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Organophosphorus Pesticides in the Malibu Creek Watershed

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Final Report

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EXECUTIVE SUMMARY

This study was conducted in order to accomplish three goals: 1) to provide pesticide and toxicity information for the Malibu Creek watershed in order to assist the RWQCB's TMDL process; 2) to assess the usefulness of a relatively low cost, rapid method of measuring pesticides (Enzyme-Linked Immuno Sorbent Assay, ELISA); and 3) to assess the practicability of volunteer-based monitoring groups to measure pesticides as part of their routine monitoring program.

Creek Analysis

Three streams in the Malibu Creek watershed were assessed for contamination by organophosphorus pesticides (including diazinon and chlorpyrifos) and toxicity to *Ceriodaphnia dubia*. Dry weather samples were collected from Malibu Creek and two of its tributaries, Las Virgenes Creek and Medea Creek at monthly intervals between June 2002 and March 2003. Two storm events were sampled at Malibu Creek in February 2003.

- Diazinon was the only organophosphorus pesticide detected in any of the creek samples. Medea Creek was the only stream with measurable amounts of diazinon during the dry weather sampling. Diazinon was also found in both of the stormwater samples from Malibu Creek, with concentrations of 0.04 and 0.06 μg/L.

- Concentrations of diazinon exceeded California Department of Fish and Game water quality criteria[1] in some samples from both Malibu and Medea Creek. The concentration of diazinon in one stormwater composite sample from Malibu Creek (0.06 μg/L) was just above the chronic criterion (0.05 μg/L). Six out of 10 samples from Medea Creek exceeded the acute criterion (0.08 μg/L) by up to a factor of nine, and the chronic criterion by up to a factor of 14.

- Toxicity was present in some of the samples from each of the streams. The frequency of acutely toxic samples was not significantly different among streams. The frequencies of reproductive toxicity at Las Virgenes Creek and Medea Creek were significantly greater than the frequency at Malibu Creek. Two out of eight dry weather samples from Malibu Creek impaired *C. dubia* reproduction, compared to eight out of ten samples from Medea Creek and ten out of ten samples from Las Virgenes Creek. The stormwater samples were not toxic to *C. dubia*.

- Most of the dry weather samples that were toxic did not contain measurable amounts of pesticides. Of the 28 dry weather stream samples collected in this study, 20 samples impaired *C. dubia* reproduction, yet only six samples exceeded the diazinon chronic criterion; two additional toxic samples had measurable amounts of diazinon that did not exceed the chronic criterion. There was, however, a significant negative relationship between creek conductivity (a measure of dissolved salts) and toxicity.

Impacted water quality, as indicated by toxicity to *C. dubia*, appeared to be most severe in Medea Creek and Las Virgenes Creek, where the incidence of reduced survival and reduced reproduction was greater than that measured in Malibu Creek. Dissolved salts such as chlorides and the OP pesticide diazinon are both contributing factors to the reduced water quality. These two constituents had different effects on water quality at the various sites, however. Diazinon appeared to be responsible for the most severe toxic effects, complete mortality in two samples from Medea Creek, but did not appear to have an impact on the observed toxicity in the other samples. Dissolved salts were shown to be the likely cause of persistent impaired reproduction of *C. dubia* in many of the samples from all three study sites, indicating that this constituent group is of broad concern throughout the watershed.

ELISA Comparison

Two approaches were used to evaluate the usefulness of ELISA methods. First, diazinon and chlorpyrifos measurements made by ELISA were compared to measurements made by the more traditional GC/MS. Second, an interlaboratory comparison was conducted with four labs using ELISA to assess the accuracy and reliability of this technique.

- There was a significant relationship between ELISA and the more traditional GC/MS method for both diazinon ($r = 0.99$, $p < 0.01$) and chlorpyrifos ($r = 1.00$, $p < 0.01$) in spiked runoff samples.

- The interlaboratory comparison with ELISA indicated this technique has low variability for both diazinon (average coefficient of variation = 18% among labs with the same sample) and chlorpyrifos (average CV = 24%). Comparisons between measured and nominal concentrations of pesticide indicated there were no matrix effects associated with spiked samples of deionized water, dry weather runoff, or stormwater runoff with ELISA.

- The interlaboratory and method comparison results indicated ELISA was an accurate and reliable method for measuring diazinon and chlorpyrifos in environmental runoff samples.

- The ELISA method was considered feasible for use in a volunteer monitoring program, but the training necessary to apply the method was determined to outweigh the benefits in this particular study.

[1]The California Department of Fish and Game water quality criteria for diazinon were developed following the procedures outlined in USEPA 1985. Water quality criteria for diazinon are currently under development by USEPA.

ACKNOWLEDGEMENTS

The diazinon and chlorpyrifos EnviroGard ELISA plate kits used in the interlab comparison were obtained at a reduced cost from Strategic Diagnostics Inc. The authors thank Kerry Ritter (SCCWRP) for guidance with the data analysis, and Shelley Luce and Mark Abramson (Heal the Bay) for site selection and sample collection. The authors also thank Phil Markle (Los Angeles County Sanitation Districts), Bryn Phillips (Marine Pollution Studies Laboratory at Granite Canyon) and Jeff Miller (AquaScience) for participating in the ELISA interlab comparison. This project was funded by California State Water Resources Control Board Agreement Number 00-194-140-0.

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INTRODUCTION

Pesticides are widely used in urban regions for public and private use. Diazinon and chlorpyrifos are two of the three most commonly used pesticides on structures and/or landscapes in California. In fact, approximately 50-60% of diazinon is used for unreported uses, like home and garden areas. These pesticides often are sprayed on asphalt and lawns, and when these surfaces become wet or saturated with water, the runoff contains the contaminant and carries it to the stormdrain system.

In South San Francisco Bay, diazinon and chlorpyrifos have been found to cause significant toxicity in runoff from largely urban watersheds. Diazinon has been detected in urban creeks at levels as high as 6 µg/L during storms. The LC50s for diazinon and chlorpyrifos for *Ceriodaphnia* are approximately 0.4 µg/L and 0.06 µg/L respectively, so receiving waters that are acutely toxic to aquatic organisms have been found in California. A similar monitoring program is necessary in the Los Angeles Region because it is apparent from ongoing monitoring efforts that there are scant data on the presence of commonly used pesticides in our local streams and storm drains.

One large problem facing the Los Angeles Regional Water Quality Control Board (RWQCB) is the lack of data on which to base management decisions that are required for total maximum daily load (TMDL) development. Special studies are necessary to collect the pertinent information to support management decision-making.

This study was conducted in order to accomplish three goals: 1) to provide pesticide and toxicity information for the Malibu Creek watershed in order to assist the RWQCB's TMDL process; 2) to assess the usefulness of a relatively low cost, rapid method of measuring pesticides (Enzyme-Linked Immuno Sorbent Assay, ELISA); and 3) to assess the practicability of volunteer-based monitoring groups to measure pesticides as part of their routine monitoring program.

METHODS

STUDY DESIGN

The first part of this study measured concentrations of organophosphorus (OP) pesticides (including diazinon and chlorpyrifos), and toxicity of streams in the Malibu Creek watershed. The Malibu Creek watershed, located in the Santa Monica Mountains, receives drainage from both urban and undeveloped areas. Three streams within the Malibu Creek watershed were selected for this study: Malibu Creek, and two of its tributaries, Las Virgenes Creek, and Medea Creek (Figure 1). Malibu Creek receives drainage from 105 square miles, which includes six man-made lakes, the Tapia Water Reclamation Facility, the Rancho Las Virgenes Composting Facility, the Calabasas Landfill, and urban runoff from Agoura Hills, Westlake Village, part of Calabasas, and part of the 101 Freeway. Malibu Creek enters the Pacific Ocean in Malibu. Las Virgenes Creek receives drainage from part of Calabasas, the Rancho Las Virgenes Composting Facility, and the Calabasas Landfill. Medea Creek receives urban runoff from most of Agoura Hills, and part of the 101 Freeway. The sampling locations selected on these creeks are existing Heal the Bay monitoring sites (see Appendix). Heal the Bay is a nonprofit environmental organization concerned with protecting human health and marine life in Santa Monica Bay.

The second part of this study evaluated the usefulness of the ELISA method to measure diazinon and chlorpyrifos concentrations in urban runoff. Two approaches were used to evaluate the accuracy and variability of this technique. First, diazinon and chlorpyrifos measurements made by ELISA were compared to concurrent measurements made by GC/MS. Second, an interlaboratory comparison was conducted among four labs using ELISA to assess the between-lab variability and accuracy of this technique.

The third part of this study assessed the practicability of the Heal the Bay Stream Team to measure pesticides as part of their routine monitoring program. The Stream Team is a volunteer monitoring program that monitors streams in the Malibu Creek watershed for dissolved oxygen, pH, nutrients and bacteria, and the health indices of benthic communities. Because OP pesticides are potentially important factors affecting the creeks, it may be beneficial to include measurement of these pesticides into a monitoring program.

FIELD SAMPLING

Samples from the Malibu Creek watershed were collected monthly by Heal the Bay Stream Team volunteers between June 2002 and March 2003. Dry weather samples were collected from Las Virgenes Creek, and Medea Creek for each month. Samples were collected from Malibu Creek each month except August and September, when Malibu Creek was dry and no samples could be collected from this stream. Two wet weather events were sampled at Malibu Creek in February 2003. The first wet weather samples were collected February 12 and 13, while the second wet weather samples were collected February 25. Composites were made from five grab samples collected during each storm event. All samples were collected by immersing a precleaned sampling container into the stream, and rinsing the container twice before filling the bottle and capping. Samples for chemical analysis were collected in 1 L glass amber jars, while samples for toxicity were collected in 1 gallon amber glass jugs. The samples were kept on ice for transport to the analytical laboratories.

CHEMICAL ANALYSIS

The stream samples were analyzed for 30 OP pesticides (Table 1). The pesticides were extracted within 7 days of collection by EPA Method 3510C, and analyzed by gas chromatography/mass spectrometry (GC/MS) following EPA Method 8141A (USEPA 1996). All holding times were met. All of the dry weather samples and the second stormwater composite sample were analyzed by GC/MS at APPL Laboratories (Fresno, CA). The first stormwater composite, and all stormwater grab samples were analyzed by ELISA at SCCWRP. ELISA is an analytical method that uses antibodies to target specific pesticides, and a color changing reaction to quantify the amount of pesticide present in a sample. Pesticide analyses by ELISA were made using Strategic Diagnostics Inc. (Newark, DE) EnviroGard plate kits.

Quality assurance procedures for analysis by GC/MS included internal standards (i.e., spiked field samples), method blanks, and lab control spiked samples. Quality assurance procedures for ELISA included ensuring a linear response for the calibration curves, and maintaining a coefficient of variation $\leq$ 20% for all optical density replicates from the plate reader.

TOXICITY TESTING

Creek samples were tested for toxicity at 100% and 50% runoff concentrations using the 7-day *Ceriodaphnia dubia* survival and reproduction test (USEPA 1989). All toxicity tests were started within 2 days of sample collection. Ten replicates were used for the survival and reproduction endpoints. A concurrent copper reference toxicant test was conducted with the creek samples. Each test included a laboratory control. Test solutions were changed on a daily basis. Dissolved oxygen, conductivity, pH, and temperature were measured each day. Alkalinity, hardness, and total ammonia were measured at the beginning of each experiment. The organisms were fed each day. All toxicity testing was conducted by AMEC Earth & Environmental Inc. (San Diego, CA).

ELISA EVALUATION

Two approaches were used to evaluate the accuracy and variability of the ELISA method for pesticide measurements. First, diazinon and chlorpyrifos measurements made by ELISA were compared to concurrent measurements made by GC/MS. The samples that were analyzed included the dry weather samples from November, December and January, the stormwater sample from the second storm event in February, and runoff samples from Cerritos Channel, Coyote Creek, and San Gabriel River that were spiked with various concentrations of diazinon and chlorpyrifos. Second, an interlaboratory comparison was conducted with four labs using ELISA. The participating ELISA labs included AquaScience, Los Angeles County Sanitation Districts, Marine Pollution Studies Laboratory at Granite Canyon, and SCCWRP. The samples that were analyzed included many of the runoff samples from the comparison between ELISA and GC/MS, but also included spiked deionized water samples. Nominal concentrations of diazinon in the interlab comparison ranged from 0.031 - 0.500 µg/L, and nominal concentrations of chlorpyrifos ranged from <0.05 - 0.900 µg/L.

DATA ANALYSIS

Significant differences in the number of samples with detectable amounts of pesticide within each creek were assessed with analysis of variance. Relationships among indicators (e.g., *C. dubia* survival vs. diazinon concentration) were assessed with Spearman rank correlation (SigmaStat statistical software version 2.03). Significant differences in diazinon and chlorpyrifos measurements among the ELISA labs were assessed using multiple regression analysis (SAS statistical software version 9.0).

Data from the *C. dubia* tests were evaluated for significant reductions in survival or reproduction using analysis of variance with Dunnett's test, or with Steel's Many-One rank test when assumptions of normality or homoscedasticity were not met. Comparisons were made against the laboratory control. In this report, both the survival and reproduction data are expressed as a percentage of the control (i.e., the survival or reproduction response of each sample, divided by the survival or reproduction response of the control, multiplied by 100). Adjusting the survival and reproduction response to the control is necessary to account for differences in organism response between experiments, when data from more than one experiment are compared. Precision of the interlaboratory comparison results among labs was described by the coefficient of variation (%CV). The %CV was calculated by dividing the standard deviation of the data by the mean, and multiplying the result by 100.

RESULTS

CHEMICAL ANALYSIS

Of the 30 OP pesticides analyzed in this study, diazinon was the only pesticide found in any of the creek samples. Medea Creek was the only stream with measurable amounts of diazinon during the dry weather sampling. Diazinon was found in Medea Creek during 8 of the 10 months this stream was sampled (Figure 2, Table 2). Concentrations of diazinon ranged from <0.05 µg/L in February and March 2003 to 0.70 µg/L in December 2002.

Diazinon was also found in both of the Malibu Creek stormwater composite samples (Figure 2, Table 2). The concentrations of diazinon in the composites were 0.06 µg/L for the February 12-13 storm event, and 0.03 µg/L for the February 25 storm event. For the individual grabs that made up each composite, the diazinon concentrations were within a factor of two; concentrations for the February 12-13 storm event ranged from 0.04-0.07 µg/L, while concentrations for the February 25 storm event ranged from 0.03-0.05 µg/L.

Concentrations of diazinon in both Malibu and Medea Creek exceeded California Department of Fish and Game water quality criteria. The first stormwater composite from Malibu Creek was 1.2 times the chronic criterion (0.05 µg/L, Sipmann and Finlayson 2000). Six of the ten samples from Medea Creek exceeded both the chronic and acute (0.08 µg/L) criteria for diazinon. Exceedances in Medea Creek samples ranged from 1.4 - 8.8 times the acute criterion, and 2.2 - 14 times the chronic criterion.

TOXICITY TESTING

There were differences in the mean survival of *C. dubia* among the streams. Dry weather samples collected from Malibu Creek had the highest mean survival (95%, control adjusted), followed by Medea Creek (74%) and Las Virgenes Creek (69%) (Table 3). Statistical analysis (analysis of variance) indicated that mean survival was significantly different among the group of three streams, but the sample size was not large enough to distinguish differences between specific locations. The wet weather samples from Malibu Creek were not toxic to *C. dubia* survival (Table 3). Water quality measurements during the test were acceptable. Most water quality measurements were consistent among the creeks (Table 4). The conductivity levels of the samples from Medea Creek and Las Virgenes Creek, however, tended to be higher than values for Malibu Creek.

One out of eight dry weather samples from Malibu Creek caused significantly reduced *C. dubia* survival, compared to two out of ten samples from Medea Creek, and four out of ten samples from Las Virgenes Creek. Toxicity to *C. dubia* survival was eliminated for all samples from Malibu Creek and Las Virgenes Creek upon dilution to 50% with control freshwater, but the strong toxicity in the Medea Creek sample collected in December persisted after dilution (Table 5). The frequency of toxic samples was not significantly different among the stations ($p = 0.69$).

There was a temporal trend in survival for the Las Virgenes Creek samples, with the lowest survival occurring in June, August and September 2002, and the highest survival occurring in February and March 2003 (Figure 3). The other two streams did not have a temporal pattern in *C. dubia* survival.

Ceriodaphnia dubia reproduction also varied among the streams (Figure 4). The reproduction rate for *C. dubia* exposed to Medea Creek and Las Virgenes Creek samples was significantly lower than the reproduction rate for those exposed to dry weather samples from Malibu Creek. The highest mean reproduction rate was associated with samples from Malibu Creek (114% of the control for wet weather samples, 104% of the control value for dry weather samples), which was twice that of Medea Creek (57%), and almost three times higher than Las Virgenes Creek (39%).

Two out of eight dry weather samples from Malibu Creek had reduced *C. dubia* reproduction, compared to eight out of ten samples from Medea Creek, and ten out of ten samples from Las Virgenes Creek. The frequencies of occurrence of reproductive toxicity at Las Virgenes Creek and Medea Creek were significantly greater than the frequency at Malibu Creek. The wet weather samples were not toxic to *C. dubia* reproduction.

There appeared to be a threshold value for *C. dubia* survival related to the concentration of diazinon in the creek samples. The highest concentration of diazinon that was not associated with poor survival was 0.32 µg/L (Figure 5). Diazinon concentrations above 0.32 µg/L were associated with low survival. This concentration is near the *C. dubia* LC_{50} for diazinon (0.44 µg/L; the concentration that causes a 50% reduction in *C. dubia* survival). However, some of the toxic stream samples did not have any detectable amounts of OP pesticides. Some of the low survival may have been related to dissolved salts in the creeks, since many of the samples contained relatively high conductivities and there was a significant negative correlation between survival and creek conductivity ($p < 0.01$, $r = -0.58$) (Figure 6).

The reduced reproduction in the creek samples did not correspond to diazinon concentration (Figure 5). Most of the samples with poor reproduction did not have any measurable amounts of OP pesticides. Reproductive toxicity appeared to be related to dissolved salts in the creek, as indicated by a high negative correlation between number of young and conductivity ($p < 0.01$, $r = -0.74$) (Figure 6). The highest conductivity in the creek samples that was not consistently associated with reduced reproduction was approximately 1900 µmhos-cm, while a conductivity of approximately 2800 µmhos-cm was always associated with reduced reproduction.

To help determine if high conductivity was causing toxicity in the stream samples, a laboratory experiment was performed using conductivity levels of 1900 and 2800 µmhos-cm (Table 6). Both of these conductivities impaired reproduction in the laboratory experiment and the magnitude of effect was similar to that observed in creek samples of similar conductivity. This suggests that dissolved constituents causing the increased conductivity may have been responsible for the reproduction impairment in the stream samples that had no measurable amounts of OP pesticides. Survival did not appear to be affected by conductivity levels in the laboratory manipulation experiment.

Concentrations of ammonia in the creek samples were not correlated with *C. dubia* toxicity (Figure 7). However, the concentration of unionized ammonia in one sample from Medea Creek (0.39 mg/L NH_3) was within the range of threshold values identified as having an effect on *C. dubia* reproduction (0.39 – 0.90 mg/L) (AMEC 2002). The conductivity level in this sample (2680 μmhos-cm), incidentally, was also high enough to have caused the toxicity. No other sample in this study had a concentration of unionized ammonia within the range of threshold effect values.

ELISA EVALUATION

There was good agreement between the ELISA and GC/MS analytical results. Measurements were highly correlated for both diazinon ($r = 0.99$, $p < 0.01$) and chlorpyrifos ($r = 1.00$, $p < 0.01$) (Figure 8).

The interlab comparison also demonstrated that the ELISA method is suitable for OP pesticide measurements. The performance objectives were usually met by all four labs (calibration curves were usually linear, and replicate variability was low), indicating the method is reliable (Table 7). The mean concentrations of all samples among the four labs correlated well with the nominal concentrations of diazinon ($r = 0.99$, $p < 0.01$) and chlorpyrifos ($r = 0.96$, $p < 0.01$). ELISA measurements in the interlab comparison did not appear to be influenced by matrix type. The relationship between nominal and measured values of diazinon or chlorpyrifos were similar between spiked lab water, dry weather runoff, and wet weather runoff samples (Figures 9 and 10).

The variability among labs (measured as % coefficient of variation) was low for both pesticides. The %CV for diazinon ranged from 7-42%, with a mean of 18%, while the %CV for chlorpyrifos ranged from 12-38%, with a mean of 24%. The concentrations of chlorpyrifos and diazinon among the labs varied by up to a factor of three, but were typically within a factor of two. The differences among laboratories were not statistically significant for diazinon ($p = 0.07$) or chlorpyrifos ($p = 0.10$) (multiple regression analysis).

DISCUSSION

The toxicity and chemical results from this study showed that there were differences in water quality throughout the Malibu Creek watershed. While toxicity was present in samples from each of the three study sites during this study, there were differences in the frequency and likely cause of effects (Table 8).

Contamination by the OP pesticide diazinon was usually present at the Medea Creek site, where eight of ten samples contained detectable levels of this pesticide. Most of the toxicity that was observed at this site, however, was not related to concentrations of diazinon. Toxicity that was likely due to diazinon was detected in two of the samples from Medea Creek, where concentrations of this pesticide were above levels known to produce mortality and there was a corresponding reduction in *C. dubia* survival. The other six toxic samples from Medea Creek produced reduced reproduction, but no increase in mortality. Although diazinon was often detected in these samples, the toxicity was more likely due to increased dissolved constituents since every toxic sample had a relatively high conductivity that was above the level shown to be toxic in a laboratory test.

Water from the Las Virgenes Creek site was always toxic, yet OP pesticides were not responsible. All of the Las Virgenes Creek samples contained high conductivity, which was sufficient to cause the observed reductions in reproduction. Six of the ten Las Virgenes Creek water samples also caused increased mortality in *C. dubia*. The cause of this mortality is uncertain; it may reflect the high conductivity of the samples, but the conductivity toxicity experiment did not test a high enough concentration to evaluate this hypothesis. The reduced survival measured at these stations may have also been due to an unmeasured chemical constituent. Only a limited chemical analysis was conducted in the samples, so the presence of additional toxic constituents cannot be excluded.

Water quality appeared to be best at the Malibu Creek site. Toxicity was only infrequently present and no OP pesticide contamination was present in the dry weather samples. Conductivity in the Malibu Creek samples was also high enough to account for the reproductive toxicity observed.

The results from this study demonstrate that water quality is impacted in portions of the Malibu Creek watershed. Impacted water quality, as indicated by toxicity to *C. dubia*, appeared to be most severe in Medea Creek and Las Virgenes Creek, where the incidence of reduced survival and reduced reproduction was greater than that measured in Malibu Creek. Dissolved salts such as chlorides and the OP pesticide diazinon are both contributing factors to the reduced water quality. These two constituents had different effects on water quality at the various sites, however. Diazinon appeared to be responsible for the most severe toxic effects, complete mortality in two samples from Medea Creek, but did not appear to have an impact on the observed toxicity in the other samples. Dissolved salts were shown to be the likely cause of persistent impaired reproduction of *C. dubia* in many of the samples from all three study sites, indicating that this constituent group is of broad concern throughout the watershed. Some instances of reduced survival and reproduction in samples from the Malibu Creek watershed could not be associated with a likely cause based on the results of this study. Possible causes for this toxicity include unmeasured contaminants such as other pesticides or the interaction of dissolved salts with other environmental factors. Additional research, including comprehensive chemical analyses and Toxicity Identification Evaluation studies are needed in order to identify other likely causes of toxicity in this watershed.

The ecological significance of the water quality impacts identified using the chemical and toxicological analyses could not be verified in this study. No bioassessment data for the same time frame were available for comparison. These sites were examined in May and October 2000 using the California Stream Bioassessment Procedure (SLSI 2000) and the results showed that each creek was in poor condition. However, the poor biological condition observed at these sites was likely due to habitat factors unrelated to the water quality parameters found to be associated with toxicity. The kinds and proportions of macroinvertebrates present at these sites were tolerant forms that have the ability to survive warm waters, thick algal growth, and mild organic pollution. The large amounts of algae present at these sites suggested these streams receive relatively high nutrient concentrations, and adequate sunlight.

This study also examined the practicability of a volunteer-based monitoring group to collect and measure pesticide samples as part of their routine monitoring program. The Heal the Bay Stream Team volunteers were successful in collecting the dry weather creek samples for this study during pre-scheduled monthly sampling events. The storm samples, however, were collected by Heal the Bay staff, because of the time constraints and unpredictable nature of stormwater sampling.

Heal the Bay volunteers did not use the ELISA technique for pesticide monitoring, as was originally planned for this project. After a training session on the ELISA methodology, Heal the Bay staff thought the method was feasible for use by volunteers but that it was not practical to commit volunteer monitors to learn and use ELISA in this study, and that GCMS analysis was preferred in a general survey project. This opinion reflected the fact that ELISA analysis provided information for only two analytes of interest (diazinon and chlorpyrifos), compared to GC/MS, which would provide information for 30 OP pesticides. Therefore, instead of volunteers analyzing the Malibu Creek watershed samples, all ELISA measurements of the watershed samples in this study were made at SCCWRP.

Measurements using ELISA and GC/MS showed a high degree of correspondence between analytical methods and the results agreed well for different matrix types (dry weather and storm water runoff). Others have reported seeing a greater difference in measured pesticide concentrations between these two techniques when different matrices are analyzed. Sullivan and Goh (2000) found a close correlation between ELISA and GC/MS with river water samples, but a poor relationship between methods with urban stormwater runoff samples. Moreover, ELISA tended to estimate a higher concentration of diazinon than did GC/MS, particularly for stormwater runoff samples (Sullivan and Goh 2000). In the current study, there did not appear to be a bias in diazinon measurements towards either technique with Malibu Creek watershed samples. Chlorpyrifos measurements, however, tended to be greater using ELISA than GC/MS for both dry and wet weather runoff samples. This difference in chlorpyrifos measurements could be due to a loss of pesticide during sample extraction for GC/MS. A consistent positive bias towards ELISA was not seen for diazinon measurements, possibly because diazinon is less hydrophobic, and not as easily lost during preconcentration and cleanup steps. One of the advantages of ELISA is that there are no sample extraction steps. Thus ELISA is a faster technique than GC/MS, and requires less manipulation of the sample. The interlaboratory and method comparison results showed that ELISA was an accurate and reliable method for measuring diazinon and chlorpyrifos in environmental runoff samples.

The variability among the four labs in the ELISA interlab comparison was similar to the variability found during an interlab comparison study of GC/MS labs that measured DDT in sediments (Gossett et al. 2002). The average coefficient of variation in the interlab ELISA comparison was 24% for chlorpyrifos measurements and 18% for diazinon. By comparison, the CV in the interlab GC/MS study was 31% for total DDT in sediments (Gossett et al. 2002).

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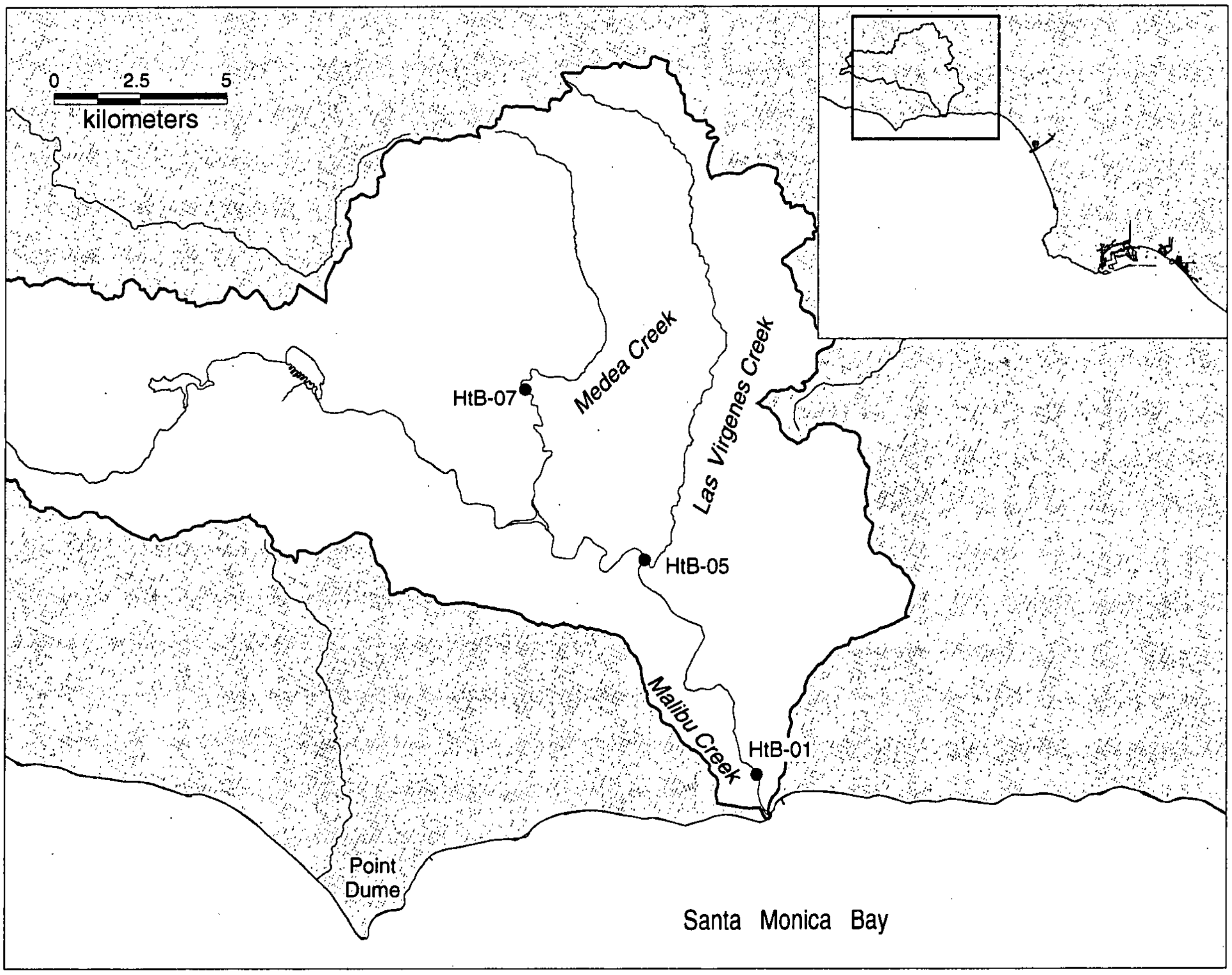


Figure 1. Stream sampling locations within the Malibu Creek watershed.

Table 1. Analyte list of organophosphorus pesticides analyzed by GC/MS in the Malibu Creek watershed samples. PQL = practical quantitation level (lowest concentration which can be consistently determined within ±20% of the nominal concentration).

Analyte	**PQL (µg/L)**
Azinphosmethyl	1
Bolstar	0.1
Chlorpyrifos	0.05
Coumaphos	0.02
Def	0.01
Demeton	0.02
Diazinon	0.05
Dichlorvos	0.2
Dimethoate	0.1
Disulfoton	0.1
EPN	0.1
EPTC	0.1
Ethion	0.1
Ethoprop	0.1
Fensulfothion	0.5
Fenthion	0.1
Malathion	0.1
Merphos	0.1
Mevinphos	0.7
Naled	0.5
Parathion, ethyl	0.1
Parathion, methyl	0.1
Phorate	0.1
Prowl (Pendimethalin)	0.1
Ronnel	0.1
Stirophos	0.1
Sulfotep	0.1
Tokuthion	0.1
Trichloronate	0.1
Trifluralin	0.1

Table 2. Diazinon concentrations in Malibu Creek watershed stream samples measured by GC/MS and ELISA. Diazinon was the only OP pesticide that was detected in any of the creek samples. All samples were collected during dry weather flow, except for the stormwater samples collected in February. NA = not analyzed. Nondetects (<0.05 µg/L GC/MS, <0.03 µg/L ELISA) were treated as equal to half the practical quantitation level and are indicated by a "u" suffix.

		Concentration of diazinon (µg/L)	
Sample	**Collection date**	**GC/MS**	**ELISA**
Malibu Creek	6/3/2002	0.025u	NA
Malibu Creek	7/22/2002	0.025u	NA
Malibu Creek	10/14/2002	0.025u	NA
Malibu Creek	11/4/2002	0.025u	0.015u
Malibu Creek	12/2/2002	0.025u	0.015u
Malibu Creek	1/13/2003	0.025u	0.015u
Malibu Creek	2/3/2003	0.025u	NA
Malibu Creek (stormwater composite)	2/12 & 2/13/03	NA	0.06
Malibu Creek (stormwater composite)	2/25/2003	0.03	0.04
Malibu Creek	3/3/2003	0.025u	NA
Las Virgenes Creek	6/3/2002	0.025u	NA
Las Virgenes Creek	7/22/2002	0.025u	NA
Las Virgenes Creek	8/5/2002	0.025u	NA
Las Virgenes Creek	9/2/2002	0.025u	NA
Las Virgenes Creek	10/14/2002	0.025u	NA
Las Virgenes Creek	11/4/2002	0.025u	0.015u
Las Virgenes Creek	12/2/2002	0.025u	0.015u
Las Virgenes Creek	1/13/2003	0.025u	0.015u
Las Virgenes Creek	2/3/2003	0.025u	NA
Las Virgenes Creek	3/3/2003	0.025u	NA
Medea Creek	6/3/2002	0.11	NA
Medea Creek	7/22/2002	0.41	NA
Medea Creek	8/5/2002	0.05	NA
Medea Creek	9/2/2002	0.32	NA
Medea Creek	10/14/2002	0.21	NA
Medea Creek	11/4/2002	0.11	0.10
Medea Creek	12/2/2002	0.70	0.82
Medea Creek	1/13/2003	0.04	0.05
Medea Creek	2/3/2003	0.025u	NA
Medea Creek	3/3/2003	0.025u	NA

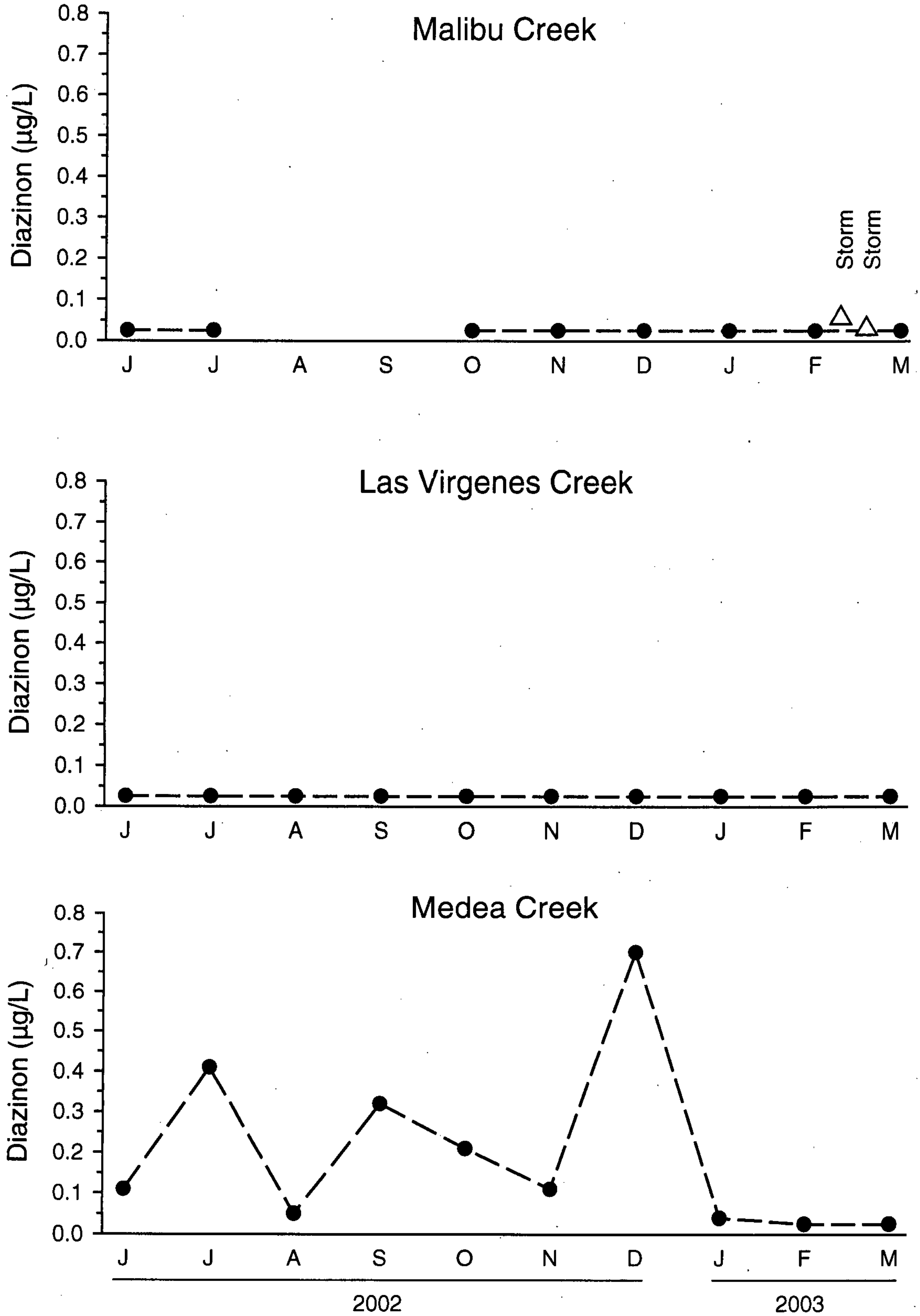


Figure 2. Concentrations of diazinon in stream samples collected from the Malibu Creek watershed between June 2002 and March 2003. All samples were collected during dry weather flow, except for the two stormwater samples collected from Malibu Creek in February. The first storm sample collected in February was analyzed by ELISA, while all other samples were analyzed by GC/MS. Nondetects (<0.05 µg/L) were treated as equal to half the practical quantitation level.

Table 3. Mean concentrations of diazinon, and *C. dubia* survival and reproduction in stream samples collected from the Malibu Creek watershed between June 2002 and March 2003. Diazinon was the only OP pesticide measured in any stream sample. All diazinon measurements were made using GC/MS, exept for one of the wet weather samples, which was analyzed by ELISA. Nondetects (<0.05 µg/L) were treated as equal to half the practical quantitation level, and are indicated by a "u" suffix.

Stream	# Samples	Mean diazinon concentration (µg/L)	Mean survival (% control)	% Samples toxic to *C. dubia* survival	Mean reproduction (% control)	% Samples toxic to *C. dubia* reproduction
Malibu Creek (dry weather)	8	0.02u	95	12	104	25
Malibu Creek (wet weather)	2	0.04	100	0	114	0
Las Virgenes Creek	10	0.02u	69	40	39	100
Medea Creek	10	0.20	74	20	57	80

Table 4. Summary of water quality conditions from the *C. dubia* toxicity tests. Mean values are followed in parentheses by the minimum and maximum daily values. Nondetects for total ammonia (<0.1 mg/L) were treated as equal to half the detection limit, and are indicated by the "u" suffix. Ammonia was measured in only one of the Malibu Creek wet weather samples.

Stream	# Samples	pH	Dissolved Oxygen (mg/L)	Temperature (°C)	Conductivity (µmhos-cm)	Total ammonia (mg/L)	Unionized ammonia (mg/L)
Malibu Creek (dry weather)	8	8.33 (7.74-8.70)	8.9 (6.3-13.5)	24.7 (23.4-26.0)	1943 (1338-3150)	0.6 (0.05u-3.8)	0.058 (0.004-0.301)
Malibu Creek (wet weather)	2	8.12 (7.85-8.40)	8.4 (6.4-9.9)	24.6 (23.9-25.5)	1002 (688-1313)	1.1	0.062
Las Virgenes Creek	10	8.21 (7.91-8.66)	8.9 (7.2-11.3)	24.7 (23.4-25.9)	3241 (2540-3510)	0.6 (0.05u-3.9)	0.039 (0.003-0.278)
Medea Creek	10	8.22 (7.81-8.69)	8.6 (7.2-11.2)	24.7 (23.7-26.0)	2715 (2240-3100)	0.7 (0.05u-4.6)	0.057 (0.003-0.389)

Table 5. *Ceriodaphnia dubia* survival and reproduction in Malibu Creek watershed samples. The samples were analyzed as 100% creek sample, and 50% creek sample diluted with laboratory control freshwater. Data are expressed as percent control. * = significantly different from control.

		Survival (% control)		Reproduction (% control)	
Sample	**Collection date**	**50%**	**100%**	**50%**	**100%**
Malibu Creek	6/3/2002	100	111	116	111
Malibu Creek	7/22/2002	100	60*	89	58*
Malibu Creek	10/14/2002	100	90	96	61*
Malibu Creek	11/4/2002	112	100	137	111
Malibu Creek	12/2/2002	100	100	131	111
Malibu Creek	1/13/2003	100	100	106	106
Malibu Creek	2/3/2003	100	90	94	81
Malibu Creek (stormwater composite)	2/12 & 2/13/03	100	100	106	114
Malibu Creek (stormwater composite)	2/25/2003	100	100	100	113
Malibu Creek	3/3/2003	111	111	195	190
Las Virgenes Creek	6/3/2002	111	44*	89	16*
Las Virgenes Creek	7/22/2002	100	70*	86*	36*
Las Virgenes Creek	8/5/2002	90	50*	92	44*
Las Virgenes Creek	9/9/2002	100	50*	76*	30*
Las Virgenes Creek	10/14/2002	101	67*	91	43*
Las Virgenes Creek	11/4/2002	90	60*	54*	18*
Las Virgenes Creek	12/2/2002	100	80	95	52*
Las Virgenes Creek	1/13/2003	100	80	84*	52*
Las Virgenes Creek	2/3/2003	100	89	81	53*
Las Virgenes Creek	3/3/2003	111	100	150	45*
Medea Creek	6/3/2002	111	67	116	68*
Medea Creek	7/22/2002	89	0*	81	0*
Medea Creek	8/5/2002	100	100	100	80*
Medea Creek	9/9/2002	100	80	99	69*
Medea Creek	10/14/2002	90	100	100	75*
Medea Creek	11/4/2002	90	100	93	50*
Medea Creek	12/2/2002	0*	0*	16*	0*
Medea Creek	1/13/2003	100	90	103	77*
Medea Creek	2/3/2003	100	100	78*	36*
Medea Creek	3/3/2003	100	100	155	115

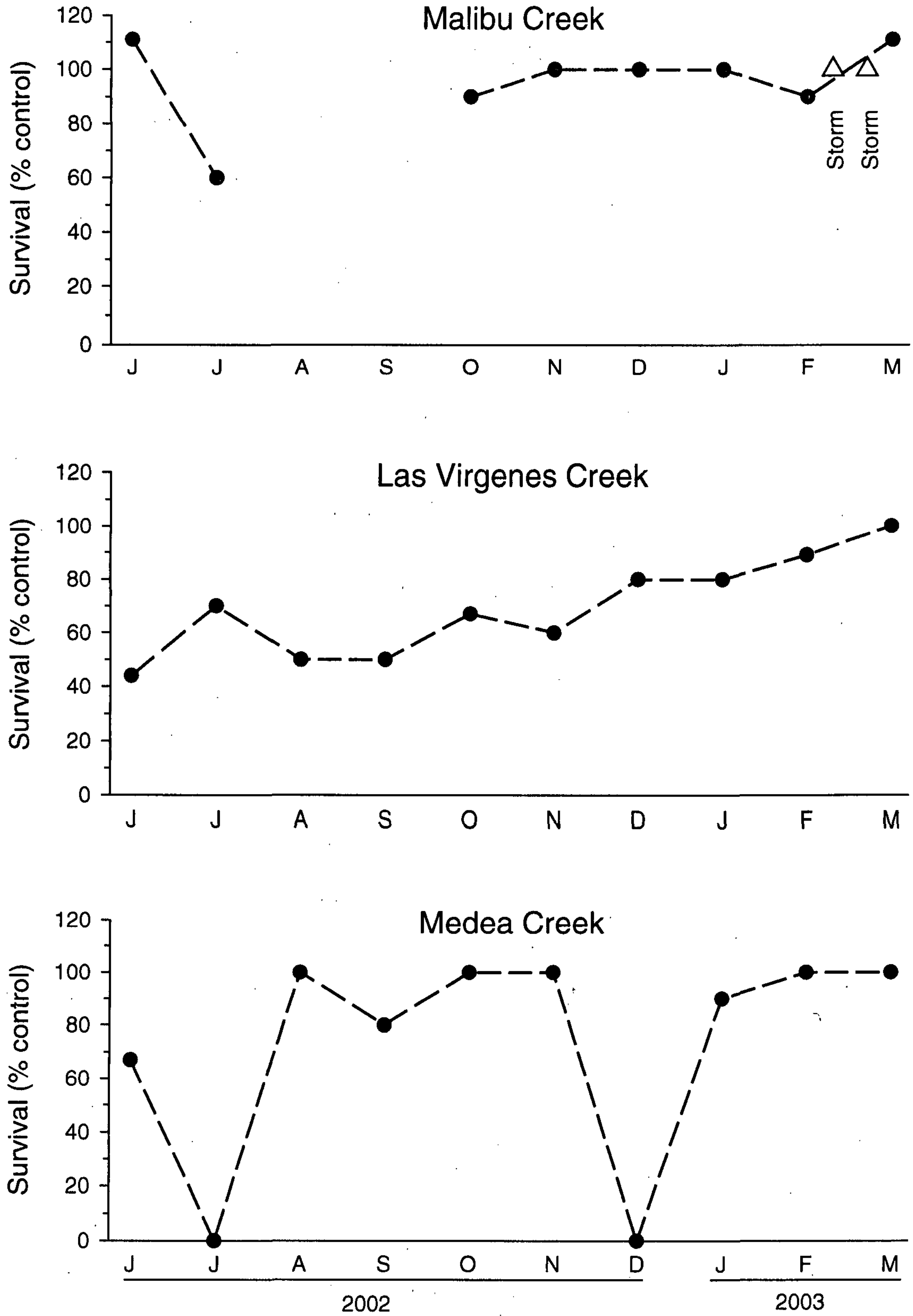


Figure 3. *Ceriodaphnia dubia* survival in 100% stream samples collected from the Malibu Creek watershed between June 2002 and March 2003. All samples were collected during dry weather flow, except for the two stormwater samples collected from Malibu Creek in February.

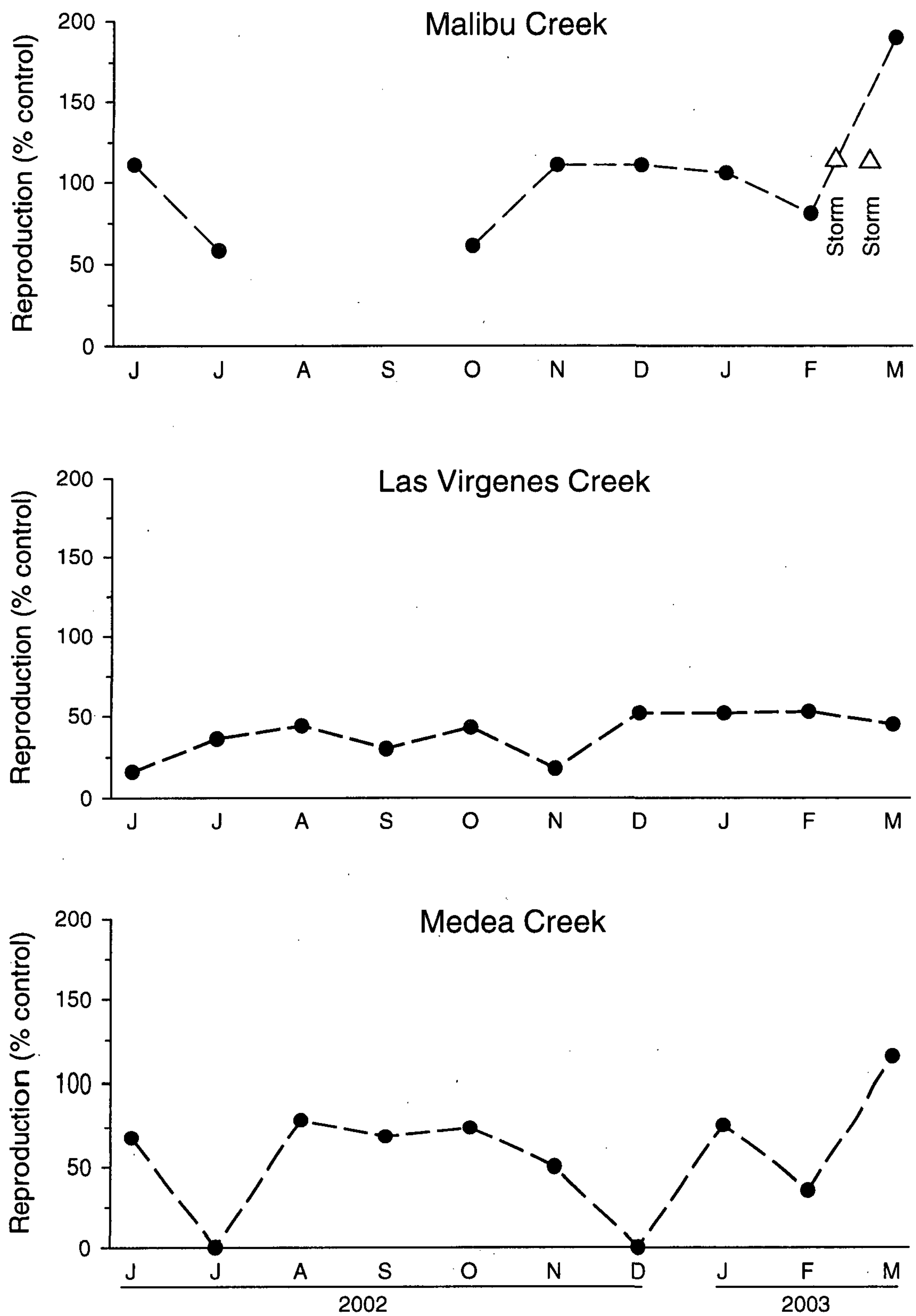


Figure 4. *Ceriodaphnia dubia* reproduction in 100% stream samples collected from the Malibu Creek watershed between June 2002 and March 2003. All samples were collected during dry weather flow, except for the two stormwater samples collected from Malibu Creek in February.

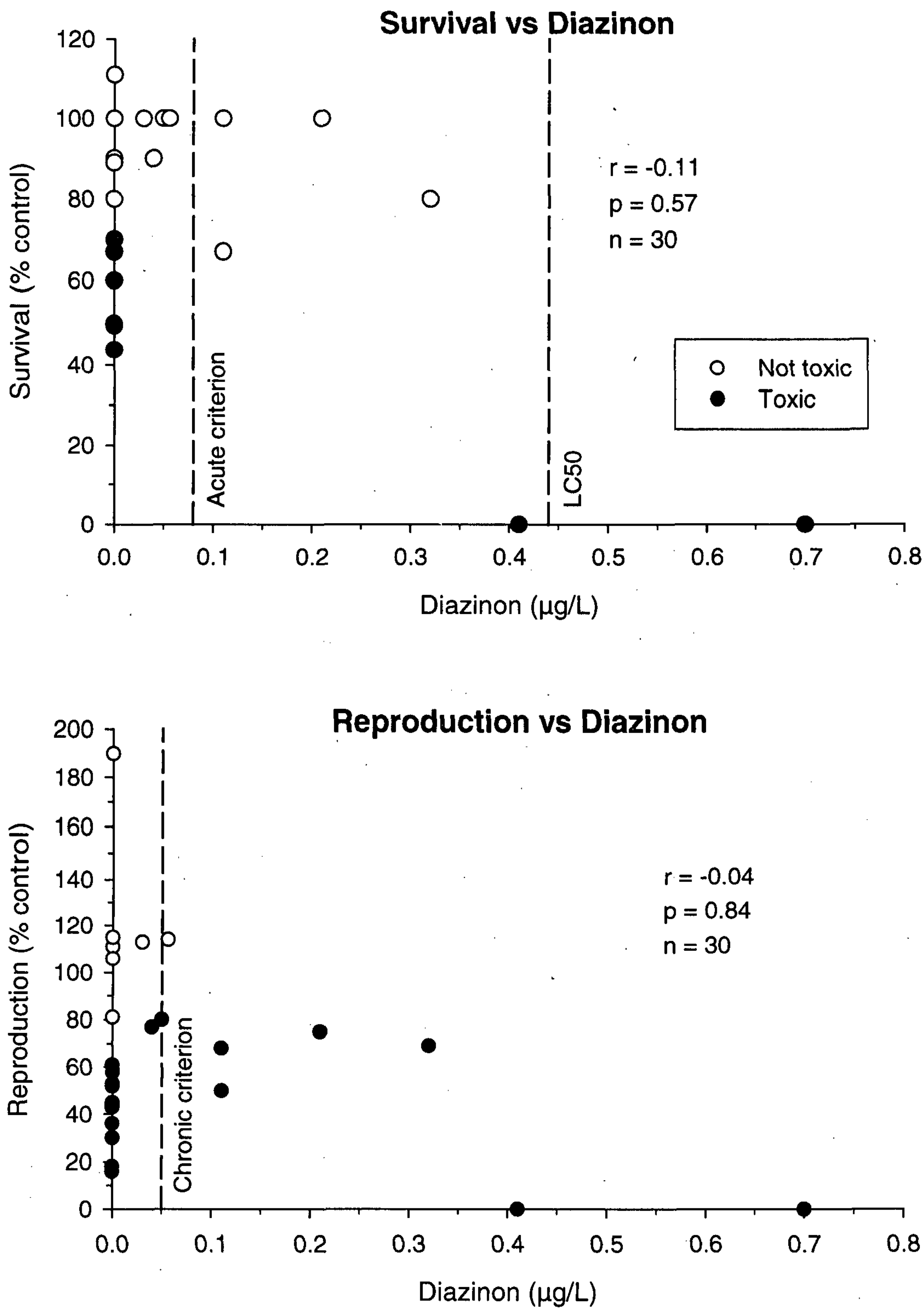
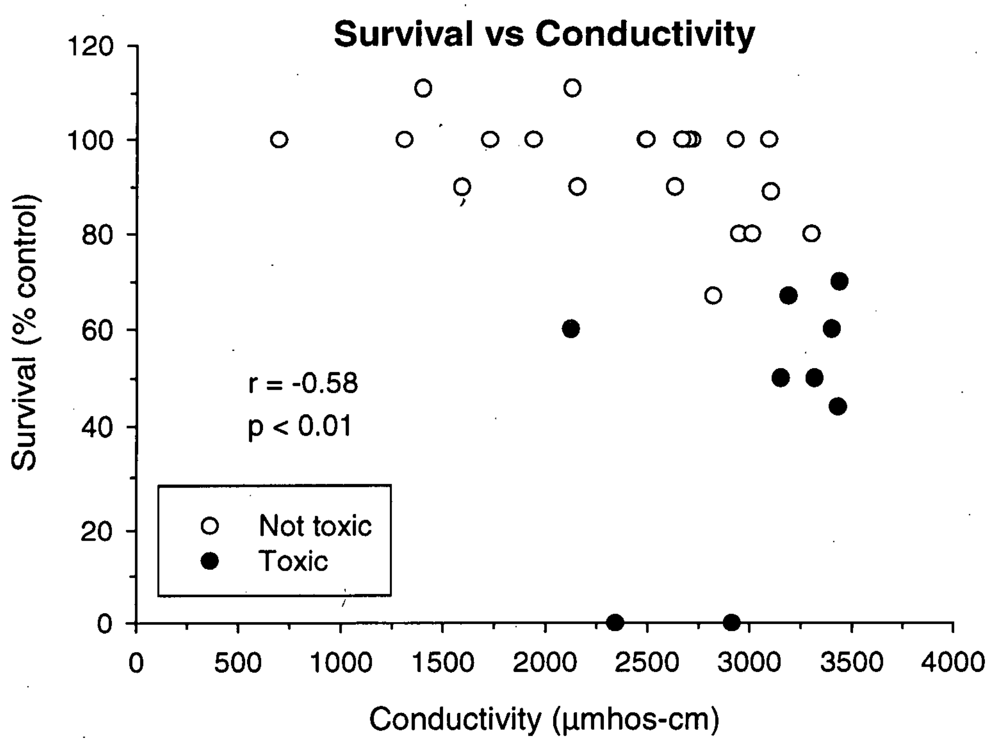


Figure 5. Relationship between diazinon and *C. dubia* survival and reproduction in 100% stream samples. Data are from all Malibu Creek watershed samples collected between June 2002 and March 2003. The data were adjusted to the percent of control in order to account for differences in organism response among experiments. Water Quality Criteria are from California Department of Fish and Game.



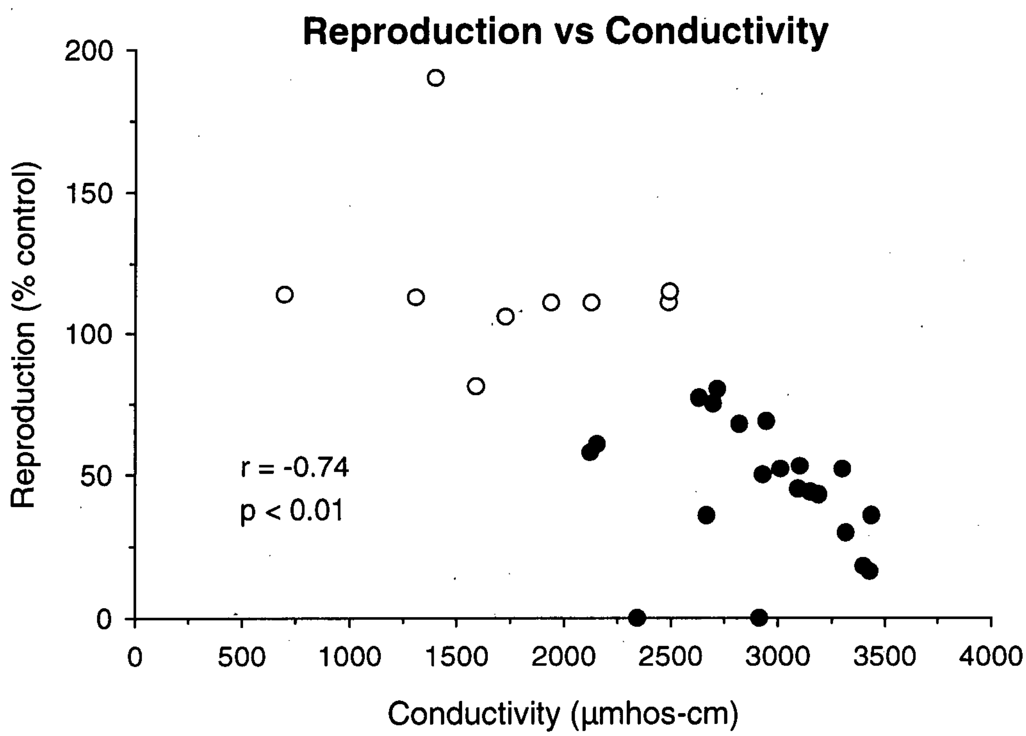
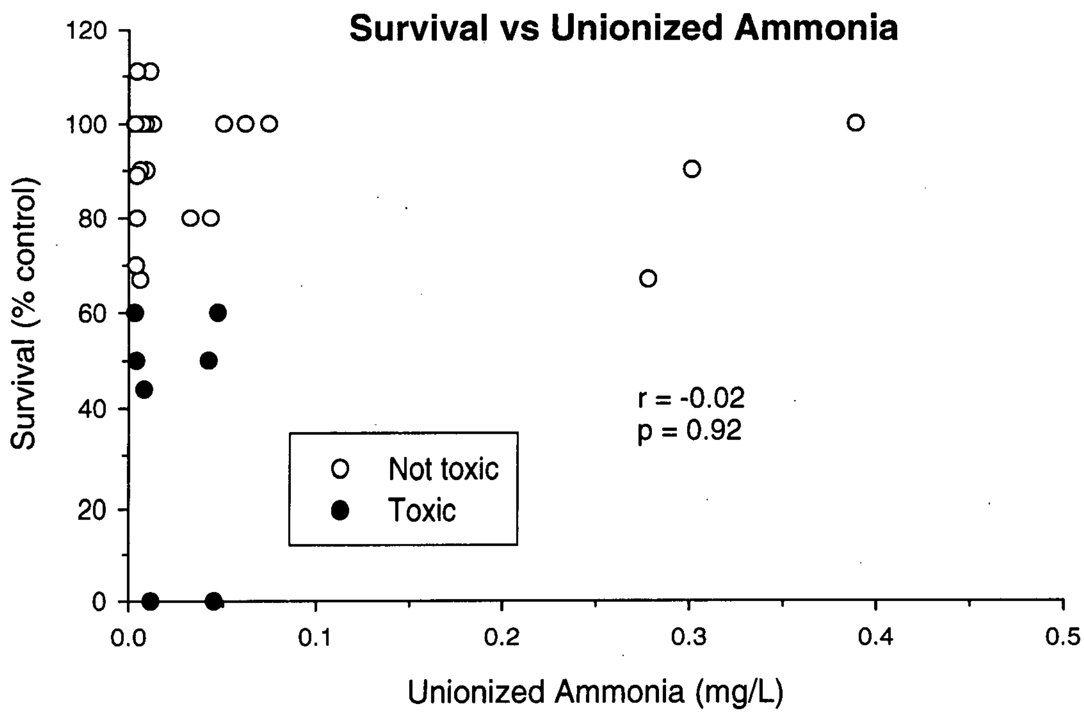


Figure 6. Relationships between conductivity and *C. dubia* survival and reproduction for 100% samples from the Malibu Creek watershed. Data are from all Malibu Creek watershed samples collected between June 2002 and March 2003.

Table 6. *Ceriodaphnia dubia* survival and reproduction in the laboratory experiment with manipulated conductivity levels conducted in March 2003. Both of the manipulated conductivity levels impaired reproduction. * = significantly different from control.

Mean Conductivity (µmhos-cm)	Survival (% control)	Reproduction (% control)
194 (Control)	100	100
1894	67	32*
2807	100	6*



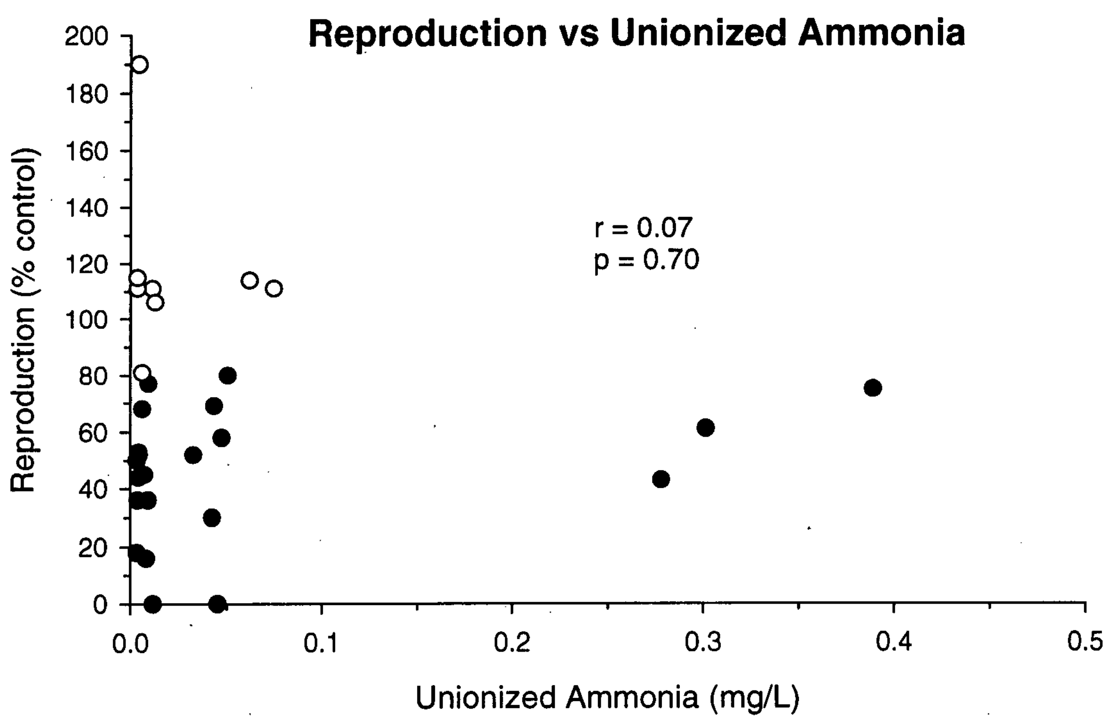
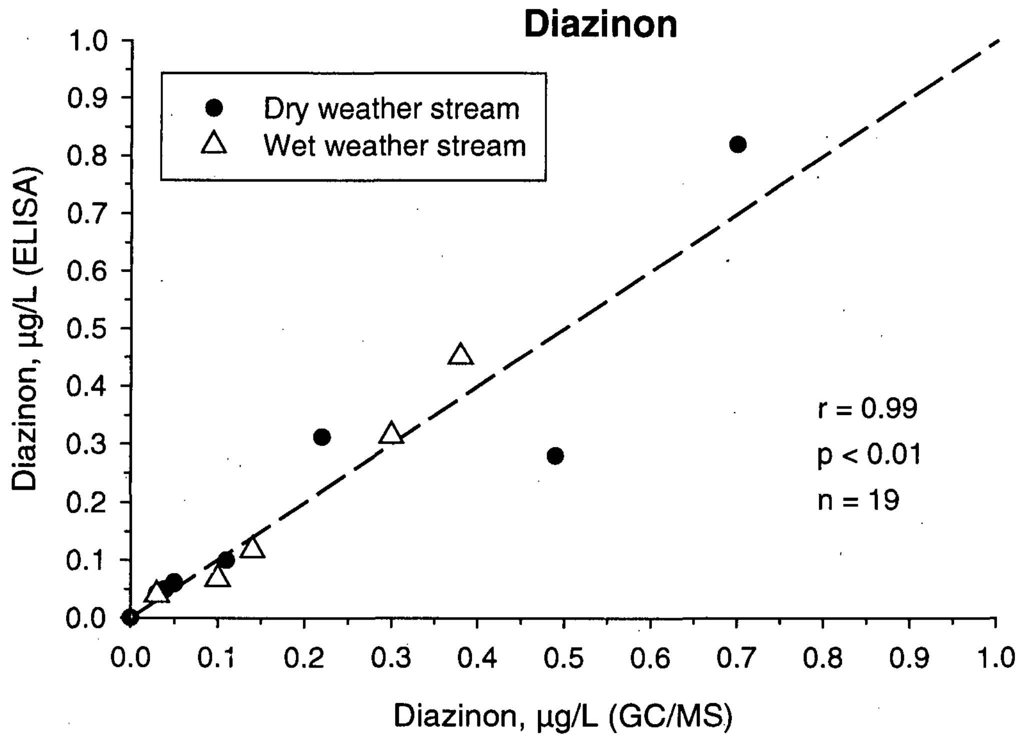


Figure 7. Relationships between the concentrations of unionized ammonia and *C. dubia* survival and reproduction for 100% samples from the Malibu Creek watershed. Data are from all Malibu Creek watershed samples collected between June 2002 and March 2003 that had associated ammonia measurements.



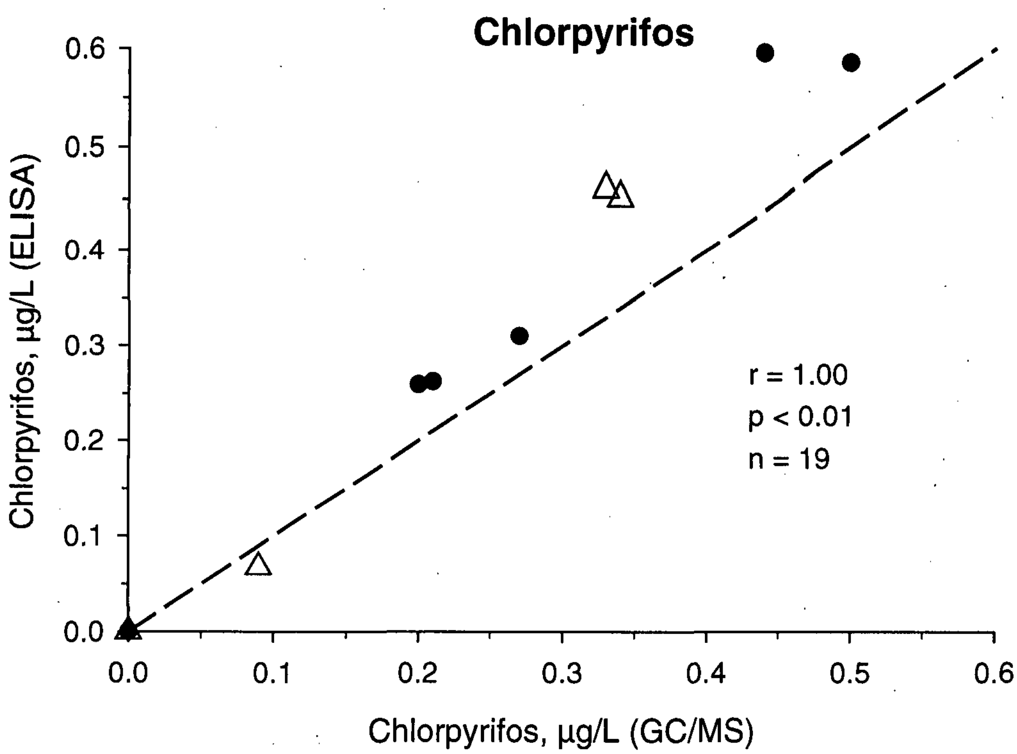


Figure 8. Comparison of ELISA measurements by SCCWRP and GC/MS measurements by APPL Labs for diazinon and chlorpyrifos. The samples included Malibu Creek watershed dry weather and stormwater samples, and spiked dry weather runoff samples from Cerritos Channel, Coyote Creek, and San Gabriel River. The dashed line indicates the theoretical one-to-one relationship between techniques.

Table 7. Performance objective results for all three rounds of ELISA analyses. The values in parentheses are the r^2 values for the standard curves.

	Proportion of analyses meeting the objective			
Analyte / Objective	Lab 1	Lab 2	Lab 3	Lab 4
Diazinon				
Standard curve goodness of fit $r^2 \geq 95\%$	3/3 (0.989, 0.979, 0.971)	3/3 (0.998, 0.995, 0.998)	3/3 (0.987, 0.985, 0.987)	2/3 (0.998, 1.000, 0.897)
Replicate optical densities $\leq 20\%$ CV	17/17	17/17	17/17	17/17
Chlorpyrifos				
Standard curve goodness of fit $r^2 \geq 95\%$	3/3 (0.999, 0.986, 0.995)	3/3 (0.994, 0.986, 0.988)	3/3 (1.000, 0.997, 0.974)	3/3 (1.000, 0.997, 0.984)
Replicate optical densities $\leq 20\%$ CV	16/17	17/17	17/17	17/17

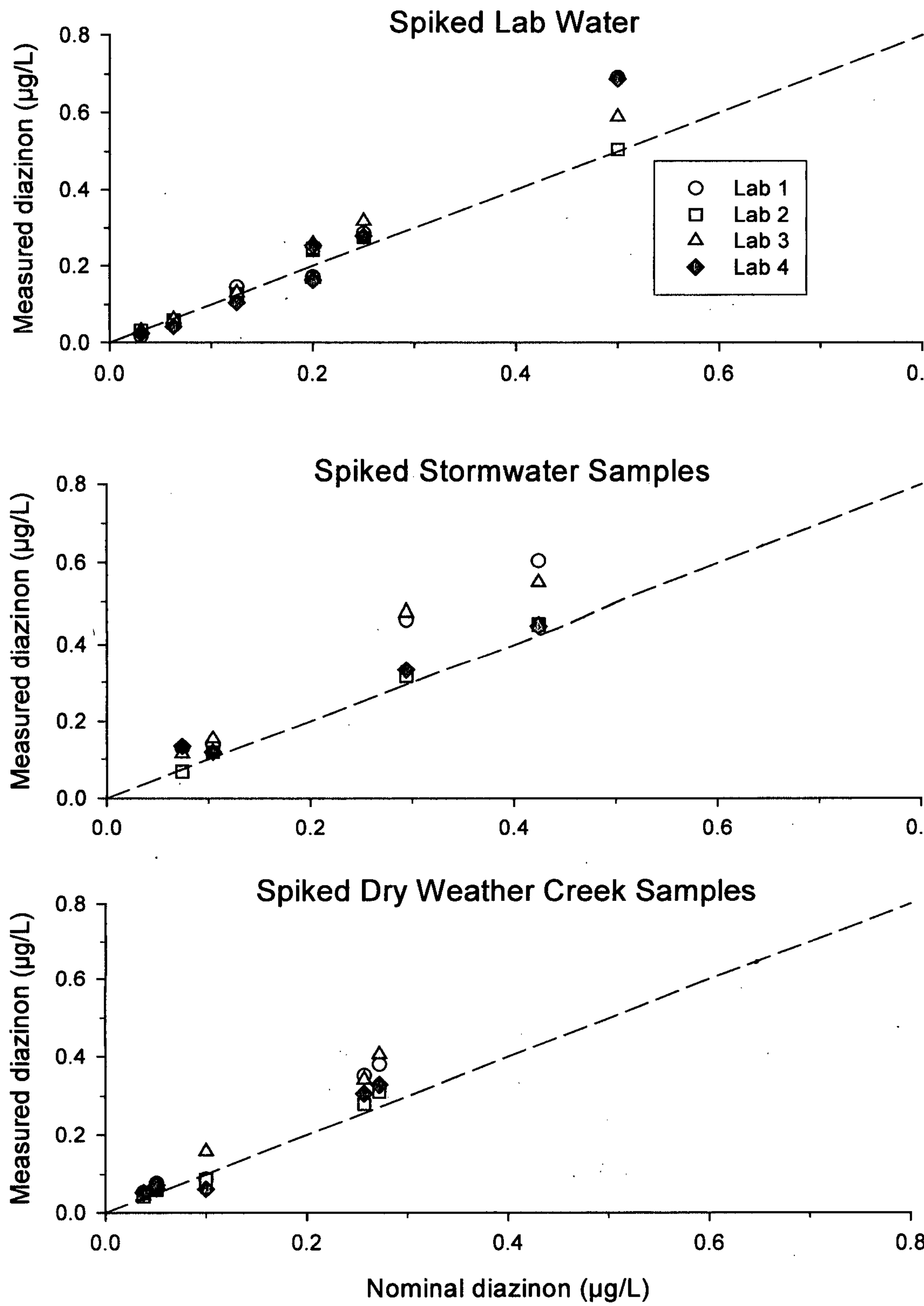


Figure 9. Measured vs nominal concentrations of diazinon associated with spiked lab water, storm water, and dry weather runoff during the ELISA interlab comparison. The dashed line represents the theoretical one-to-one relationship between measured and nominal concentrations.

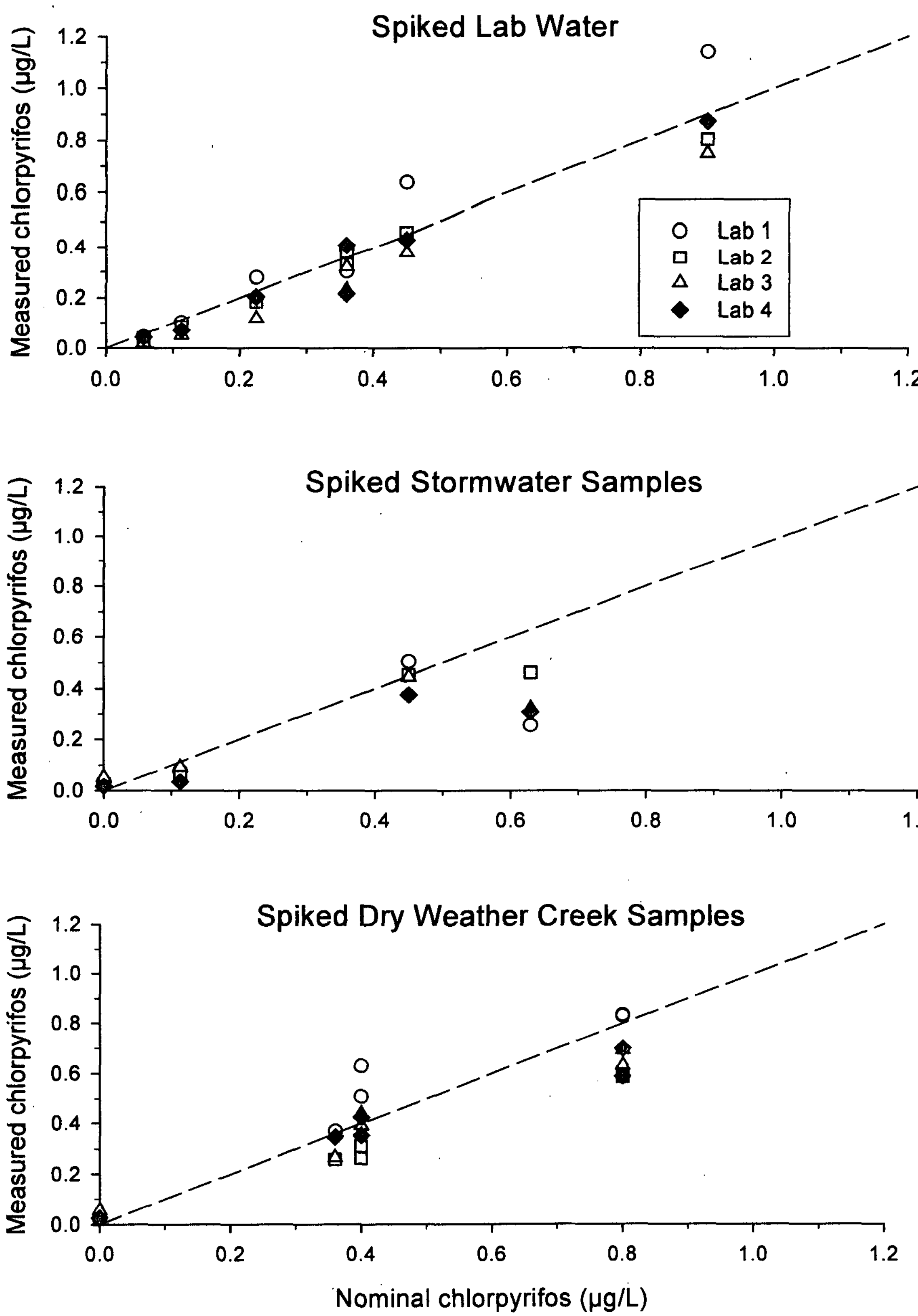


Figure 10. Measured vs nominal concentrations of chlorpyrifos associated with spiked lab water, storm water, and dry weather runoff during the ELISA interlab comparison. The dashed line represents the theoretical one-to-one relationship between measured and nominal concentrations.

Table 8. Summary of organophosphorus (OP) pesticides, *C. dubia* toxicity, and bioassessment results for the Malibu Creek watershed study sites.

Stream	OP pesticides above levels of concern[a]	Conductivity above toxic levels	Toxicity present (proportion of samples)		Biota impact[d]	OP pesticide related impact
			Survival	Reproduction		
Malibu Creek (dry weather)	None	occasionally	Yes (1/8)	Yes (2/8)	Yes[e]	Unlikely
Malibu Creek (wet weather)	Diazinon[b]	no	No (0/2)	No (0/2)		
Las Virgenes Creek	None	always	Yes (4/10)	Yes (10/10)	Yes[e]	Unlikely
Medea Creek	Diazinon[c]	always	Yes (2/10)	Yes (8/10)	Yes[e]	Possible

[a]For any creek sample in this study.
[b]Above California Department of Fish and Game Chronic Water Quality Criterion of 0.05 µg/L (Siepmann and Finlayson 2000).
[c]Above California Department of Fish and Game Acute (0.08 µg/L) and Chronic Water Quality Critera (0.05 µg/L) (Siepmann and Finlayson 2000).
[d]Data from 2000 only (SLSI 2000).
[e]Probably due to habitat modification.

APPENDICES

Appendix A. Sampling locations within the Malibu Creek watershed.

Stream	Heal the Bay station designation	Latitude	Longitude
Malibu Creek	HtB-01	34.04289103	-118.6842165
Las Virgenes Creek	HtB-05	34.09724331	-118.7208819
Medea Creek	HtB-07	34.13931295	-118.7593906

Appendix B. *Ceriodaphnia dubia* survival and reproduction in Malibu Creek watershed samples. The samples were analyzed as 100% creek sample, and 50% creek sample diluted with laboratory control freshwater. * = significantly different from control.

		Mean percent survival			Mean number of neonates		
Sample	**Collection date**	**Control**	**50%**	**100%**	**Control**	**50%**	**100%**
Malibu Creek	6/3/2002	90	90	100	19	22	21
Malibu Creek	7/22/2002	100	100	60*	36	32	21*
Malibu Creek	10/14/2002	100	100	90	28	27	17*
Malibu Creek	11/4/2002	80	90	80	19	26	21
Malibu Creek	12/2/2002	100	100	100	36	47	40
Malibu Creek	1/13/2003	100	100	100	31	33	33
Malibu Creek	2/3/2003	100	100	90	32	30	26
Malibu Creek (stormwater composite)	2/12 & 2/13/03	100	100	100	35	37	40
Malibu Creek (stormwater composite)	2/25/2003	100	100	100	38	38	43
Malibu Creek	3/3/2003	90	100	100	20	39	38
Las Virgenes Creek	6/3/2002	90	100	40*	19	17	3*
Las Virgenes Creek	7/22/2002	100	100	70*	36	31*	13*
Las Virgenes Creek	8/5/2002	100	90	50*	26	24	12*
Las Virgenes Creek	9/9/2002	100	100	50*	40	30*	12*
Las Virgenes Creek	10/14/2002	89	90	60*	23	21	10*
Las Virgenes Creek	11/4/2002	100	90	60*	28	15*	5*
Las Virgenes Creek	12/2/2002	100	100	80	40	38	21*
Las Virgenes Creek	1/13/2003	100	100	80	31	26*	16*

Sample	Collection date	Mean percent survival			Mean number of neonates		
		Control	50%	100%	Control	50%	100%
Las Virgenes Creek	2/3/2003	100	100	89	32	26	17*
Las Virgenes Creek	3/3/2003	90	100	90	20	30	9*
Medea Creek	6/3/2002	90	100	60	19	22	13*
Medea Creek	7/22/2002	90	80	0*	33	29	0*
Medea Creek	8/5/2002	100	100	100	35	35	28*
Medea Creek	9/9/2002	100	100	80	36	35	25*
Medea Creek	10/14/2002	100	90	100	24	24	18*
Medea Creek	11/4/2002	100	90	100	28	26	14*
Medea Creek	12/2/2002	100	0*	0*	37	6*	0*
Medea Creek	1/13/2003	100	100	90	31	32	24*
Medea Creek	2/3/2003	100	100	100	36	28*	13*
Medea Creek	3/3/2003	90	100	100	20	31	23

Appendix C. Water quality measurements from daily renewals in the *C. dubia* toxicity tests. Mean values are followed in parentheses by the minimum and maximum values. Alkalinity and hardness were only measured once for each experiment.

Sample	Collection Date	Alkalinity (mg/L CaCO3)	Hardness (mg/L CaCO3)	Conductivity (µmhos-cm)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Total Ammonia (mg/L)
Malibu Creek	06/03/02	273	830	2127 (2110-2140)	24.8 (24.2-25.6)	9.4 (8.1-10.8)	8.37 (8.03-8.59)	0.1
Malibu Creek	07/22/02	253	833	2122 (2080-2180)	25.0 (24.2-25.8)	9.3 (7.9-11.1)	8.42 (8.24-8.70)	0.4
Malibu Creek	10/14/02	251	979	2153 (2140-2170)	24.7 (24.1-25.5)	8.8 (7.4-10.4)	8.20 (7.80-8.59)	3.8
Malibu Creek	11/04/02	282	>1000	2487 (2250-3150)	25.1 (24.1-26.0)	8.7 (7.5-10.3)	8.15 (7.74-8.66)	<0.1
Malibu Creek	12/02/02	220	700	1938 (1831-2010)	24.6 (23.4-25.0)	8.7 (7.2-11.1)	8.42 (8.26-8.61)	0.6
Malibu Creek	01/13/03	213	629	1726 (1620-1819)	24.4 (24.0-25.4)	8.9 (7.5-10.8)	8.44 (8.19-8.62)	0.1
Malibu Creek	02/03/03	200	566	1590 (1546-1700)	24.4 (23.9-25.0)	8.6 (7.4-13.5)	8.41 (7.86-8.59)	<0.1
Malibu Creek (stormwater)	02/12 & 2/13/03	125	269	696 (688-700)	24.7 (24.2-25.5)	8.6 (7.4-9.6)	8.04 (7.85-8.28)	1.1
Malibu Creek (stormwater)	02/25/03	190	575	1308 (1303-1313)	24.4 (23.9-25.5)	8.3 (6.4-9.9)	8.21 (8.02-8.40)	Not analyzed
Malibu Creek	03/03/03	199	503	1399 (1338-1470)	24.5 (23.7-25.8)	8.6 (6.3-11.3)	8.26 (8.18-8.32)	<0.1
Las Virgenes Creek	06/03/02	399	>1000	3427 (3400-3470)	24.9 (24.1-25.6)	9.4 (8.0-10.8)	8.32 (8.05-8.66)	0.6
Las Virgenes Creek	07/22/02	406	>1000	3435 (3410-3460)	24.8 (24.4-25.4)	9.1 (7.9-10.6)	8.18 (8.09-8.36)	<0.1
Las Virgenes Creek	08/05/02	374	>1000	3150 (3090-3420)	24.8 (24.0-25.7)	9.0 (7.2-10.5)	8.22 (8.06-8.40)	<0.1
Las Virgenes Creek	09/09/02	586	>1000	3314 (2540-3510)	24.4 (23.8-25.5)	8.9 (7.8-10.6)	8.25 (8.07-8.39)	0.5
Las Virgenes Creek	10/14/02	389	>1000	3187 (3160-3220)	24.7 (24.1-25.9)	8.5 (7.4-9.8)	8.15 (7.95-8.34)	3.9
Las Virgenes Creek	11/04/02	443	>1000	3397 (3160-3530)	25.1 (24.0-25.9)	8.7 (7.7-9.8)	8.11 (7.91-8.32)	<0.1
Las Virgenes Creek	12/02/02	378	>1000	3011 (2820-3100)	24.5 (23.4-25.5)	8.8 (7.6-11.3)	8.22 (8.03-8.39)	0.4
Las Virgenes Creek	01/13/03	410	>1000	3298 (3060-3420)	24.5 (24.0-25.4)	8.8 (7.5-10.2)	8.26 (8.07-8.38)	<0.1
Las Virgenes Creek	02/03/03	388	>1000	3101 (3020-3280)	24.4 (23.9-25.4)	8.6 (7.4-10.0)	8.25 (8.13-8.39)	<0.1
Las Virgenes Creek	03/03/03	397	1560	3093 (2950-3290)	24.6 (23.7-25.9)	8.8 (7.2-11.1)	8.16 (8.08-8.32)	0.1

Appendix C. continued

Sample	Collection Date	Alkalinity (mg/L CaCO3)	Hardness (mg/L CaCO3)	Conductivity (μmhos-cm)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Total Ammonia (mg/L)
Medea Creek	06/03/02	290	>1000	2820 (2790-2800)	25.0 (24.1-26.0)	9.2 (7.9-10.1)	8.07 (7.92-8.69)	0.1
Medea Creek	07/22/02	329	>1000	2915 (2880-2930)	25.0 (24.6-25.4)	8.2 (7.7-9.0)	8.29 (8.03-8.63)	0.1
Medea Creek	08/05/02	290	>1000	2716 (2670-2960)	24.7 (24.0-25.2)	8.6 (7.2-10.9)	8.22 (8.06-8.40)	0.6
Medea Creek	09/09/02	316	>1000	2946 (2740-3000)	24.3 (23.8-24.9)	8.4 (7.7-9.4)	8.40 (8.03-8.64)	0.4
Medea Creek	10/14/02	331	>1000	2696 (2680-2710)	24.7 (24.1-25.8)	8.4 (7.5-9.4)	8.23 (7.92-8.49)	4.6
Medea Creek	11/04/02	359	>1000	2930 (2470-3100)	25.2 (24.1-25.9)	8.5 (7.7-9.8)	8.09 (7.81-8.36)	<0.1
Medea Creek	12/02/02	295	970	2343 (2240-2450)	24.9 (24.1-25.6)	8.8 (7.5-11.2)	8.17 (8.01-8.48)	0.6
Medea Creek	01/13/03	326	>1000	2630 (2470-2770)	24.6 (24.0-25.4)	8.7 (7.5-10.2)	8.28 (8.02-8.52)	0.1
Medea Creek	02/03/03	363	>1000	2666 (2600-2840)	24.4 (23.9-24.9)	8.5 (7.2-9.4)	8.27 (8.10-8.43)	0.1
Medea Creek	03/03/03	358	1172	2491 (2380-2630)	24.5 (23.7-25.8)	8.6 (7.3-10.9)	8.16 (8.06-8.31)	<0.1

Appendix D. Diazinon measurements (µg/L) for all three analysis rounds (events) of the interlab ELISA comparison.

Sample	Sample Type	Nominal	Lab 1	Lab 2	Lab 3	Lab 4
Round 1 – 1	Spiked lab water	0.500	0.691	0.505	0.589	0.687
Round 1 – 2	Spiked lab water	0.250	0.286	0.274	0.318	0.278
Round 1 – 3	Spiked lab water	0.125	0.146	0.128	0.131	0.105
Round 1 – 4	Spiked lab water	0.063	0.047	0.059	0.062	0.042
Round 1 – 5	Spiked lab water	0.031	0.017	0.032	0.032	0.024
Round 2 – 1	Wet weather creek	0.074	0.129	0.068	0.114	0.134
Round 2 – 2	Spiked wet weather creek	0.294	0.462	0.315	0.477	0.332
Round 2 – 3	Spiked wet weather creek	0.104	0.138	0.118	0.154	0.118
Round 2 – 4	Spiked wet weather creek	0.424	0.605	0.450	0.550	0.447
Round 2 – 5	Dry weather creek	0.100	0.088	0.087	0.158	0.062
Round 2 – 6	Spiked lab water	0.200	0.172	0.239	0.258	0.163
Round 3 – 1	Spiked dry weather creek	0.257	0.352	0.280	0.342	0.307
Round 3 – 2	Dry weather creek	0.051	0.073	0.062	0.072	0.063
Round 3 – 3	Spiked dry weather creek	0.272	0.380	0.312	0.406	0.329
Round 3 – 4	Dry weather creek	0.051	0.077	0.059	0.069	0.072
Round 3 – 5	Dry weather creek	0.038	0.054	0.042	0.045	0.053
Round 3 – 6	Spiked lab water	0.200	0.168	0.246	0.166	0.253

Appendix D. continued. Chlorpyrifos measurements (µg/L) for all three analysis rounds of the interlab ELISA comparison.

Sample	Sample Type	Nominal	Lab 1	Lab 2	Lab 3	Lab 4
Round 1 – 1	Spiked lab water	0.900	1.141	0.804	0.750	0.872
Round 1 – 2	Spiked lab water	0.450	0.638	0.458	0.380	0.432
Round 1 – 3	Spiked lab water	0.225	0.279	0.185	0.119	0.204
Round 1 – 4	Spiked lab water	0.113	0.104	0.086	0.052	0.071
Round 1 – 5	Spiked lab water	0.056	0.046	0.040	0.020	0.043
Round 2 – 1	Wet weather creek	<0.050	0.029	0.027	0.054	0.017
Round 2 – 2	Spiked wet weather creek	0.450	0.504	0.452	0.446	0.376
Round 2 – 3	Spiked wet weather creek	0.113	0.077	0.068	0.093	0.035
Round 2 – 4	Spiked wet weather creek	0.630	0.255	0.461	0.322	0.308
Round 2 – 5	Dry weather creek	<0.050	0.024	0.021	0.056	0.026
Round 2 – 6	Spiked lab water	0.360	0.302	0.382	0.229	0.216
Round 3 – 1	Spiked dry weather creek	0.360	0.370	0.259	0.267	0.346
Round 3 – 2	Spiked dry weather creek	0.800	0.830	0.596	0.631	0.699
Round 3 – 3	Spiked dry weather creek	0.400	0.630	0.263	0.391	0.352
Round 3 – 4	Spiked dry weather creek	0.400	0.509	0.310	0.441	0.425
Round 3 – 5	Spiked dry weather creek	0.800	0.835	0.586	0.692	0.588
Round 3 – 6	Spiked lab water	0.360	0.392	0.333	0.321	0.410