A preliminary study to investigate the possible effect of the common pesticide carbaryl on the copepod *Euchaeta elongate* and the copepod community of Hood Canal, Washington.

Brianna Fox
School of Oceanography
Box 357940
University of Washington
Seattle, WA 98195-7940
seabjf@aol.com
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Non-Technical Summary

In 2004, a court-ordered pesticide buffer was imposed around West Coast waterways that are known to support endangered salmon species in an effort to protect salmon from the dangers of direct exposure to pesticides. One of the court listed pesticides, carbaryl, has been measured at elevated levels in Seattle area waters. This project considers potential indirect effects on salmon from the presence of the common pesticide carbaryl in Puget Sound, Washington, by describing the effect of exposure to this chemical on copepods, a major food source for juvenile salmon. After collecting samples of copepods, individual copepods from the species *Euchaeta elongate*, as well as samples of a mix of copepod species, were exposed to a wide range of carbaryl concentrations. Copepod collection and exposure to the chemical took place in late March, 2007. Results for all experiments suggest that short term exposure to carbaryl, at levels that have been measured in Seattle area waters, has no effect.

Abstract

This project considers potential indirect effects on salmonids from the presence of the pesticide carbaryl in Puget Sound, Washington, by quantifying the acute toxicity of carbaryl on a salmonid food source, the copepod *Euchaeta elongate* and the Hood Canal copepod community. Zooplankton samples were collected from Hood Canal, in Puget Sound, Washington, in late March, 2007. From those samples, individual *Euchaeta* adults, along with representative samples of the Hood Canal copepod community were exposed to a wide range of carbaryl concentrations to determine whether levels they may encounter in Puget Sound are potentially harmful. For both the adult copepod and the copepod community experiments, increased mortality was not observed until concentrations of 1 ppm and 100 ppb, respectively, 200X or higher of that measured in Seattle area waters. Results suggest levels measured in Seattle area waters have no short term harmful effects. This study is an effort to improve our understanding of how urban populations directly and indirectly impact salmon and other aquatic animals.

Introduction

In 2004, a court ordered pesticide buffer was imposed around west coast waterways that are known to support endangered salmon species. The court order prohibits the ground application of three dozen pesticides within twenty yards of "salmon supporting waters", according to the US Environmental Protection Agency's (EPA) website. The court's primary concern is the direct effect these chemicals will have on endangered salmon. Monitoring of Seattle area watersheds by the US Geological Survey (USGS) has found elevated levels of numerous pesticides following storm events. In the Seattle area watershed of Thornton Creek, the pesticide carbaryl was measured at 0.48 ppb, 24X the recommended maximum aquatic concentration suggested by the National Academy of Science and Environment Canada. The presence of carbaryl and other pesticides in these waterways is likely due to yard runoff following storms.

In addition to direct effects, pesticides may have potentially negative indirect effects once they feed into downstream marine waters. Copepods and other zooplankton represent a major food source for small and juvenile fish (Mauchline 1998), including salmonids, that may be equally at risk in Puget Sound. Studies have shown that even at low concentrations pesticides can potentially be devastating to a single species (Hanazato and Hirokawa 2004; Forget-Leray et al. 2005) or to entire communities (Medina et al. 2004; Relyea 2005; Tidou et al. 1992). Either impact can alter community composition (Tidou et al. 1992) and biodiversity (Medina 2004; Relyea 2005). Such changes in community structure are potentially damaging to entire trophic systems. Copepods in general hold an important role in marine trophic systems serving as a link between primary production and higher trophic levels (Mauchline 1998; Sackmann 2000). This project considers a potential indirect effect on salmon by examining the acute toxicity of carbaryl to the copepod *Euchaeta elongate* and the copepod community of Hood Canal, Washington. *E. elongate* is a carnivorous coastal calanoid copepod species (Mauchline 1998) found in waters of the northern Pacific (Park 1995) and throughout Puget Sound (Strathmann 1987).

The insecticide carbaryl is widely used for commercial agriculture and aquaculture (Dumbauld et al. 2006), and home lawn and garden maintenance. It is a popular pesticide for use around waterways due to its rapid breakdown in waters above pH 7. If pH is close to 9, carbaryl will breakdown within a matter of hours according to California's Department of Pesticide Management. In typical marine waters, which have pH close to 8, the time in which carbaryl has the potential to be damaging is possibly very short. Alternatively, in waters with pH below 7, such as fresh river water, carbaryl may take years to break down increasing the time span over which it can damage aquatic communities. If a storm event were to carry carbaryl downstream to the surface waters of Puget Sound, the potential impact on invertebrates, such as copepods, that feed and reproduce in surface waters (Mauchline 1998; Strathmann 1987), could be devastating. Carbaryl affects invertebrates by inhibiting acetylcholine esterase (Dumbauld et al. 2006; Relyea 2005) leading to over stimulation at nerve synapses (Nachmansohn and Neumann 1975) and eventually death.

Studies have shown pesticides to have significant negative impacts on populations of aquatic organisms (Ma et al. 2006; Relyea 2005; Tidou et al. 1992). This study will examine whether or not *E. elongata* and the copepod community of Hood Canal will be impacted negatively from exposure to carbaryl. Negative response in this study is defined as an increase in mortality within a population.

Methods

Eighty liters of seawater was collected from Discovery Bay, Puget Sound, Washington (Station 1, Fig. 1) on March 22, 2007, from the *R/V Thomas G. Thompson*. Water was collected using 8 – 10 liter Niskin bottles mounted on a CTD-rosette. Forty liters was collected from each 35 m and 5 m depth. Once aboard the ship, water from both depths was filtered through a 73 µm screen, combined in equal proportions in carboys equipped with spigots, and stored in an 8ºC environmental chamber.

Zooplankton samples were collected twice from Hood Canal. The first collection was from aboard the *R/V Thomas G. Thompson* on March 22, 2007 (Station 2, Fig.1), in central Hood Canal. Collection involved using a 505 µm mesh, ½ meter opening bongo net (Fig. 2) with weights taped to the bottom of the cod ends in an effort to keep the nets vertically oriented. Using this net, we towed the entire water column (~150 m) at approximately 30 m min^{-1} (Runge 1985: Sackmann 2000), twice. Contents of the net tows were diluted into 8 – one gallon sized jars filled ¾ of the way full with unfiltered seawater collected from 35 m at the same station. These jars were equipped with aeration systems and stored in an 8ºC environmental chamber (Omori and Ikeda 1984; Sackmann 2000).

The second sample of zooplankton was collected in Dabob Bay, Hood Canal, Washington (Station 3, Fig.1), on March 27, 2007, from the *R/V Clifford A. Barnes*. Collection was done using a ½ meter opening net with 217µm mesh. The contents of the net tow were diluted into three gallon size jars and kept in a cooler to maintain ambient temperature.

Within a few hours of sample collection, adult *Euchaeta* were isolated. Using a turkey baster, zooplankton were transferred from the jars to a large Petri dish and adult *Euchaeta* were sorted out into a jar of filtered seawater. Once isolated, the adults were incubated individually in varied concentrations of carbaryl, in acid-washed (as described by Omori and Ikeda 1984) Linbro® 4-well tissue culture trays (M. Rasmussen pers. comm.; Pierson, Halsband-Lenk and Leising 2005) in a cooler to maintain samples at 12ºC (Pierson, Halsband-Lenk and Leising 2005; Sackmann 2000). Carbaryl used in this project was from the liquid product Sevin (22.5% carbaryl by weight). Carbaryl concentrations used were based on a log scale ranging from 1 to 100,000 ppb. For a control a sub-sample of adult *Euchaeta* was incubated in the same fashion in seawater without carbaryl.

This project included a total of 3 experiments. The first experiment involved adult *Euchaeta* from the sample collected from station 2 in central Hood Canal. The number of adults available was limited and so only 4 individual *Euchaeta* were used per treatment. The experiment ran for a total of 24 hours, with monitoring of *Euchaeta* condition at 13 and 24 hours. Condition was recorded as *dead* or *alive*.

The second experiment involved adult *Euchaeta* from the sample collected from station 3 in Dabob Bay. Twelve individual *Euchaeta* were used per treatment. This experiment ran for 72 hours, with monitoring of their condition and behavior every 12 hours. At those times condition was recorded as *dead* or *alive*, while behavior was recorded into one of 6 categories: 1) dead, 2) very sluggish with prodding or questionable dead, 3) sluggish with prodding, 4) active with prodding, 5) active without prodding or very active with prodding, or 6) very active without prodding (Table 1). From the mortality data collected in these first two experiments an LD_{50} (lethal dose required to kill 50% of a population; Relyea 2005) was determined at 24 hours for both experiments and 72 hours for the second experiment only. On the final day of this experiment water samples from the waters that had been used for incubating the individual copepods, the pure seawater without any copepods, and pure seawater with copepods were tested to measure pH.

For the third experiment, mixed samples from the copepod community of Hood Canal were exposed to carbaryl to explore their response. Copepods used were from the samples collected from Dabob Bay. In lab, clean 950 ml glass jars were prepared with 500 ml of carbaryl contaminated seawater. The same carbaryl concentrations used in the adult *Euchaeta* experiments were used for this experiment. An additional amount of the chemical was added appropriately to each treatment jar to compensate for the dilution that would occur when the zooplankton were added. Then, in order to insure uniformity in the amount and variety of types of zooplankton to be added to each treatment, a turkey baster was used to mix the container of zooplankton before extracting it. Immediately following mixing, the turkey baster was inserted to the 1 ounce mark (~ 4 inches) and approximately 1 ounce (~ 30 ml) of the zooplankton mixture was extracted and added to a treatment jar. This step was repeated until each treatment jar had approximately 60 ml of the zooplankton mixture added to it. This resulted in samples sizes ranging from 20 to 71 individuals. After 22-25 hours of incubation in a 12ºC environmental chamber copepods were counted and their condition was noted as dead or alive.

Results

Results for the first experiment suggest an LD_{50} at 24 hours of 1ppm (Fig. 3). In treatments below 1 ppm there was zero mortality, in treatments above 1ppm there was 100% mortality, in the 1ppm treatment there was 50% mortality at 24 hours. Results for the second experiment suggest a higher LD_{50} at 24 hours (Fig. 4) occurring between 10 ppm and 100 ppm. At 24 hours all treatments except the control population and the highest treatment, 100 ppm, had zero mortality. At 72 hours, the 1ppm treatment was the only treatment to have zero mortality, while the 10 ppm treatment had just over 50% mortality, suggesting the LD_{50} at 72 hours was greater than 1 ppm but less than 10 ppm. Expanding the description of behavior in this second experiment to all 6 levels of activity (outlined in Methods) results show that the populations in the mid-level concentrations (10 ppb – 1 ppm) consistently had the highest levels of activity during the 72 hour experiment (Fig. 5). Additionally, in the second adult Euchaeta experiment, after 3 days of incubating in the carbaryl contaminated water, three individuals in the 10 ppm treatment developed black fungus like material on their antennae and appendages. It is unknown what the black material was or why it only developed on individuals in the 10 ppm treatment. PH measurements of the contaminated waters that contained copepods, uncontaminated water that contained copepods and a sample of the pure seawater were 7.88±.01, 7.86 and 7.74, respectively.

For the copepod community experiment, samples were composed of several species, sizes and life stages of copepods. Mortality was directly related to carbaryl concentration above 100 ppb (Fig. 6). The lowest mortality occurred in the 100 ppb treatment, while highest mortality occurred in the 100 ppm treatment.

Discussion

Results for the two adult *Euchaeta* experiments vary greatly. The first experiment had 100% mortality above, zero mortality below, and 50% mortality in the 1 ppm treatment (Fig. 3). The second experiment had no mortality at or below, but 100% mortality above the 10 ppm treatment (Fig. 4). The discrepancy seen when comparing these two experiments might be explained by the sample collection sites. Because the populations of copepods used in these two experiments were from different locations, it is possible that one population experienced a stress prior to collection that the other did not. In this case, the population of *Euchaeta* collected from central Hood Canal may have experienced or been experiencing a stressor at the time of collection, reducing their ability to endure exposure to the carbaryl at the same levels as the Dabob Bay population. Studies have shown that the addition of another stressor such as food limitation (Pieters et al 2005) or an additional pesticide (George and Liber 2007) can make a population less able to endure pesticide exposure. This then raises the question as to what the response would be from populations of copepods in areas closer to urban centers where they are sure to be exposed to both higher concentrations as well as more numerous chemicals that are known to be detrimental to the health of aquatic organisms. If the copepod population collected from central Hood Canal was indeed experiencing a chemical type stress that the Dabob Bay population was not, and that is why mortality occurred at a much lower carbaryl treatment, then copepod populations in waters near urban centers, too, would possibly experience mortality at an even lower level of carbaryl increasing the danger such a chemical poses to aquatic communities.

A similar idea might explain the black fungus-like material observed on the copepods in the 10 ppm treatment, the highest treatment that did not experience 100% mortality, of the second adult *Euchaeta* experiment. Diseases are thought to be a result of a culmination of several factors as described by the "disease triangle concept". This concept describes three conditions that need to be satisfied in order for disease to develop: 1) the disease pathogen needs to be present, 2) certain environmental conditions must be achieved, and 3) the host must be susceptible to invasion by the pathogen (Lucas 2004; Scholthof 2007). Because the same water source was used for incubating all individuals in all treatments, it is reasonable to assume that all of the copepods experienced the same pathogens and environmental conditions. Instead, it is likely that the additional stress of exposure to the high level of pesticide (10 ppm) made the copepods in this treatment susceptible to the infection.

In the second experiment, the observations of increased activity in the copepods in the mid level treatments might be explained by the nature of carbaryl. Acetylcholine esterase is an enzyme in the body that regulates activity at nerve synapses (Nachmansohn and Neumann 1975). Carbaryl inhibits acetylcholine esterase (Dumbauld et al. 2006; Relyea 2005) leading to over stimulation and eventual death. The increased behavior in the 10 – 1000 ppb treatments might be a result of this over stimulation, and may in fact suggest they are nearer to death than the control and 1ppb treatments.

The mortality data from the second and third experiments at 24 hours showed very dissimilar results (Fig. 4, 6). In the second experiment at 24 hours only the control and the 100 ppm treatments had any mortality, whereas all treatments in the third experiment had experienced some mortality. However, the second experiment at 72 hours and the third experiment at 24 hours showed very similar results, with both experiments showing mortality in all treatments, except the 1 ppm treatment in the second experiment. One possible reason for this lag in response from the copepods in the second experiment is that the *Euchaeta* are a very large and hearty species of copepod. The copepods in the community experiment where predominantly smaller species and juveniles of smaller species of copepods, which may be more sensitive to chemical exposure (Forget-Leray et al. 2005). If this study were repeated, copepods should be classified to species and life stage to better understand if there is any variation in response between species and/or life stages.

There are several other considerations that were not made, but may have been contributing factors, in these experiments. Were this study to be repeated, larger containers should be used and a food source should be supplied. The 42 ml wells were not of sufficient size to accommodate a copepod the size of *Euchaeta*, and the increased stress on the animal from hunger could have contributed to agitation in the animal. If this is true then the true LD_{50} at 24 and 72 hours is possibly much higher than measured in this study.

Another aspect that could be added to the study if it were repeated would be a recovery effort. Carbaryl is known to degrade quickly in marine waters with pH greater than 8, so it is likely that copepods in pelagic regions would experience the chemical for only a short time. If the copepods were exposed for only a day or two would they recover?

Conclusion

Results from this project suggest that adult *Euchaeta* do not appear to experience any short term negative effects from exposure to carbaryl at levels they are likely to experience in Puget Sound. Other copepod species and the copepod community should be studied further to determine if smaller species or juvenile copepods are more susceptible to negative effects from exposure to carbaryl. Also, the possibility of recovery after exposure should be investigated to understand a more realistic response to this chemical.

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Table 1. Descriptions of behavior in the second adult *Euchaeta* experiment.

Activity level	**Description of behavior**
0	Dead
1	Questionably dead or very sluggish with prodding
2	Sluggish with prodding
3	Active with prodding
4	Active without prodding or very active with prodding
5	Very active without prodding

Figures:

Figure 1. Partial map of Puget Sound, Washington, with all three stations labeled. Station 1, in Discovery Bay, N 48º05.50, W 122º52.99. Station 2, in central Hood Canal, N 47º32.79, W 123º00.49. Station 3, in Dabob Bay, Hood Canal, N 47º46.14, W 122º50.10.

Figure 2. Basic shape of a bongo net.

Figure 3. Mortality of adult *Euchaeta* in the first experiment, expressed as the percentage of individuals within the populations of each treatment.

Figure 4. Mortality of adult *Euchaeta* in the second experiment, expressed as the percentage of individuals within the populations of each treatment. Activity levels with the descriptions of dead and questionable dead or very sluggish with prodding were combined into one category described as "dead" in this figure; all other activity levels were combined into one category described as "alive" in this figure.

Figure 5. Activity levels of the adult *Euchaeta* in the second experiment, expressed as a percentage of the population within each activity level. Activity levels correspond to descriptions of activity as described in Table 1.

Figure 6. From the copepod community experiment, copepod mortality expressed as the percentage of individuals within the populations of each treatment.

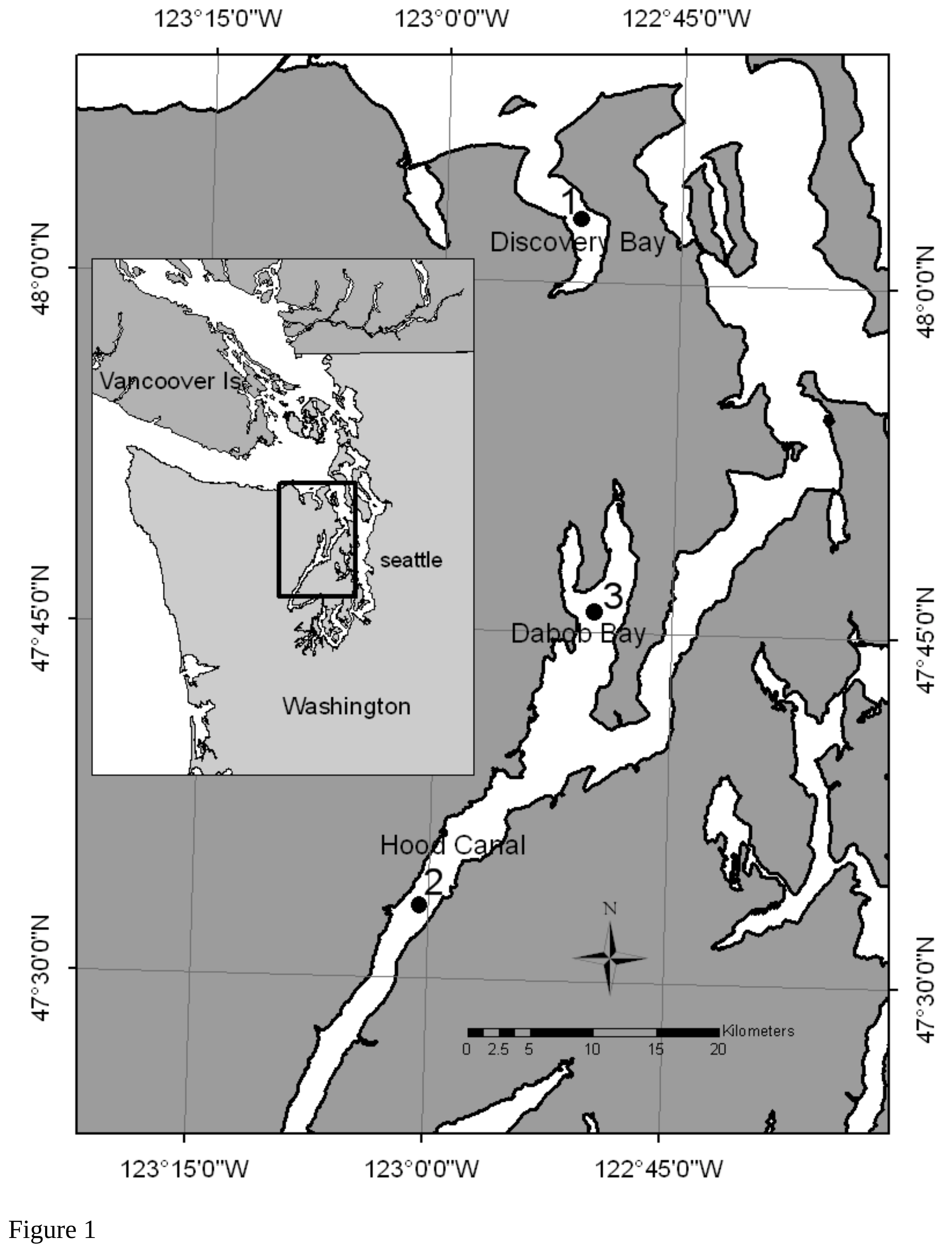
123°15'0"W
123°0'0"W
122°45'0"W
1
Discovery Bay
48°0'0"N
Vancoover Is.
seattle
3
Dabob Bay
47°45'0"N
Washington
Hood Canal
2
N
47°30'0"N
Kilometers
0 2.5 5 10 15 20

Figure 1

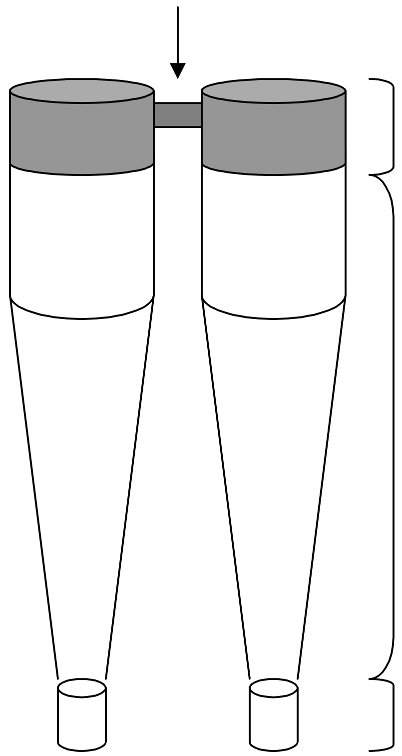

Figure 2

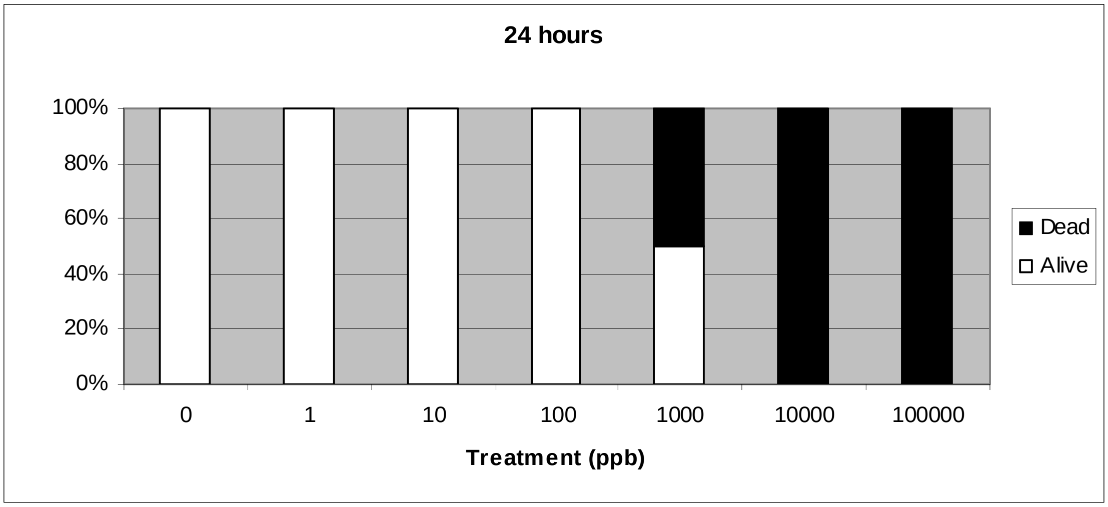
24 hours
100%
80%
60%
40%
20%
0%
0
1
10
100
1000
10000
100000
Treatment (ppb)
Dead
Alive

Figure 3

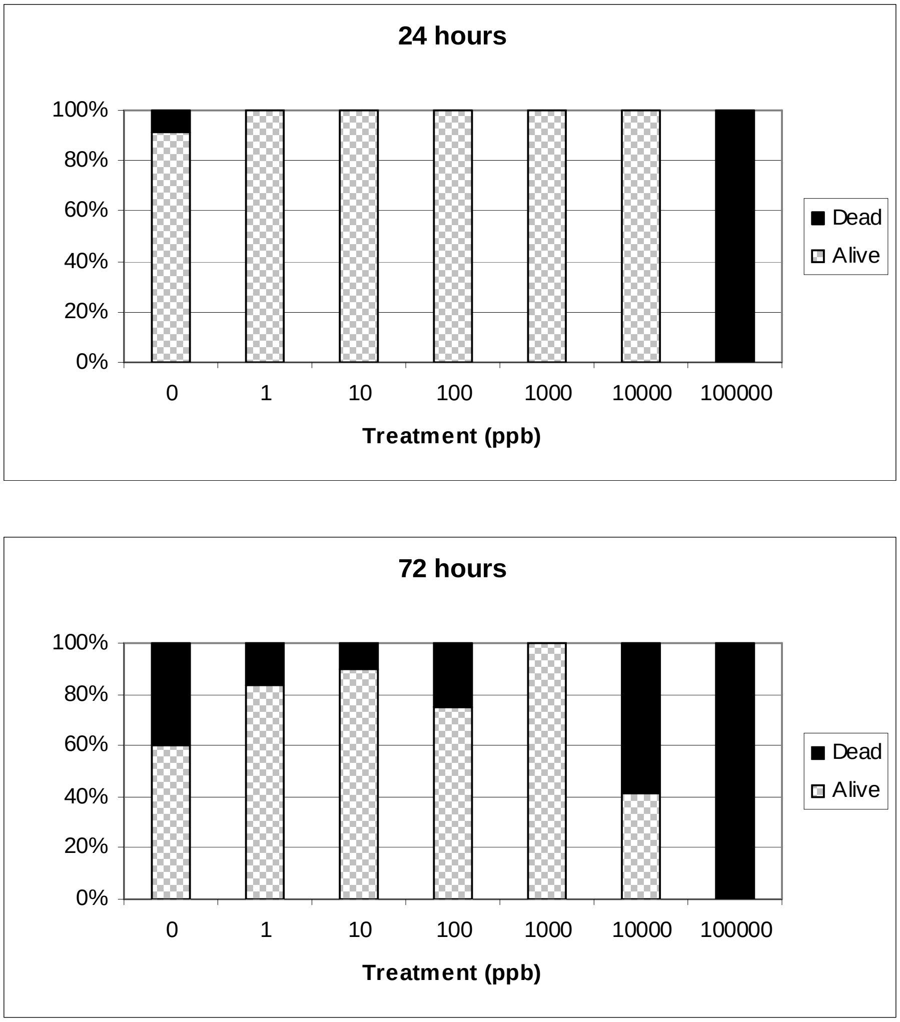
24 hours
100%
80%
60%
40%
20%
0%
0
1
10
100
1000
10000
100000
Treatment (ppb)
Dead
Alive
72 hours
100%
80%
60%
40%
20%
0%
0
1
10
100
1000
10000
100000
Treatment (ppb)
Dead
Alive

Figure 4

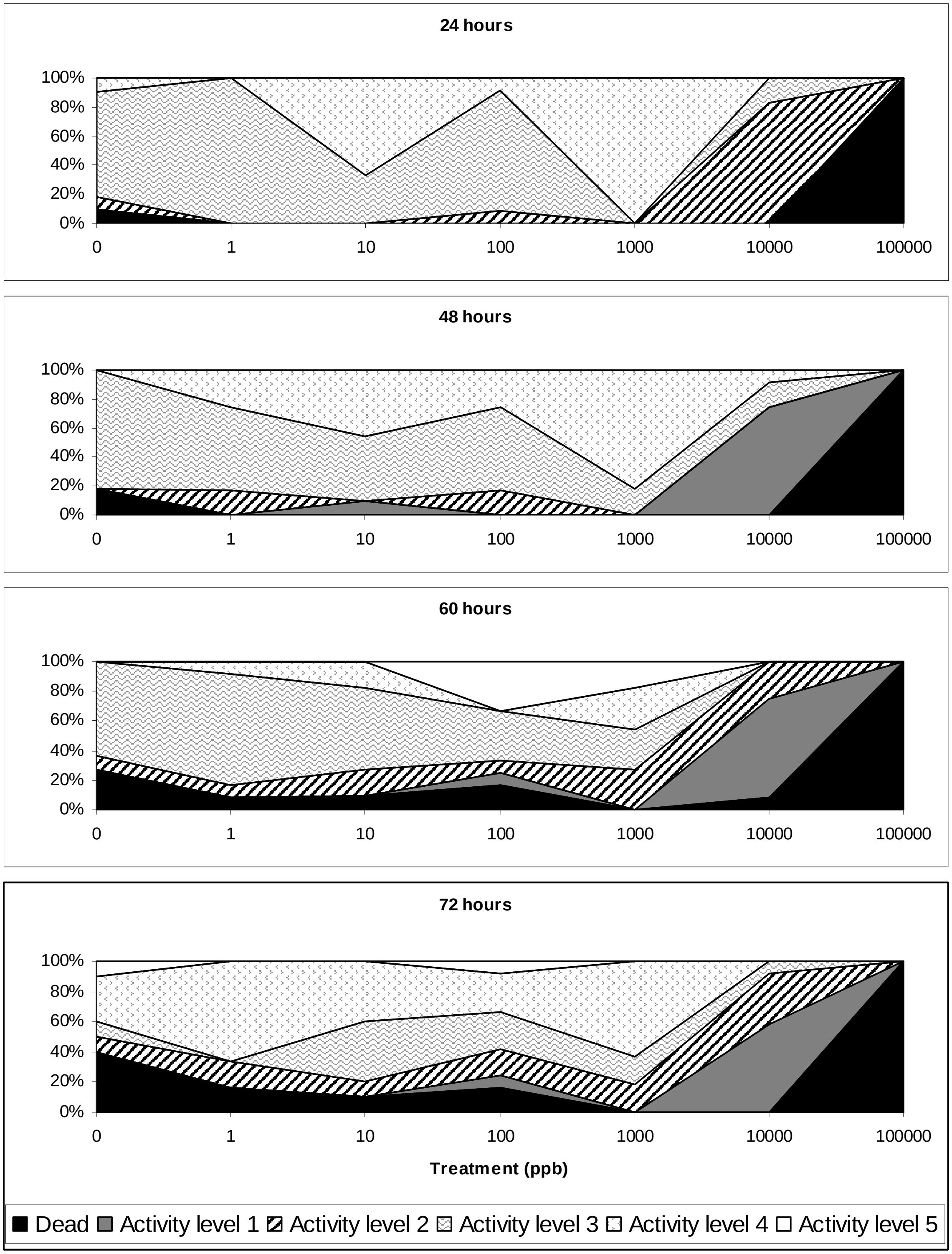
24 hours
100%
80%
60%
40%
20%
0%
0
1
10
100
1000
10000
100000
48 hours
60 hours
72 hours
Treatment (ppb)
Dead
Activity level 1
Activity level 2
Activity level 3
Activity level 4
Activity level 5

Figure 5

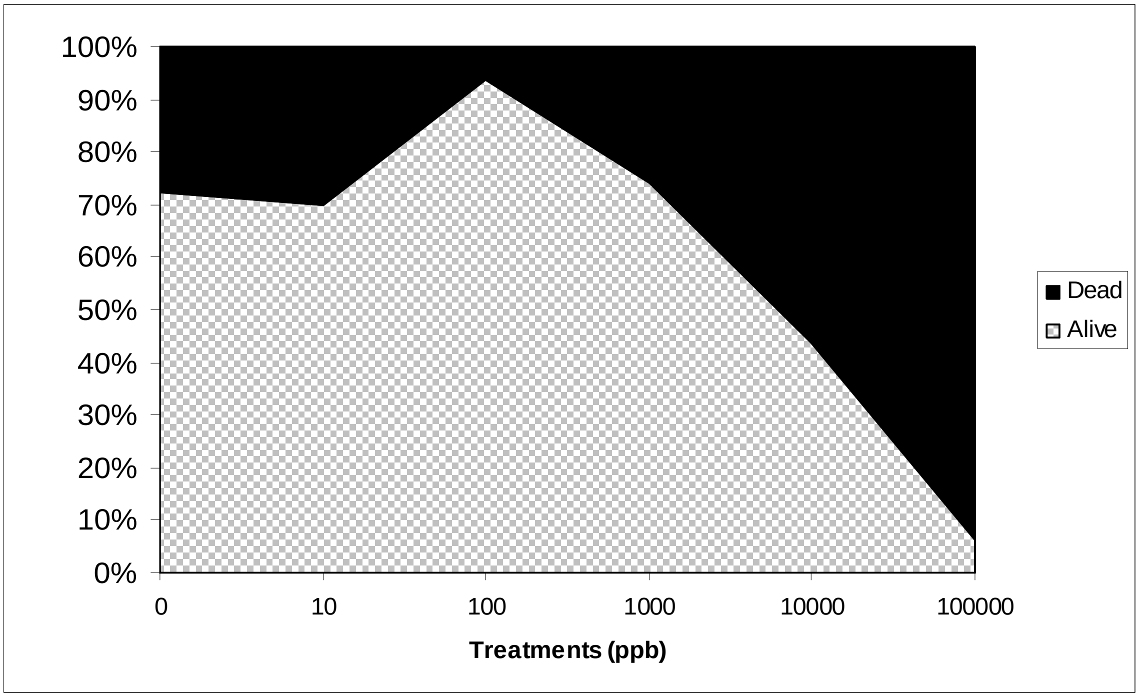
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
10
100
1000
10000
100000
Treatments (ppb)
Dead
Alive

Figure 6