Sediment LC$_{50}$ Analyses of the Pyrethroid Pesticides Cyfluthrin, Deltamethrin, and Lambda-Cyhalothrin

Nicole Ureda

Abstract Pyrethroid compounds are quickly becoming popular replacements for increasingly regulated organophosphate pesticides. Many of the newest pyrethroids have not undergone thorough toxicity testing, however, a fact that underscores their potential dangers and has repercussions in the development of both pesticide policies and practices. Additionally, many pyrethroids have been analyzed for toxicity in water, an unsuitable test medium given the high hydrophobicity of the compounds and their preferential adhesion to organic carbon in aquatic sediments. This study sought to address the inadequacies in pyrethroid toxicity research by determining the LC$_{50}$ levels of the pesticides deltamethrin, lambda-cyhalothrin, and cyfluthrin in sediment tests, following EPA protocol and using the test species *Hyalella azteca*. Additionally, all pesticides were tested in sediment samples from two different Central Valley, CA, locations, and their LC$_{50}$ levels compared to those from a previous test run with a third sediment sample. Each of the pesticides' LC$_{50}$ levels were standardized against sediment total organic carbon (TOC) levels, using linear regression, to see if a direct relationship between the two could be found. Data analysis yielded LC$_{50}$ values and trends comparable to those from similar sediment tests run with different pyrethroid compounds. Regression analyses were able to confirm the existence of a linear relationship between LC$_{50}$ values and TOC for cyfluthrin only. The emergence of this sediment toxicity data provides pesticide industry representatives and regulators with a better understanding of the environmental implications of pyrethroid use, and more realistic standards against which pyrethroid exposure limits may be set.

Introduction

Pyrethrin pesticides were first derived from flowers in the chrysanthemum genus in the 1970s (Bateman, 2000), though the widespread agricultural use of pyrethrins, and their synthetic analogues, the pyrethroids, did not begin until the 1990s (Weston 2004, pers. comm.). The rise in pyrethrin/pyrethroid application followed increasing U.S. Environmental Protection Agency (EPA) restriction on organophosphate compounds, such as malathion and diazinon, which were found to be toxic to humans and have high rates of environmental persistence. Like the organophosphates, pyrethroids are known to be potent insecticides effective on a wide range of animal species (Smith and Stratton 1986, Bradbury and Coats 1989). Although their avian and mammalian toxicities are significantly reduced in comparison to organophosphates, certain laboratory studies indicate that pyrethroids remain markedly lethal to aquatic organisms, particularly arthropods and fish (Siegfried 1993). The body of scientific literature on the new compounds, however, remains incomplete.

Much of the available data on pyrethroids as a chemical class has been largely inferred from the analysis of a handful of compounds (e.g. cypermethrin) that has taken place over the last decade (Maund *et al*. 1998, Solomon *et al*. 2001, Maund *et al*. 2002). Pyrethroids are widely recognized as being strongly lipophilic, and thus highly hydrophobic (Hill 1989, Muir *et al*. 1994, Maund *et al*. 1998, Solomon *et al*. 2001), adsorbing almost exclusively to organic carbon molecules in water-sediment slurries within 24 hours (Maund *et al*. 2002). Furthermore, pyrethroids have shorter chemical half-lives than their organophosphate predecessors, ranging from several days (Muir *et al*. 1994) to around one month (in aerobic sediments; Weston *et al*, 2004).

The details of pyrethroids' ecological effects, yet, are largely unknown, and toxicity data for many of the compounds is particularly scarce. Often, toxicity information that is available is restricted entirely to the basal data set required for chemical registration, and in some cases, specifically among the newest synthetic compounds, quantified exposure data does not exist at all (Solomon *et al*. 2001). Among available toxicity data sets are reports gauging pyrethroids' effects according to amounts of the pesticides dissolved in the water column (Hill 1989). Due to the difficulties involved in distinguishing between pyrethroids adhered to dissolved organic carbon (DOC) and those actually dissolved in water, however, such models are misleading at best (Maund *et al*. 2002).

Recent research has indicated that the distribution of a given amount of a pyrethroid between water and sediment in a benthic system is governed by an equilibrium partitioning coefficient based on the total amount of organic carbon (TOC) available to the system (Hoke *et al*. 1994, Hoke *et al*. 1995). Hence, by these models, sediment organic carbon plays a critical factor in determining the bioavailability of a given pyrethroid in a particular aquatic system, and accordingly, the pyrethroid's potential toxic effects (Maund *et al*. 2002). What have been needed are aquatic pyrethroid studies that take this factor into consideration, assessing pesticide toxicity in direct relation to sediment TOC.

In areas where agriculture and aquatic ecosystems are found together, such as California's Central Valley, the need for accurately assessed toxicity information becomes particularly pronounced. Furthermore, it is important that a range of toxicity data becomes available, detailing information on both particular pesticides and particular benthic species. Without realistic data on pyrethroids' effects in aquatic environments, water-quality monitors and legislators have fewer tools with which they may make informed decisions on acceptable pesticide exposure limits within these systems. The assessment of toxicity levels for these pesticides can therefore contribute to the development of the most appropriate regulatory actions in protection of local, national, and global environmental health.

This research was designed to determine the lowest observable effect concentrations (LOECs) and LC_{50}s of three pyrethroids, cyfluthrin, deltamethrin, and lambda-cyhalothrin, in two separate aquatic sediment samples, with each sediment sample having a different TOC level. An LC_{50} level is the chemical concentration at which 50% mortality in an exposed population is observed. LOECs are the lowest concentrations of a chemical at which effects on the growth levels in an exposed population are recognized. These markers are among the most common standards for gauging comparative toxicity levels. Finally, this research sought to explore the nature of the TOC- LC_{50} relationship.

Methods

This test was designed to determine the LOECs and LC_{50} for the arthropod *Hyalella azteca* (Crustacea: amphipoda), exposed to cyfluthrin, deltamethrin, and lambda-cyhalothrin, three pyrethroid compounds frequently used in agriculture. Tests were conducted according to standard EPA protocols for sediment toxicity testing with aquatic invertebrates (US EPA 2000).

H. azteca is a common environmental test species, and is known to be significantly more sensitive to pesticides than other species. Subsequently, pesticide exposure levels that are safe for *H. azteca* should be acceptable as safe for other environmental organisms.

Sediment Preparation Each of the compounds was tested in sediments previously sampled by members of the Weston Laboratory (Amweg 2004, pers. comm.). These samples came from two Central Valley locations, Del Puerto Creek and Pacheco Creek, which were chosen for their representativeness of Central Valley ecosystems. Sediments were collected upstream of any agricultural inputs to decrease the likelihood that they would be pre-contaminated with insecticidal compounds, and were sieved through a one millimeter sieve to achieve a rough consistency in sediment sample grain sizes. Sub-samples of the sediments were analyzed for a suite of 26 pesticides and their degradation products by GC ECD prior to commencement of the LC-50 tests (Hewlett Packard 6890 Series Gas Chromatograph System, HP6890GC, with electron capture detector) consistent with EPA standards (US EPA Method #8080A), and were confirmed clean of all detectable pesticides. The sediments were further analyzed for grain size, total solids content, and total organic carbon.

Experimental Procedure Sediment samples were each homogenized with an electric drill fitted with a steel mixing accessory. At the beginning of the tests, sediments were divided into 5 groups, one per pesticide plus one control and one solvent control. Sediments were spiked with pesticide dissolved in an acetone carrier, using a glass syringe to minimize adsorption. Seven spiking concentrations were chosen based on sediment TOC and the ballpark LC_{50} results from previous tests with a different sediment (Amweg 2004, pers. comm), to yield a theoretical survival range from 0% to 100%. A solvent control group was spiked only with acetone to rule out its potential effects on *H. azteca* growth and survival rates. Sediments and pesticides were re-homogenized with the electric mixer, and then aged for 11 days at 4°C to allow the pesticides to associate with sediment carbon.

Three days prior to initiation of the Del Puerto Creek test, *H. azteca* juveniles from a mature culture were sieved through a 500nm sieve and retained on 350nm sieves, and the retained individuals incubated at 23°C, with 10mL yeast, trout chow, and cyanobacteria (YCT) slurry daily feedings, until the tests commenced. *H. azteca* were sieved but not incubated for three days prior to the Pacheco Creek test due to low recovery among incubated individuals in the Del Puerto Creek test, thus ensuring the availability of sufficient individuals for the Pacheco Creek

test. One day before initiation of the tests, 50mL sediment samples from each of the pesticide-concentration groups, and from the control and solvent control groups, were placed in each of three replicate 350mL, labeled beakers. Moderately hard water, reconstituted by addition of salts to Milli-Q de-ionized water, was added to the 200mL mark on the beakers, and the beakers were allowed to equilibrate overnight until commencement of the tests. At day zero, water in the tests was removed and the beakers re-filled to the 300mL mark, and ten *H. azteca* were collected under a microscope and placed within each of the beakers. Beakers were then incubated at 23°C for ten days on a 16:8 hr light:dark cycle. Beakers were fed daily with 1.0mL YCT, and received an 80% water change every other day. On days two and ten of the tests, water samples from three beakers were tested for anomalies in temperature, dissolved oxygen, pH, conductivity, alkalinity, hardness, and ammonia concentration. On the tenth day of the tests, each of the beakers was carefully sieved over 425μm mesh, and surviving *H. azteca* counted and collected in labeled containers. Hyalella were dried overnight in a 70°C oven and weighed to find mean biomass weights per individual.

Data Analysis Survival and growth data among the pesticides and sediments were analyzed with ToxCalc (Version 5.0 for Microsoft® Excel under Windows™ 3.1 or Apple® Macintosh®, ©1992-1994, Tidepool Scientific Software). LC_{50}s and growth LOECs were determined using maximum likelihood probit analyses and trimmed Spearman-Karber tests, and Bonferroni's adjusted t-test, respectively. Measured LC_{50} levels from these tests and a previous test were then linearly regressed to sediment TOC to levels to analyze the relationship between the factors.

Results

Sediment analysis showed that Del Puerto Creek had a TOC level of 11.4 g organic carbon per kilogram of dry sediment weight, or 1.14%; Pacheco Creek had a TOC level of 6.51%.

The Del Puerto Creek test yielded survival data almost consistently inversely proportional to the concentrations of pesticides in the sediment samples, with zero survival at the highest concentrations and 100% survival at the lowest (Fig. 2 growth data). Survival rates were also generally consistent among all three replicate beakers per pesticide concentration. Survival rates within Pacheco Creek test groups were less regular, with typically far lower survival rates at higher concentrations and much less survival consistency among pesticide concentration

replicate beakers. The non-parametric trimmed Spearman-Karber LC_{50} analyses were chosen to describe survival data due to the inconsistencies within the Pacheco Creek test data.

LC_{50} results for both sediments were on the same order of magnitude, at .46-1.06 μg/g organic carbon for Del Puerto Creek and .20-.34 μg/g organic carbon for Pacheco Creek. Table 1 details this LC_{50} data, and includes data from a previous test run in an American River sediment sample (Amweg 2004, pers. comm). LC_{50} values were found to rise with sediment TOC, as expected, though the regression analyses for LC_{50} vs. TOC gave a significant value for cyfluthrin only (Fig. 1; note LC_{50} data given per kilogram of dry sediment vs. per gram organic carbon).

Ten-day biomass analyses showed generally consistent trends between both sediments, with decreasing biomass at increasing pesticide concentrations (Fig. 2). The pesticide LOEC trends varied between the sediments, with higher LOEC values for cyfluthrin and deltamethrin in the Del Puerto Creek sediment, but equal LOEC values for Lambda-Cyhalothrin in both sediments (Table 2).

Pesticide	Del Puerto Creek LC-50 (μg/g organic carbon)	Pacheco Creek LC-50 (μg/g organic carbon)	American River LC-50 (μg/g organic carbon)
Cyfluthrin	1.06 (0.93-1.22)	0.34 (0.29-0.39)	1.07 (0.96-1.2)
Deltamethrin	0.87 (0.75-1.02)	0.20 (0.15-0.28)	0.71 (0.60-0.83)
Lambda-Cyhalothrin	0.46 (0.40-0.53)	0.20 (0.18-0.23)	0.43 (0.38-0.49)

Table 1: LC_{50} Levels with 95% CIs per Pesticide-Sediment Group

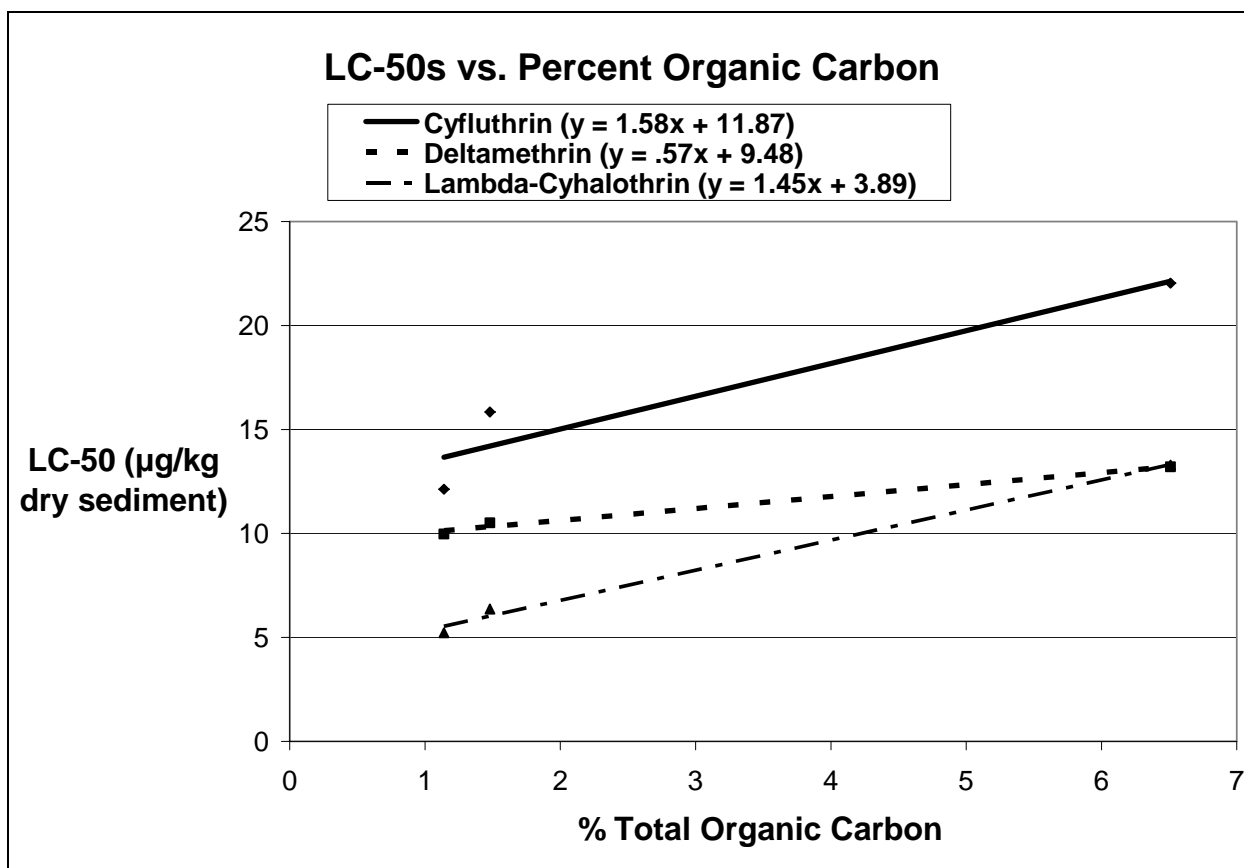**Figure 1:** LC-50 Levels vs. Total Organic Carbon Levels. <u>Cyfluthrin:</u> R^2 = .8893, σ = 2.24, P-value = .045
<u>Deltamethrin:</u> R^2 = .9901, σ = .244, P-value = .366 <u>Lambda-Cyhalothrin:</u> R^2 = .9945, σ = .457, P-value = .212

Pesticide	Del Puerto Creek LOEC (µg/g organic carbon)	Pacheco Creek LOEC (µg/g organic carbon)
Cyfluthrin	0.77	0.17
Deltamethrin	1.57	0.12
Lambda-Cyhalothrin	0.23	0.23

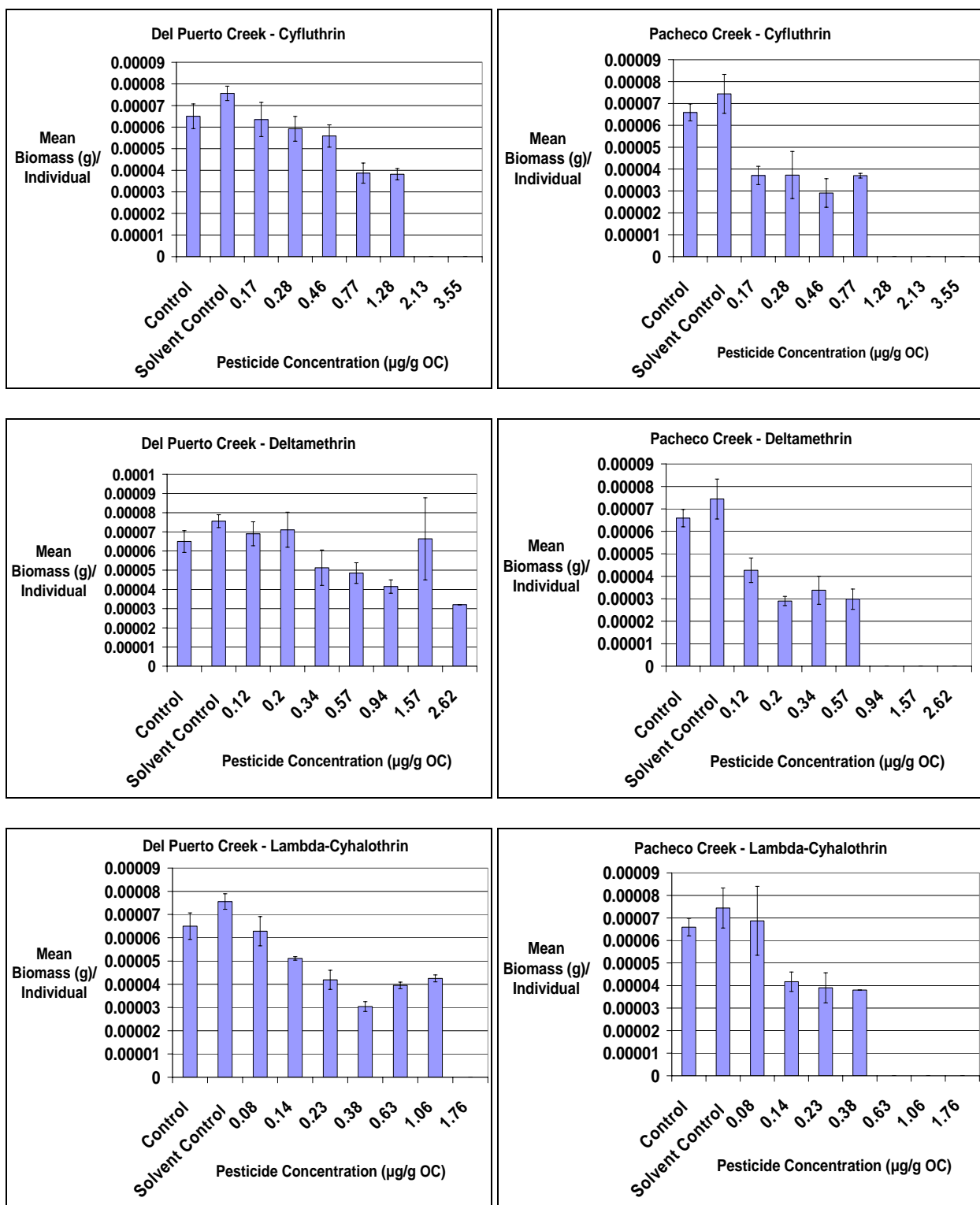**Table 2:** LOEC Data per Pesticide-Sediment Group

Figure 2: Growth LOEC data for Del Puerto Creek and Pacheco Creek Sediments

Discussion

The analysis of data in this study yielded a number of trends in agreement with expectations. As shown in Table 1 above, LC_{50} data among tests is relatively consistent; particularly so between the Del Puerto Creek and American River tests. Such replication indicates that the standardization of LC_{50}s to TOC is a sensible choice, and that these LC_{50} estimates are reliable figures. These LC_{50} values are also generally concordant with a previously conducted 10-day LC_{50} analysis of the pyrethroid cypermethrin with *H. azteca*, in 1.0% TOC sediment, which yielded a value of 3.6 μg/kg sediment (Weston *et al*. 2004). Additionally, they contrast with a 10-day LC_{50} analysis of the organophosphate DDT, with *H. azteca*, yielding a value of 260 μg/g OC (Weston *et al*. 2004).

LC_{50} data were plotted against TOC values (Fig. 1) to analyze the strength of the direct TOC-LC_{50} relationship, and to produce an approximate tool for establishing LC_{50} levels according to sediment TOC in future endeavors. The positive slopes of the regression lines are congruent with the conclusions of previous research reports, which have documented a broadly linear decrease in pyrethroid bioavailability with increasing TOC (Maund *et* al. 2002). Cyfluthrin data produced the only statistically significant regression line in this model, however, and the existence of a generalized, linear relationship between mortality and TOC among these pyrethroid compounds cannot be confirmed. Instead, the potential influences of an individual organism's health, general environmental conditions, and other factors on growth and mortality must continue to be regarded in TOC-LC_{50} analyses, until such time as they may be decisively ruled out, if at all. The lack of significance in the deltamethrin and lambda-cyhalothrin regressions may be due to the limited sample sizes, and a replication of the studies using more data points would expectedly produce a more certain result.

Biomass data detailed declining trends in growth with increasing pesticide concentrations (Fig. 2) for each sediment-pesticide test. This result is sensible, as one would expect the stress of exposure to sub-lethal pesticide concentrations to tax the metabolic capacities of a sensitive species such as *H. azteca*, and consequently inhibit its general growth and vitality. Growth LOEC values (Table 2) for Pacheco Creek were smaller than for Del Puerto Creek. This result is interesting given the LC_{50} trends for all sediments, and given the higher TOC content of the Pacheco Creek sediment, which would characteristically be associated with a decrease in pesticide bioavailability. While it may be suggested that other contents of the Pacheco Creek

sediment led to the decreases in lowest effect concentrations, it should also be noted that the growth trends in the Pacheco Creek test were also less consistent than in the Del Puerto Creek samples, and that due to high mortality there were fewer replicate samples with which the Pacheco Creek LOECs could be statistically analyzed.

The variance between the Del Puerto Creek/American River LC_{50}s and the Pacheco Creek data, and between the growth responses in the Del Puerto Creek and Pacheco Creek sediments, may be explained by several factors. First, differences between LC_{50} reports in different sediments are commonly off by a factor of 2-3, even after accounting for OC, and so the results provided by this study may not be as erroneous as suggested at first sight (Amweg, 2004). Secondly, the size of the TOC particles in the Pacheco Creek sediment could account for survival and growth data inconsistencies; as noted by Maund *et al.* (2002), a higher number of smaller organic particles, with more collective surface area, are available to bind with more pyrethroid content than are a smaller number of larger organic particles, having less surface area; if the organic carbon units present in the Pacheco Creek sediments were significantly larger than those in the American River or Del Puerto Creek sediments, relatively less pyrethroid chemical content may have been bound to them, and relatively more of the pesticide may have been available for toxic affect. Accordingly, this could account for some of the differences in the respective LC_{50} and growth data between the sediment samples.

Third, most of the *H. azteca* used in the control and Lambda-cyhalothrin replicate beakers in the Pacheco Creek test were sieved only with a 500nm sieve, and were not transferred into the beakers under observation with a microscope. For these reasons, miniscule juvenile *Hyalella* were added to the beakers in addition to the ten more mature individuals. While these juveniles maintained their small size relative to the larger adults, and were excluded from survival tallies as much as possible, their potential inclusion in control survival estimates could have possibly skewed comparative survival data, and could have significantly skewed comparative LOEC data. Finally, pure water was mistakenly added to the Pacheco Creek beakers during the day four water change of the Pacheco Creek test, rather than moderately hard water. While this water was again changed two hour later, it is possible that it could have contributed to lower growth rates and higher-than-average mortality rates among *Hyalella* in higher pesticide concentration beakers.

A more detailed reproduction of the trials could yield more precise data, above all with regard to improving LOEC analysis. In particular, a reproduction of tests with a more finely divided series of pesticide concentrations between the highest concentrations at which no observable effects on growth took place, the LOECs, and the next, higher concentrations would give a more accurate estimate of true LOECs for these pesticides. For example, the LOEC for cyfluthrin in the Del Puerto Creek test was found to be at .77μg/gTOC, so a test run with a finer gradation of pesticide concentrations between .46μg/gTOC and 1.28μg/gTOC could yield a more precise LOEC. In addition, a replication of Pacheco Creek tests with more finely divided concentrations around the LC_{50} determined by this study could potentially yield different data consistent with the Del Puerto Creek and American River assays.

Tests run in 2002 and 2003 on sediments from the Central Valley detected pyrethroids in 75% of 23 sites sampled, with lambda-cyhalothrin being the fourth most frequently found compound (Weston *et al.* 2004). In addition, pyrethroid concentrations in the sediments were sufficiently high to have contributed to mortality in 70% of samples toxic to *H. azteca* (Weston *et al.* 2004). Thirty-nine percent to 100% mortality among *H. azteca* was observed in three independent sediment samples from Del Puerto Creek (taken downstream of sampling locations for this study), taken between June and October 2002 (Weston *et al.* 2004). Officials have recorded deltamethrin levels between 4.0μg/L and 24.0μg/L in water, 3μg/kg to 5μg/kg in sediments, and 3.0μg/kg and 50μg/kg in animals in Canadian agricultural areas. These facts and figures indicate the prevalence of pyrethroids in agricultural areas, and when taken with the LC_{50} and LOEC data determined by this study, and the knowledge that *H. azteca* may not be the most sensitive animal in aquatic-agricultural environments, underscore the great need for relevant toxicity data among environmental stewards and students.

The LOEC and LC_{50} levels found in this study would not ideally translate directly into regulatory environmental standards, as the concentrations of pesticides they represent would likely be too high to maintain healthy ecosystem functioning in exposed areas. The use of LC_{10}s or LC_{20}s, for instance, might be more appropriate in such an endeavor. Regardless, accurate growth and LC_{50} information on environmental toxicants provide important assistance in the development of healthy environmental standards.

For the protection of global agricultural ecosystems and their multitude of inhabitants, more sediment-associated research is needed on the growth effects and toxicities of pyrethroid

pesticides. The numbers measured here are among the first of their kind being offered within the scientific community, and provide a sound range for future environmental studies of pyrethroid sediment toxicities and their ecological implications.

Acknowledgements

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