Organophosphorus pesticides in the Malibu Creek Watershed

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ABSTRACT - To assess the persistence and magnitude of pesticides in the water column, three streams in the Malibu Creek watershed were sampled for organophosphorus (OP) pesticide (including diazinon and chlorpyrifos) contamination 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, with measurable amounts in most of the dry-weather samples from Medea Creek, and both of the stormwater samples from Malibu Creek. Concentrations of diazinon in some samples exceeded the California Department of Fish and Game acute water quality criterion by up to a factor of 9, and the chronic criterion by up to a factor of 14. Toxicity was present in some of the samples from each of the streams. 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 contributed to the reduced water quality, but these two constituents had different effects on water quality at the various sites. Diazinon had the most severe toxic effects (complete mortality in two samples from Medea Creek), but did not impact the observed toxicity at Malibu Creek or Las Virgenes Creek. 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.

INTRODUCTION

Diazinon and chlorpyrifos were two of the three most commonly used pesticides for structural pest control in California during 2000 (CDPR 2000). These pesticides often are sprayed on asphalt and lawns; when these surfaces become wet or saturated with water, runoff carries the contaminant to the storm drain system.

Organophosphorus (OP) pesticides (including diazinon and chlorpyrifos) are a major cause of impaired water quality in California. In northern California, diazinon and chlorpyrifos have been found to cause significant toxicity in runoff from largely urban watersheds (Bailey *et al.* 2000). In Orange County, diazinon has been detected in runoff at levels as high as 15.5 µg/L (CDPR 2001), exceeding the California Department of Fish and Game acute toxicity threshold level for this pesticide (0.08 µg/L, Siepmann and Finlayson 2000). Moreover, the contamination appears to be widespread in surface waters throughout southern California. OP pesticides have been found in agricultural runoff in Imperial County (De Vlaming *et al.* 2000), in stormwater runoff in Los Angeles County (LACDPW 2002, Schiff and Sutula 2003), and in surface runoff throughout Orange County (CDPR 2001).

OP pesticide contamination in the Malibu Creek watershed has become a concern in recent years, as reflected by the current plans to develop total maximum daily loads (TMDLs) for this watershed. Diazinon has been found sporadically in stormwater from Malibu Creek (LACDPW 1999, 2000), but the persistence and magnitude of OP pesticides in dry-weather flow is unknown. In addition, the prevalence of these pesticides in the tributaries of the Malibu Creek watershed has not been characterized, and no toxicity data exist. Consequently, the extent of OP pesticide contamination and potential adverse effects on water quality are not known.

This study had two objectives: The first was to assess the contamination of OP pesticides in streams located in the Malibu Creek watershed. The second objective was to measure the potential impacts of OP pesticide contamination to organisms living in these streams using toxicity tests.

METHODS

Study Design

Three streams within the Malibu Creek watershed were sampled: Malibu Creek and two of its tributaries, Las Virgenes Creek and Medea Creek (Figure 1). Malibu Creek receives drainage from 105 square miles of urban and undeveloped areas, including 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.

One sampling site was located on each of the three creeks. Las Virgenes Creek and Medea Creek are tributaries to Malibu Creek, and therefore the Malibu Creek site reflects contributions from the entire watershed. The Malibu Creek site was approximately 5 km downstream of the Los Angeles County Department of Public Works (LACDPW) monitoring site.

Dry-weather samples were collected at monthly intervals between June 2002 and March 2003. Ten dry-weather samples were collected from Las Virgenes Creek and Medea Creek. Eight dry-weather samples were collected from Malibu Creek, which was dry during August 2002 and September 2002.

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.

Laboratory Analysis

The samples were analyzed for 30 organophosphorus (OP) pesticides, including diazinon and chlorpyrifos (Table 1). All dry-weather samples and the second stormwater composite sample were analyzed by gas chromatography/mass spectrometry (GC/MS). The pesticides were extracted within 7 d of collection by EPA Method 3510C, and analyzed by GC/MS following EPA Method 8141A (U.S. EPA 1996). All holding times were met.

All dry-weather and wet-weather creek samples were tested for toxicity at 100% and 50% runoff concentrations using the 7-d *Ceriodaphnia dubia* survival and reproduction test (U.S. EPA 1994). All toxicity tests were started within 2 d of sample collection. Ten replicates were included in each test. The test endpoints were percent of survival and the number of offspring. 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. Water quality measurements during the test met the test recommended ranges. The organisms were fed each day.

A separate laboratory experiment was performed to determine whether elevated creek conductivity was

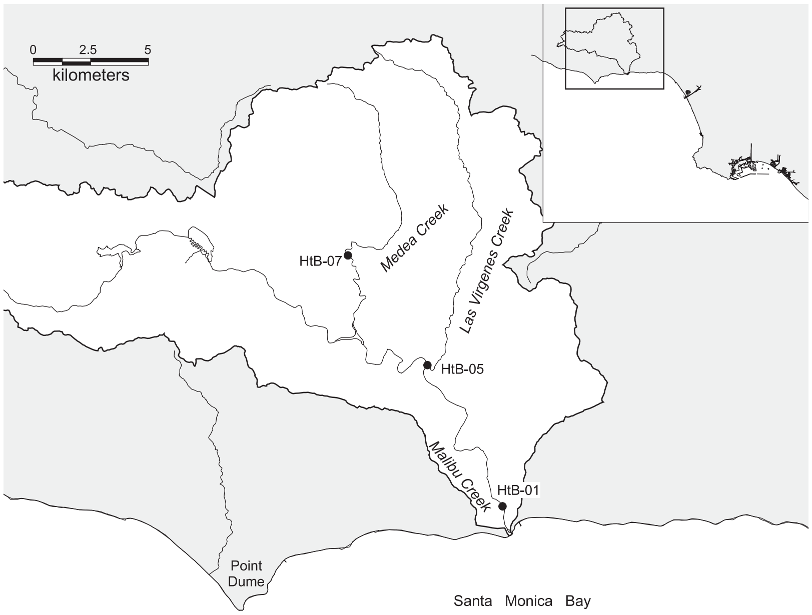

Figure 1. Stream sampling locations within the Malibu Creek watershed.

Table 1. 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

affecting *C. dubia* survival and reproduction. Test organisms were exposed to dilution water conductivity levels of 1,900 and 2,800 µmhos-cm. The conductivity levels were adjusted by adding reagent grade salts to dilution water (U.S. EPA 1994). This experiment used the same testing procedures (7-d exposure, 10 replicates, daily feeding and water change) and endpoints (survival/reproduction) as the tests with the creek samples.

Data Analysis

Significant differences in the number of samples with detectable amounts of pesticide within each creek were assessed with analysis of variance. Samples with detectable pesticides were coded with a “1”, while samples with no detectable pesticides were coded with a “0” for the analysis of variance (ANOVA). Relationships among indicators (e.g., *C. dubia* survival vs. diazinon concentration) were assessed with Spearman rank correlation (SigmaStat statistical software version 2.03).

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. 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).

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). 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). 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.

Concentrations of diazinon in some samples from both Malibu and Medea creeks exceeded California Department of Fish and Game water quality criteria. The first stormwater composite from Malibu Creek (0.06 µg/L) was roughly equivalent to

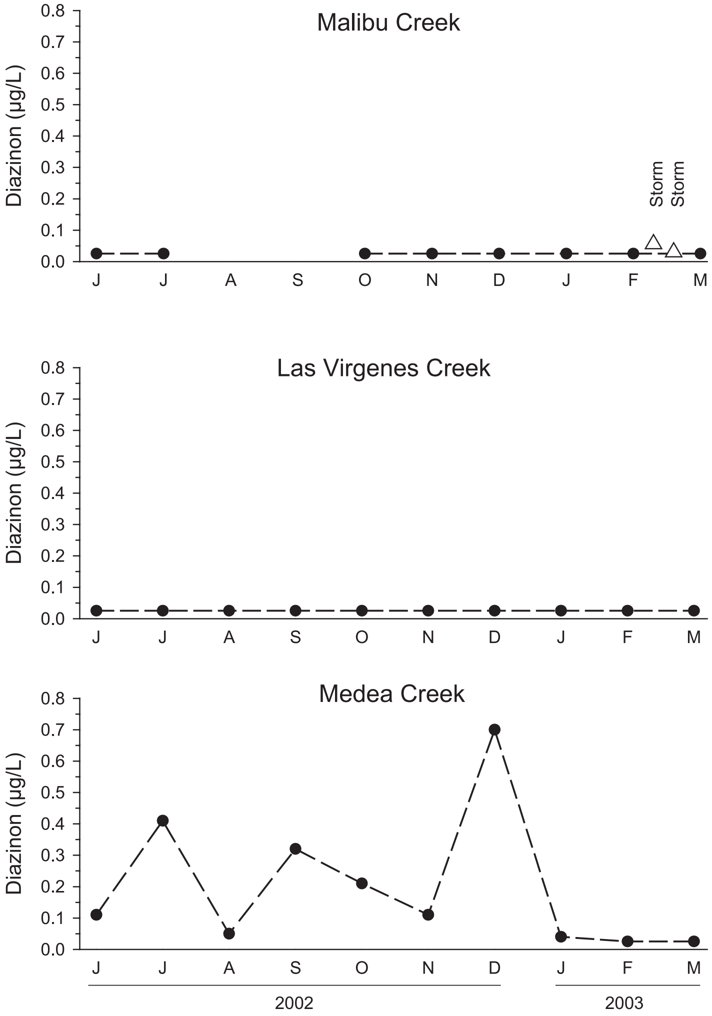

Figure 2. Concentrations of diazinon in stream samples collected from the Malibu Creek watershed between June 2002 and March 2003. All samples were collected during dry-weather flow, except for the two stormwater samples collected from Malibu Creek in February. Nondetectable values (<0.05 µg/L) were treated as equal to half the practical quantitation level. Due to sample availability, the first stormwater composite was analyzed by enzyme-linked immunosorbent assay (ELISA).

the chronic criterion (0.05 µg/L; Siepmann and Finlayson 2000). The concentration of diazinon in the second stormwater composite (0.03 µg/L) was below both the chronic and acute (0.08 µg/L; Siepmann and Finlayson 2000) criteria. Six of the ten dry-weather samples from Medea Creek exceeded both water quality criteria for diazinon. Exceedances in Medea Creek samples ranged from 1.4 to 8.8 times the acute criterion, and 2.2 to 14 times the chronic criterion.

Toxicity Testing

Dry-weather samples collected from Malibu Creek had the highest mean survival rate (95%, control adjusted), followed by Medea Creek (74%) and Las Virgenes Creek (69%) (Table 2). The mean survival rate was significantly different among the group of three streams, but the sample size was not large enough to distinguish differences between specific locations. Most water quality measurements were consistent among the creeks (Brown and Bay 2003). However, the conductivity levels of the samples from Medea Creek (2,491 to 2,946 µmhos-cm) and Las Virgenes Creek (3,011 to 3,435 µmhos-cm) tended to be higher than the dry-weather samples from Malibu Creek (1,399 to 2,487 µmhos-cm). The wet-weather samples from Malibu Creek were not toxic to *C. dubia* survival (Table 2).

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 toxicity in the Medea Creek sample collected in December persisted after dilution. The frequency of toxic samples was not significantly different among the stations ($p = 0.69$).

There was a temporal trend in survival rates for the Las Virgenes Creek samples, with the lowest survival rate occurring in June, August, and September 2002, and the highest survival rate occurring in February and March 2003 (Figure 3). All samples from Las Virgenes Creek were dry-weather samples. 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

Table 2. 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 detected in any stream sample. Nondetectable values (<0.05 µg/L) were treated as equal to half the practical quantitation level, and are indicated by a "u" suffix.

Stream	No. of Samples	Mean Diazinon Concentration (µg/L)	Mean Survival (percent control)	Percent of Samples Toxic to *C. dubia* Survival	Mean Reproduction (percent control)	Percent of 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

samples, 104% of the control value for dry-weather samples). This rate 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 of diazinon related to *C. dubia* survival. 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 rates. 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 rates may have been related to dissolved salts in the creeks, since many of the samples contained relatively high conductivities. There was a significant negative correlation between survival rate 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 was 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 1,900 µmhos-cm. A conductivity of approximately 2,800 µmhos-cm was always associated with reduced reproduction.

Both of the conductivities in the laboratory experiment with manipulated conductivity levels significantly impaired reproduction. Reproduction for the 1,900 and 2,800 µmhos-cm samples were 32% and 6% of the control, respectively. Conductivity in the control was measured at 200 µmhos/cm. The magnitude of reproductive impairment in the manipulated samples was similar to that observed in creek samples of similar conductivity. Survival rate, how-

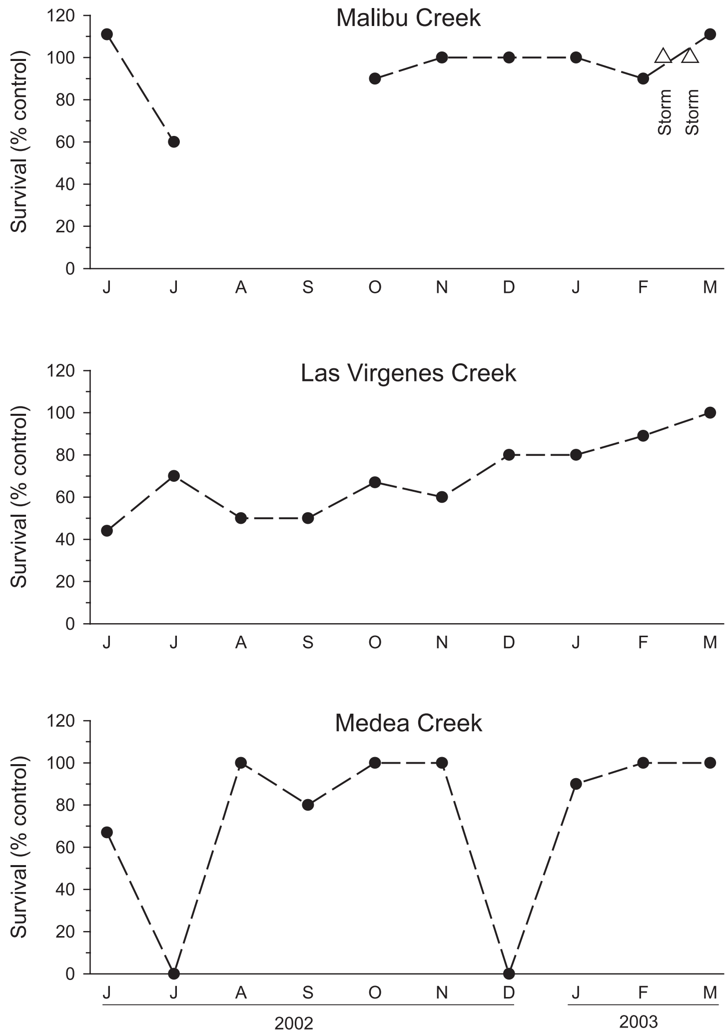

Figure 3. Ceriodaphnia dubia survival in 100% stream samples collected from the Malibu Creek watershed between June 2002 and March 2003. All samples were collected during dry-weather flow, except for the two stormwater samples collected from Malibu Creek in February.

ever, did not appear to be affected by the conductivity levels in the laboratory manipulation experiment (1,900 µmhos/cm = 67% of control survival, 2,800 µmhos/cm = 100% of control).

Concentrations of ammonia in the creek samples were not correlated with reduced survival (r = -0.02, p = 0.92) or reproduction (r = 0.07, p = 0.70). 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 (2,680 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.

DISCUSSION

The toxicity and chemical results from this study showed that there were differences in water quality in 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 3). Impacted water quality, as indicated by toxicity to *C. dubia*, was 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 were 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. Other studies have identified conductivity as an indicator of urban runoff (e.g., Walsh *et al.* 2001). However, in the Malibu Creek watershed, the dissolved salts are presumably from natural sources (Melinda Becker, LARWQCB, personal communication). Diazinon was 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 at Malibu Creek or Las Virgenes Creek.

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 (e.g., pyrethroids) or the interaction of dissolved salts with other environmental factors. Additional research, including comprehensive chemical analyses and toxicity identification evaluation studies, are needed to identify other likely causes of toxicity in this watershed.

One of the stormwater events sampled at Malibu Creek in this study (February 11-13, 2003) was also sampled by the LACDPW. Diazinon was found during this storm event in both studies, but the meas-

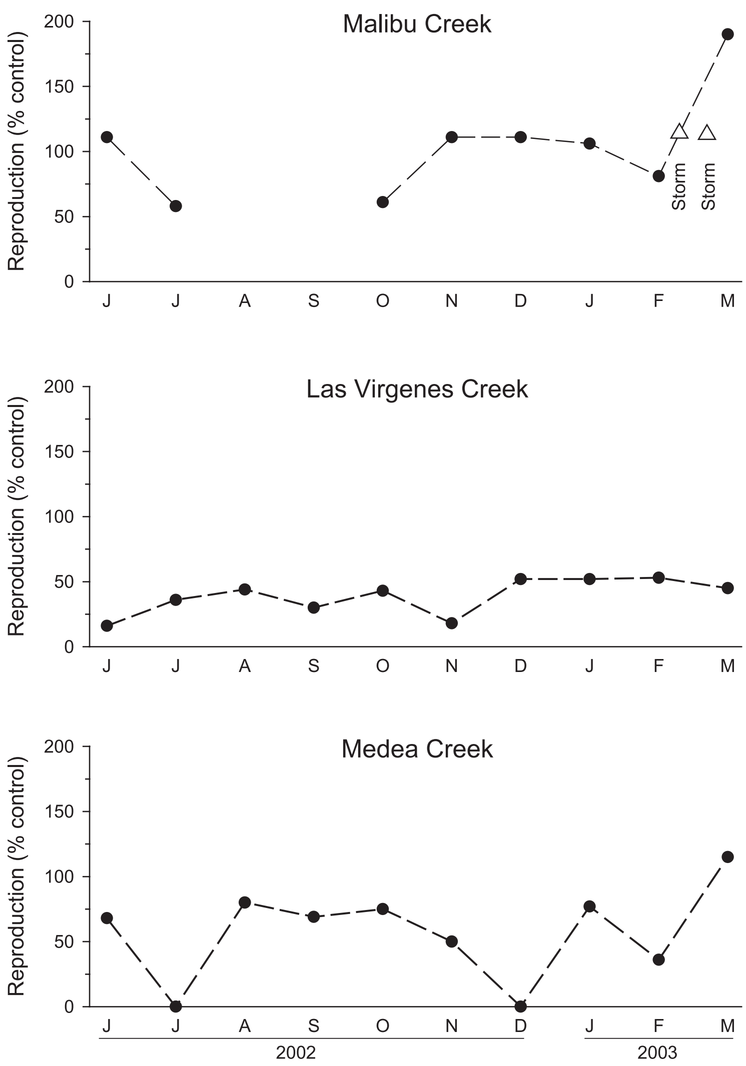

Figure 4. Ceriodaphnia dubia reproduction in 100% stream samples collected from the Malibu Creek watershed between June 2002 and March 2003. All samples were collected during dry-weather flow, except for the two stormwater samples collected from Malibu Creek in February.

ured values differed by a factor of 10. The concentration of diazinon in the present SCCWRP study was 0.06 µg/L, compared to 0.57 µg/L in the LACDPW sample (LACDPW 2003). This difference demonstrates how variable diazinon concentrations can be over time, since the LACDPW sample was collected one day earlier than the SCCWRP sample.

The California Department of Fish and Game's acute criterion appeared to be protective in this study. Concentrations of diazinon below the acute criterion, and up to four times this value, did not adversely affect *C. dubia* survival. Concentrations above the chronic criterion tended to affect reproduction, but the protectiveness of the chronic criterion in this study is unclear because of the elevated conductivity levels in the creek samples.

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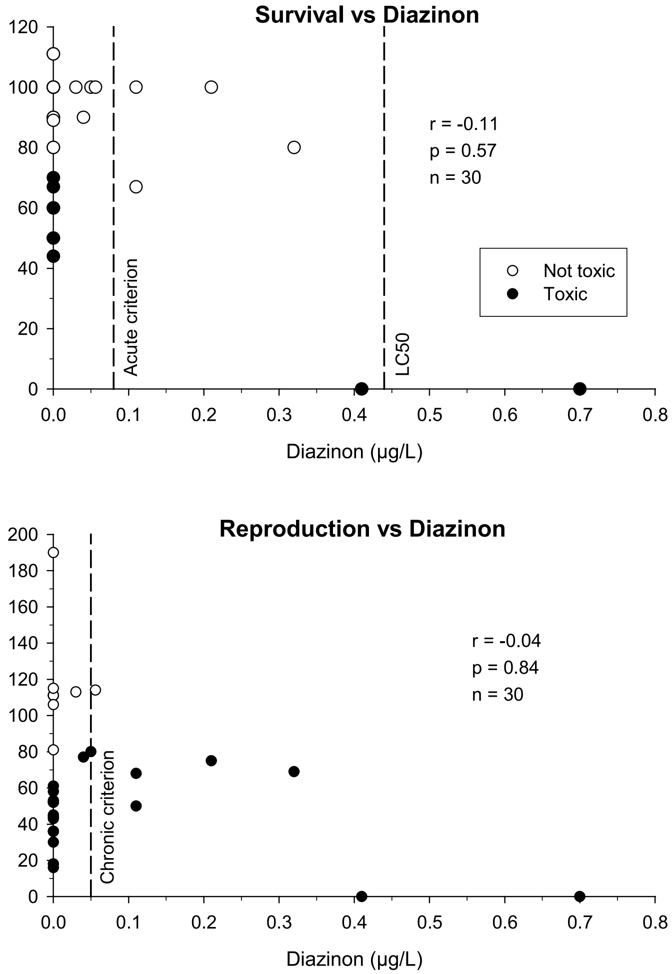

Figure 5. Relationship between diazinon and C. dubia survival and reproduction in 100% stream samples. Data are from all Malibu Creek watershed samples collected between June 2002 and March 2003. The data were adjusted to the percent of control in order to account for differences in organism response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

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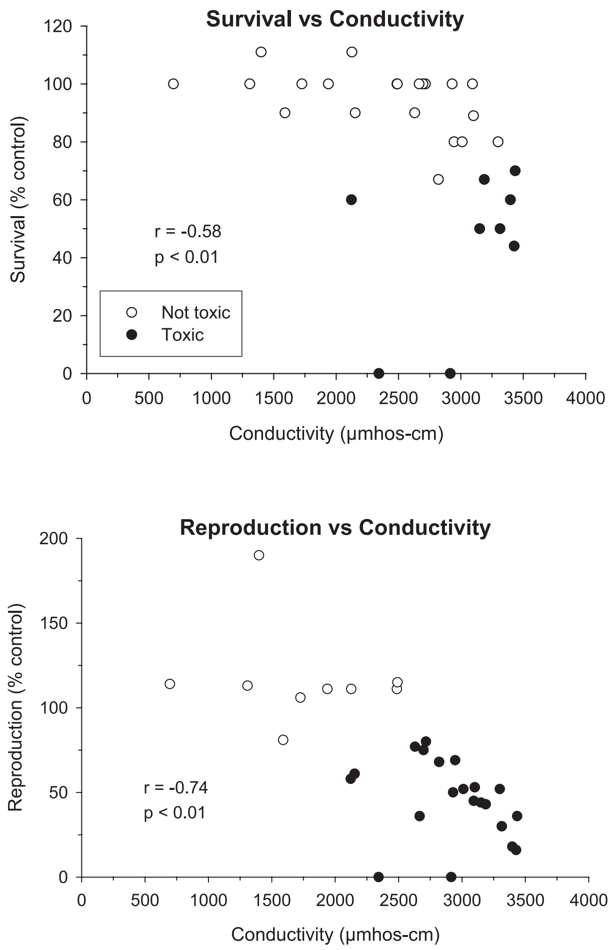

Figure 6. Relationships between conductivity and C. dubia survival and reproduction for 100% samples from the Malibu Creek watershed. Data are from all Malibu Creek watershed samples collected between June 2002 and March 2003.

Table 3. Summary of organophosphorus (OP) pesticides, *C. dubia* toxicity, and bioassessment results for the Malibu Creek watershed study sites.

			Toxicity Present (proportion of samples)		
Stream	OP Pesticides Above Levels of Concern[a]	Conductivity Above Toxic Levels	Survival	Reproduction	OP Pesticide-related Impact
Malibu Creek (dry weather)	None	Occasionally	Yes (1/8)	Yes (2/8)	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)	Unlikely
Medea Creek	Diazinon[b,c]	Always	Yes (2/10)	Yes (8/10)	Probable[d]

[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 Water Quality Criterion of 0.08 µg/L (Siepmann and Finlayson 2000).
[d] Probable impact to survival.