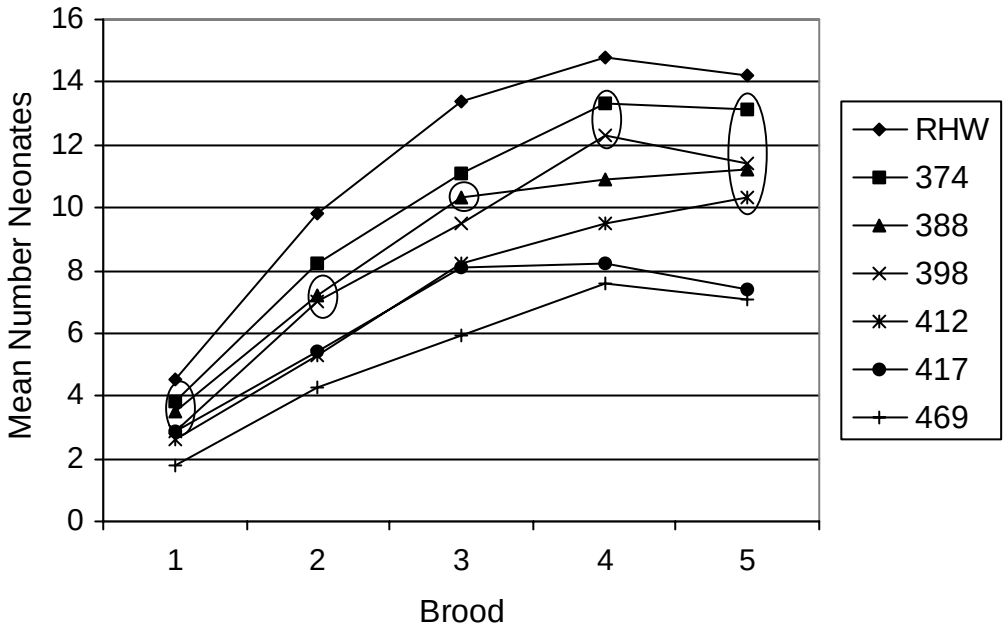

Figure A.7. Mean number of neonates produced per adult female for each individual brood: 5-minute exposure. Error bars not shown for clarity. Ovals contain concentrations found not to be significantly less than controls.

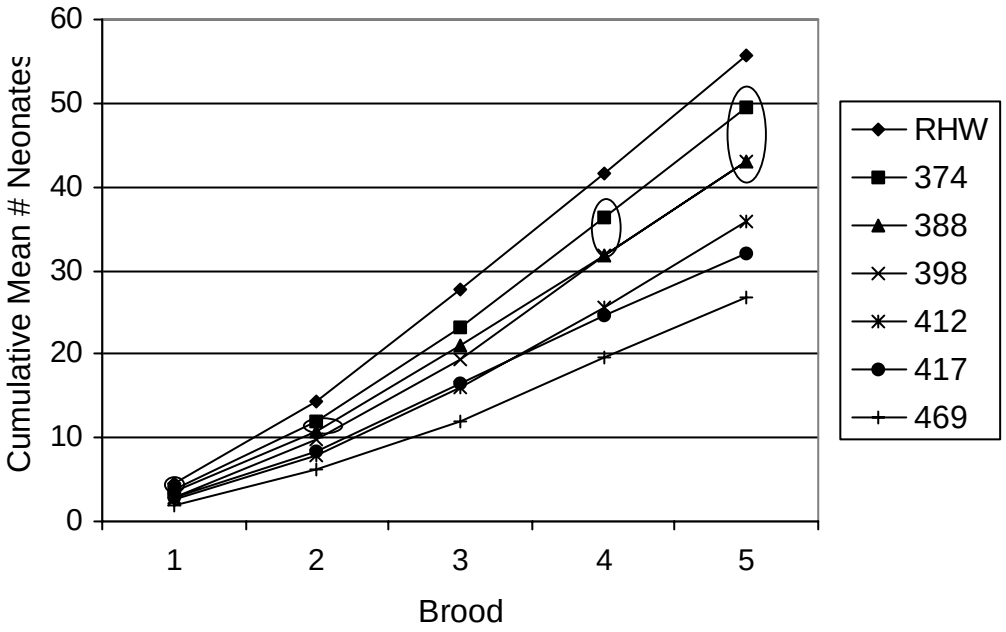

Figure A.8. Cumulative mean number of neonates produced per adult female following each subsequent brood: 5-minute exposure. Error bars not shown for clarity. Ovals contain concentrations found not to be significantly less than controls. 388 µg/L was significantly less than controls after four broods although it is contained in the oval.

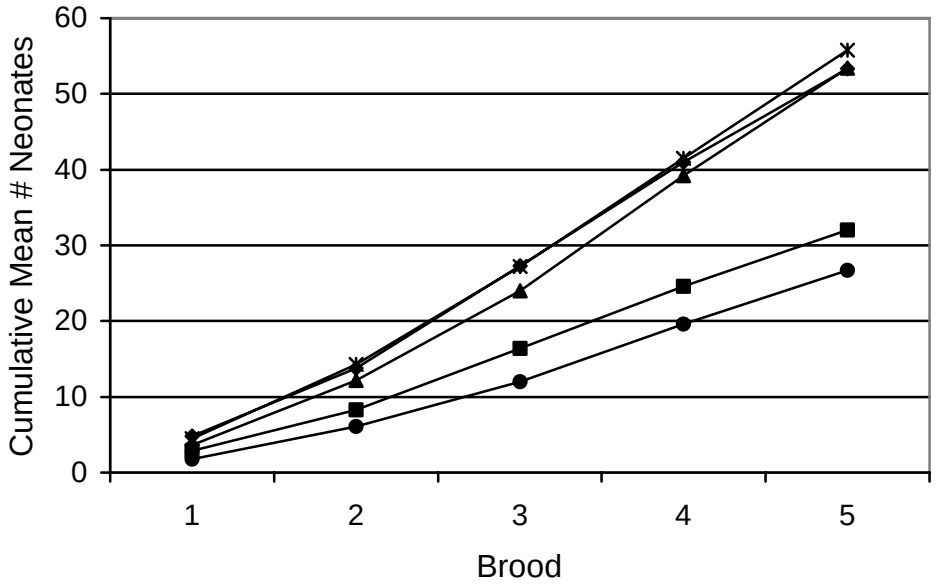

Figure A.9. Cumulative mean number of neonates produced at 417 and 469 µg/L when deceased adults were counted as zero (n=10) neonates produced (squares and circles, respectively) and when neonates of survivors only (n=6 and 5, respectively) were counted (diamonds and triangles, respectively): 5-minute exposure. RHW reference (star).

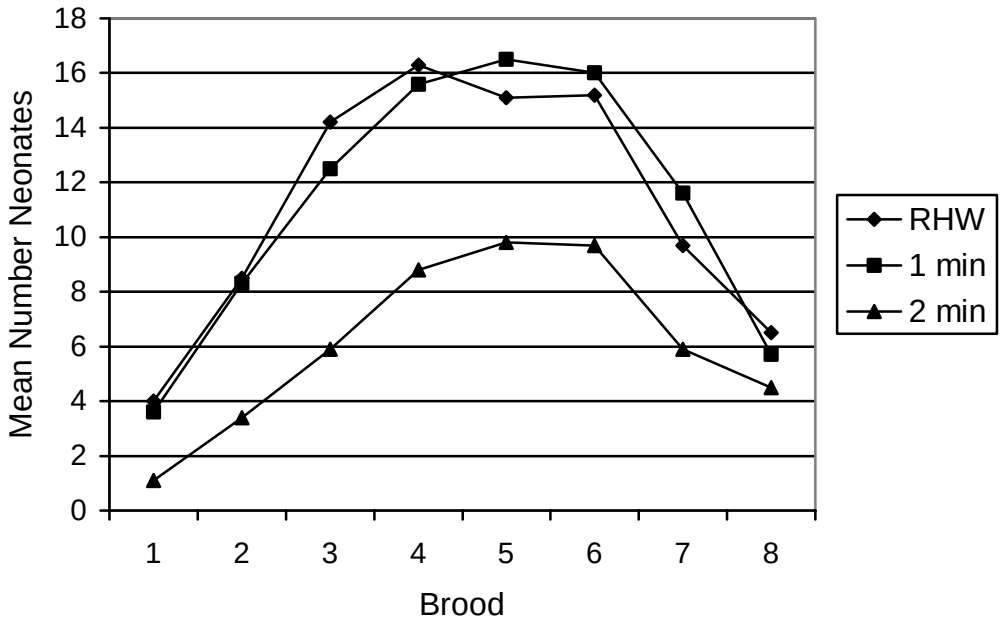

Figure A.10. Mean number of neonates produced per female for each individual brood: 600 µg/L exposure Error bars not shown for clarity. Neonate production was significantly less than controls from only the 1-minute exposure, third brood.

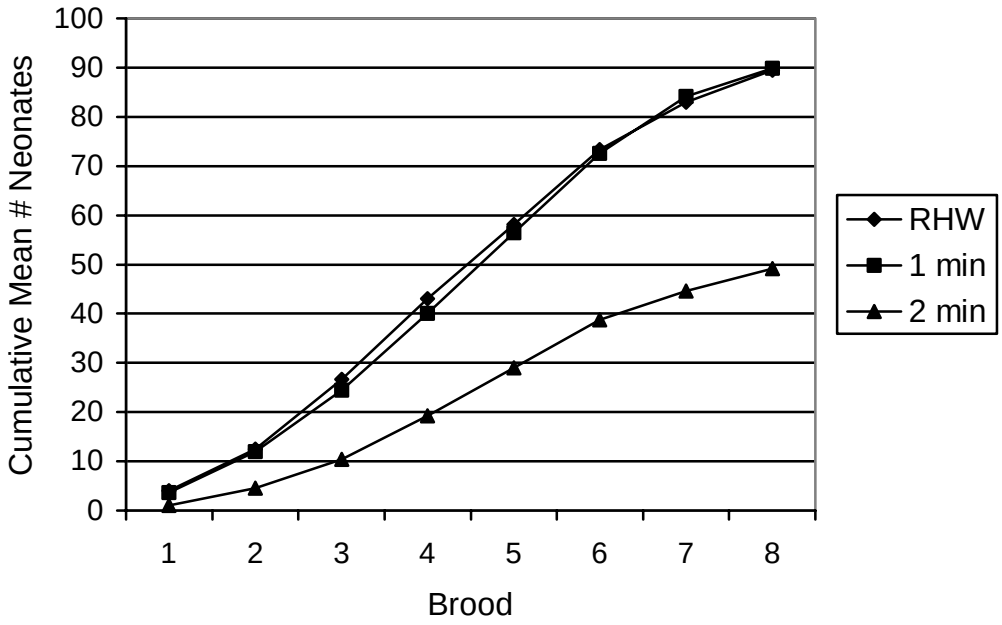

Figure A.11. Cumulative mean number of neonates produced per female following each subsequent brood: 600 µg/L exposure. Error bars not shown for clarity. Neonate production was significantly less than controls following only broods three and four from the 1-minute exposure.

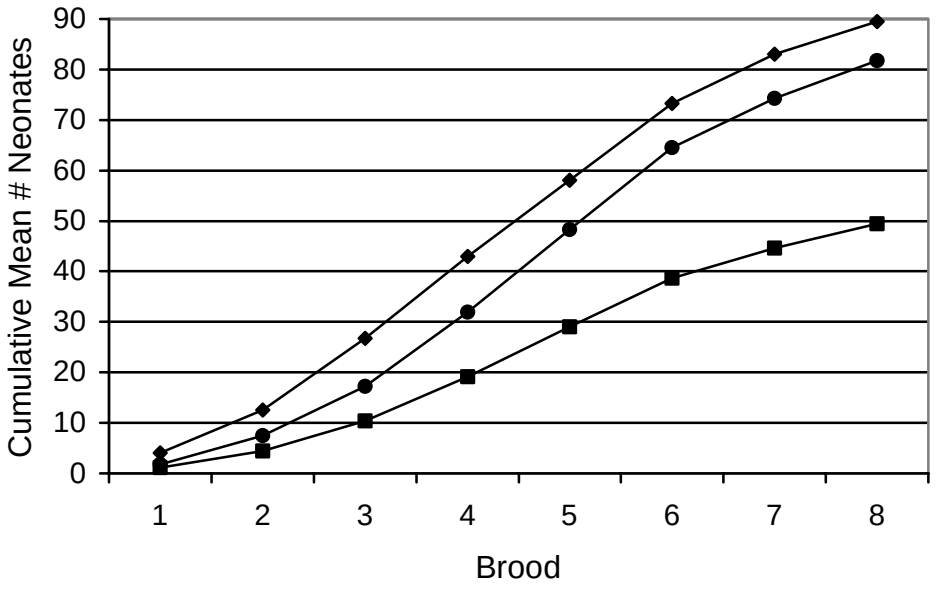

Figure A.12. Cumulative mean number of neonates produced from 2-minute exposure when deceased adults were counted as zero (n=10) neonates produced (squares) and when neonates of survivors only (n=6) were counted (circles): 600 µg/L exposure. RHW reference (diamond).

Table A.2. Mean (1 SD) number of neonates produced from second generation individuals following 24-hour exposure (second experiment).

Brood	Concentration (µg/L)							
	RHW	MSD PMSD [1]	77	93	116	133	150	171
Second Generation from Parent First Brood[2]								
1	4.7 (0.82)	1.3 (28.4)	3.5 (1.78)	3.6 (1.43)	3.8 (0.79)	**2.9** (1.20)	**3.0** (1.41)	**3.5** (0.58)
2	9.6 (1.35)	2.4 (25.3)	8.0 (3.06)	**6.5** (3.50)	7.4 (1.43)	6.6 (2.50)	**7.4** (0.97)	7.25 (1.26)
1-2	14.3 (2.06)	3.4 (23.5)	11.5 (4.40)	**10.1** (4.48)	11.2 (1.81)	**9.5** (3.47)	**10.4** (1.96)	10.8 (1.71)
3	14.2 (1.14)	3.6 (25.3)	11.8 (4.32)	**9.8** (5.39)	**10.6** (2.01)	**11.0** (4.14)	**10.9** (1.52)	**10.8** (1.26)
1-3	28.6 (1.51)	6.6 (23.0)	**23.3** (8.46)	**19.9** (9.67)	**21.8** (2.74)	**20.5** (7.44)	**21.6** (3.31)	**21.5** (2.52)
Second Generation from Parent Second Brood[3]								
1	5.0 (0.71)	1.0 (20.1)	4.6 (0.55)	4.2 (0.45)	4.0 (0.71)	4.6 (0.89)	4.2 (0.45)	5.0 (0.71)
2	10.2 (0.84)	1.6 (16.0)	9.6 (1.52)	9.8 (0.45)	**8.0** (1.41)	8.6 (1.14)	9.6 (1.14)	8.6 (0.55)
1-2	15.2 (0.84)	2.0 (13.0)	14.2 (1.92)	14.0 (0.71)	**12.0** (1.22)	**13.2** (1.92)	13.8 (0.84)	13.6 (0.89)
3	12.8 (0.84)	2.1 (16.2)	12.4 (0.89)	11.8 (1.92)	13.8 (1.48)	13.2 (0.84)	**10.6** (1.67)	**10.6** (1.34)
1-3	28.0 (1.00)	3.3 (11.6)	26.6 (2.30)	25.8 (2.49)	25.8 (1.79)	26.4 (2.61)	**24.4** (2.30)	**24.2** (1.92)
Second Generation from Parent Third Brood[3]								
1	4.2 (0.45)	0.9 (20.4)	ne	ne	3.2 (0.84)	4.2 (0.45)	4.0 (0.71)	4.0 (2.34)
2	9.2 (0.84)	1.9 (20.6)	ne	ne	9.6 (1.14)	10.0 (0.71)	7.6 (1.34)	**7.2** (2.05)
1-2	13.2 (0.84)	3.2 (24.4)	ne	ne	12.8 (1.64)	14.2 (0.84)	11.6 (1.34)	11.2 (4.32)
3	13.6 (1.68)	4.4 (32.5)	ne	ne	13.2 (1.30)	13.2 (1.64)	12.4 (1.52)	10.8 (6.06)
1-3	27.0 (1.87)	7.3 (26.9)	ne	ne	26.0 (2.12)	27.4 (2.30)	24.0 (2.74)	22.0 (10.20)

Values highlighted-bold were significantly less than respective controls.
[1] MSD = minimum significant difference. PMSD = percent MSD.
[2] n = 10 for all concentrations except 171 µg/L where n = 4. PMSDs for 171 µg/L were 37.6, 33.4, 31.1, 33.5 and 30.5, respectively.
[3] n = 5 for all concentrations. ne = not examined.

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