California Environmental Protection Agency
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Environmental Monitoring and Pest Management
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STUDY 179: PROTOCOL FOR MONITORING ACUTE AND CHRONIC TOXICITY IN THE SAN JOAQUIN RIVER WATERSHED, WINTER 1998-99

November 23, 1998

I. INTRODUCTION

In the San Joaquin Valley, the organophosphorus insecticides diazinon, chlorpyrifos, or methidathion are generally applied with a dormant oil on nut and stone fruit trees to control peach twig borer, San Jose scale, European red mite, and brown mite pests. The best time to achieve control of these pests is December through February, when trees are dormant and better pesticide coverage is possible (Zalom *et al.*, 1995). This dormant orchard spray application period, however, coincides with seasonal rainfall. Thus, these pesticides have the potential to wash off target areas and migrate with runoff waters to the San Joaquin River. This study is designed to monitor toxicity of pesticides in the San Joaquin River watershed during winter months when dormant insecticidal sprays are being applied to orchards.

From 1988 to 1990, the Central Valley Regional Water Quality Control Board conducted an aquatic toxicity survey in the San Joaquin Valley. Surface water samples collected from certain reaches of the San Joaquin River watershed during this survey were acutely toxic to the water flea, *Ceriodaphnia dubia* (Foe and Connor, 1991). The cause of toxicity was not determined but was attributed to pesticides in general. Further study was conducted in the Valley during the winter of 1991-92, and the resultant toxicity was attributed to the presence of chlorpyrifos and diazinon (Foe and Sheipline, 1993; Foe, 1995; Kuivila and Foe, 1995). The toxicity found in these studies was in violation of the Central Valley Regional Water Quality Control Board's narrative water quality objective (Foe, 1995). The toxicity objective states that, "All waters shall be maintained free of toxic substances in concentrations that produce detrimental physiological responses in human, plant, animal, or aquatic life" (CVRWQCB, 1994).

The Department of Pesticide Regulation (DPR) monitored the San Joaquin River watershed during the winters of 1991-92 and 1992-93 and reported the detection of chlorpyrifos, diazinon, and methidathion in 10, 72, and 18 percent of the 108 water samples collected, respectively (Ross, 1997). Of these positive samples, 2, 13, and 1 percent exceeded the LC_{50} for *C. dubia*,

respectively, indicating potential acute toxicity. In addition, diazinon concentrations in the San Joaquin River at Vernalis ranged from 0.148 to 1.07 µg/L, on 12 consecutive days in 1993, and the authors concluded that chronic toxicity due to diazinon might be problematic at this site (Kuivila and Foe, 1995). During the 12 days, diazinon exceeded the 96 hour LC_{50} for *C. Dubia* four times (Table 1). Diazinon was also detected at levels acutely toxic to *C. dubia* in Orestimba Creek, a tributary to the San Joaquin River, during the 1992-93 dormant spray period (Domagalski, 1995). Consequently, programs to reduce the mass of dormant orchard spray insecticides leaving target areas have been under investigation by DPR and growers (Ross, 1997; Ando, 1996; Anonymous, 1996; Biermann, 1996).

During the winter of 1996-97, DPR sampled at two sites along the San Joaquin and Sacramento Rivers to monitor dormant spray insecticides in those watersheds. This was the first year of a multi-year monitoring study. The San Joaquin River at Vernalis and Orestimba Creek were chosen as sites in the San Joaquin River watershed for conducting toxicity testing and pesticide monitoring (Bennett et.al., 1998). The first half of winter was unusually wet with flooding followed by unseasonably dry weather during the second half of winter. Water samples from Orestimba Creek contained residues of diazinon, carbofuran, and dimethoate in 20, 13, and 7 percent of the 15 samples collected, respectively. The maximum diazinon, carbofuran, and dimethoate concentrations detected were 0.092, 0.238, and 0.082 ug/L, respectively. Twelve percent of the 24 water samples from the San Joaquin River near Vernalis contained diazinon residues, with a maximum concentration of 0.070 ug/L. The concentrations detected did not exceed LC_{50}s listed for these insecticides (Table 1). *Ceriodaphnia dubia* survival ranged from 40 to 100 percent for acute toxicity tests from Orestimba Creek. Only one of these samples collected on January 29, 1997 was significantly different from the control. However, there were no pesticides detected in the sample splits for this sample. No Chronic toxicity was detected in the 8 weekly sets of water samples collected from the San Joaquin River near Vernalis.

The same two locations were again sampled during the winter of 1997-98 for organophosphate and carbamate insecticides (Ganapathy, 1998). As part of an ongoing effort to gain information about pesticide residues in state surface waters, (as recommended in the memo "Category and recommendation of currently registered pesticides for surface water monitoring during FY 97-98" (Goh, 1997)), additional samples were collected and analyzed for triazine, bromacil and diuron herbicides. The SJR and Orestimba Creek discharge and rainfall was higher than normal in 1997-98, much like the year before.

Of the eight organophosphates analyzed, chlorpyrifos, diazinon and methyl parathion were detected in Orestimba Creek. Chlorpyrifos and methyl parathion were each detected once at

0.093 and 0.19 ppb in the same sample. Diazinon was detected in three of 18 samples (17%) with concentrations ranging from 0.059 to 0.14 ppb. Four of the nine herbicides analyzed for were also detected. Bromacil was detected in three of the 18 samples (17%) with concentrations ranging from 0.066 to 0.012 ppb. Cyanazine was detected in one (6%) sample at 0.25 ppb. Diuron was detected in six samples (33%) ranging from 0.078 to 0.39 ppb and simazine was detected in five (28%) ranging from 0.063 to 0.71 ppb. Three of the samples contained three different herbicides. For acute toxicity tests, three of 18 samples had significantly reduced survival compared to the control. Diazinon, diuron and simazine were detected in one of the three samples.

Diazinon and methidathion were the two organophosphates detected in the SJR at Vernalis. Diazinon was detected in 10 of 30 samples (33%) with the detections in two groupings. Four of the five samples collected January 7, 1998 through January16 had detectible diazinon at concentrations ranging from 0.063 to 0.102 ppb. The second group of detections began on January 30 and continued through February 11 with concentrations ranging from 0.042 to 0.093 ppb. Methidathion was detected in three samples (10%) ranging from 0.053 to 0.11 ppb and coincided with diazinon detections. The herbicides bromacil, diuron, cyanazine and simazine were also detected in the SJR. Bromacil and cyanazine were detected in three (10%) and two of 30 samples (7%), respectively. Diuron was detected in all samples at concentrations ranging from 0.056 to 3.0 ppb. Simazine was detected in 16 samples (53%) with levels ranging from 0.050 to 0.47 ppb. There was only one chronic toxicity test with significant mortality. There was 0% *C. dubia* survival in the test sample collected during the week of February 2 while the corresponding control had 100% survival. The pesticide analysis sample collected on February 2 had detectible levels of diazinon, methidathion, diuron and simazine. All other chronic toxicity tests had survival ranging from 80% to 100% with corresponding control survival from 80% to 100%.

In this third year of monitoring we will continue to look at acute toxicity to *C. dubia* in a small watershed where the discharging waters do not contain municipal or industrial contaminants. We will also investigate the potential for chronic toxicity in a reach of the San Joaquin River downstream from major orchard and tributary inputs in the watershed. Due to the lack of water quality criteria with which to compare detected concentrations with, long-term monitoring of acute and chronic toxicity will provide an assessment of the effects on aquatic life and will help scientists at DPR to evaluate the effectiveness of programs designed to decrease the runoff of dormant spray insecticides. Due to the frequency of detections last winter, we will continue to sample for herbicides at these two locations.

II. OBJECTIVE

The objective of this study is to monitor the occurrence of acute and chronic toxicity in the San Joaquin River watershed during the dormant spray season. Additionally, levels of specific organophosphate and carbamate insecticides and selected herbicides that have potential to enter the San Joaquin River with surface runoff will also be monitored. A companion study will be conducted to monitor toxicity and pesticide levels in the Sacramento River.

III. PERSONNEL

This project will be conducted by the Environmental Hazards Assessment Program (EHAP) under the general direction of Don Weaver, Ph.D., Senior Environmental Research Scientist (Supervisor). Key personnel are listed below:

Project Leader: Carissa Ganapathy
Field Coordinator: Johanna Walters
Senior Scientist: Lisa Ross, Ph.D.
Statistician: Terrell Barry, Ph.D.
Contractor (toxicity tests): Charlie Huang, Ph.D., California Department of Fish & Game
Chemists: Jorge Hernandez, Jean Hsu, Hsiao Feng, Duc Tran, and Jane White- CDFA
Agency and Public Contact: Kevin Bennett

Questions concerning this monitoring project should be directed to Kevin Bennett at (916) 324-4200. Fax: (916) 324-4088.

IV. STUDY PLAN

The sampling sites for this years monitoring will be the same as the previous years. Acute toxicity sampling will be conducted at Orestimba Creek, a western tributary to the San Joaquin River, as this site receives runoff that is predominantly agricultural (Figure 1). Sampling for chronic toxicity will be conducted on the San Joaquin River at Vernalis, as this site receives discharges from all of the River's major agricultural tributaries, including the Merced, Tuolumne, and Stanislaus Rivers. Discharge records for both the Orestimba Creek and Vernalis sampling sites are available from collocated gauging stations. This information will be used to correlate any changes in chemical concentrations to fluctuations in flow and may be useful for modeling efforts, should they be undertaken.

Monitoring will commence prior to the onset of the dormant spray season (December 1998) and continue through the middle of March 1999. Background samples will be collected for one week, beginning prior to dormant spray applications, then monitoring will resume once applications have begun in the watershed. Monitoring will occur weekly and continue until no later than March 20, 1999.

V. SAMPLING METHODS

Acute toxicity sampling will be conducted on Monday and Wednesday of each week. Sampling for chronic toxicity will be conducted weekly. One chronic sample constitutes the collection of a sample on days zero, two, and four of each week (e.g. Mon., Wed., and Fri.). Water collected on those days will be delivered the following day to the laboratory for testing and sample renewal. Chemical analyses will also be performed on each sample collected for both acute and chronic tests. Selected organophosphate and carbamate pesticides will be analyzed using three chemical analytical screens (Table 2). The herbicides are not expected to reach levels that would contribute to *C. dubia* toxicity (Table 1) but will be monitored to look for their possible effects on other aquatic life.

A center channel water sample will be collected from bridges at each site. This will be done using a depth-integrated sampler (D-77) with a 3-liter Teflon® bottle and nozzle. Surface water subsamples will be composited temporarily in a stainless steel container until the appropriate volume of water has been collected. This composited sample will then be stored on wet ice until delivered to the processing facility in West Sacramento. Immediately upon arrival at West Sacramento, the samples will be split into amber glass bottles using a Geotech® 10-port splitter then sealed with Teflon®-lined lids. Samples to be analyzed for organophosphate and carbamate pesticides will be acidified with 3N hydrochloric acid to a pH between 3.0 and 3.5. At this pH, most of the organophosphate and carbamate pesticides are sufficiently preserved with the exception of diazinon. Diazinon degrades more rapidly at an acidic pH and therefore will be analyzed from a separate, unacidified, split sample. Samples submitted for herbicide analysis and toxicity tests will not be acidified. Sufficient water will be collected during each sampling event to provide approximately four liters for chemical analysis, two liters each for both acute and chronic toxicity tests, and any additional water required for quality control samples. All samples will be stored at 4°C until delivered to the laboratories for toxicity testing and chemical analyses. In addition, samples submitted for toxicity testing will be delivered within 24 hours of collection.

Dissolved oxygen, pH, specific conductance, and water temperature will be measured *in situ*, at each site, during each sampling period. As part of the toxicity testing, the California Department of Fish and Game's Aquatic Toxicology Laboratory will measure and record other parameters of

the delivered toxicity samples, including totals of alkalinity, hardness, ammonia, and specific conductivity. These measurements will be made in the laboratory, thus they will not directly reflect on-site conditions.

VI. TOXICITY TESTING AND CHEMICAL ANALYSIS

Toxicity testing, conducted by the California Department of Fish and Game's Aquatic Toxicology Laboratory, will follow current U.S. Environmental Protection Agency procedures using the cladoceran *Ceriodaphnia dubia* (U.S. EPA, 1993). The Aquatic Toxicology Laboratory has been accredited by the California Department of Health Services' Environmental Laboratory Accreditation Program. Acute toxicity will be determined using a 96-hour, static-renewal bioassay in undiluted sample water. Chronic toxicity will be determined using a seven-day bioassay with *C. dubia* in undiluted sample water, and will follow current U.S. Environmental Protection Agency guidelines (U.S. EPA, 1994). For example, test organisms used in chronic testing will be subjected to sample water from day zero on the following day (day 1). Sample water collected on days two and four will then replace test water on days three and five, respectively. All bioassays will commence within 36 hours of sample collection. In addition, data will be reported to the project leader as percent survival on each day for the duration of the tests.

Chemical analysis will be performed by the California Department of Food and Agriculture Center for Analytical Chemistry. The reporting limit will be used to record the lowest concentration of analyte that the method can detect reliably in a matrix blank. The method titles and reporting limits for the study are listed in Table 2. Comprehensive chemical analytical methods will be provided in the final report.

Number of Toxicity Tests		
2 acute/week x 11 weeks of study		22
1 chronic sample per week x 11 weeks of study		11
	Total	33
Number of Chemical Analyses		
4 (OP, CB, diazinon and herbicides) per acute toxicity sample: 4 analyses x 2 acute toxicity sampling events/week x 11 weeks		88
4 (OP, CB, diazinon and herbicides) per chronic toxicity sampling event: 4 analyses x 3 chronic sampling events (=1 chronic sample)/week x 11weeks		132
	Subtotal	220
Quality Control		
Continuing QC (approx. 10% of total chemical analyses)		22
	Total number of chemical analysis samples	242

VII. QUALITY ASSURANCE/QUALITY CONTROL

Chemical Analysis

Quality control will be conducted in accordance with Standard Operating Procedure QAQC001.00. Ten percent of the total number of primary analyses will be submitted with field samples as blind matrix spikes and rinse blanks. The total number of samples is presented above.

VIII. DATA ANALYSIS

Toxicity data will be used to establish baseline information on the occurrence of acute or chronic events at the Orestimba and Vernalis sites. A correlation matrix will be generated to investigate potential relationships between measured environmental parameters, discharge, toxic events, and chemical concentrations. Measured concentrations will be compared to various established water

quality criteria including California Quantitative Response Limits, U.S. EPA objectives, and California Department of Fish and Game suggested criteria. Pesticide concentrations will also be compared with acute and chronic LC_{50}s for *C. dubia* to aid in interpreting toxicity test results.

With a fixed monitoring schedule, sample collection during storm events, when pesticide levels are typically highest, may not occur. Therefore, records of storm events will be kept, and an analysis of discharge, chemical data, and toxicity will be discussed in relation to sampling periods. Depending on the results of the preliminary analysis, further analysis may include multivariate analysis.

IX. TIME TABLE

Field Sampling - December 1998 and application onset through March 1999
Toxicity Testing and Chemical Analysis - December 1998 through April 1999
Preliminary Memorandum - August 1999
Final Report - January 2001

X. REFERENCES

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Table 1. Relative 96 hour LC_{50} of pesticides in the insecticide and triazine herbicide screens. This table is for reference only and does not represent an exhaustive search of the literature. All concentrations are in ug/L (ppb). When 96 hour LC_{50} were not available, 48 hour tests were substituted and are italicized.

	Organism						
Insecticides	*Ceriodaphnia dubia*	*Daphnia magna*	*Daphnia pulex*	*Pteronarcys californica*	Rainbow Trout	Fathead Minnow	Bluegill
Carbaryl	*12e*	*5.6b*	*6.4b*	4.8b	1300a	1400e	8200b
Carbofuran		**39d**	*(15)a*		22,000-29,000a	870-1900b	1750a
Chlorpyrifos	0.08e	*0.21c*	*(17)a*	10b	3a	55e	3b
Diazinon	*0.5e*	*1.2e*	*0.8b*	25b	2,600-3,200a	7,800d	16,000a
Dimethoate		*2500c*		43b	6200a		6000a
Fonofos	0.26e	*2c*	*(1,000)a*		50a	1,090e	28a
Malathion		*1b*	*1.8b*	10b	7-230b	86-110b	100a
Methidathion	2e	*3c*			10a	8,900d	20a
Methyl parathion		*4.8c*	*(7.3)a*		2,700a	8,900b	4,400b
Phosmet		*5.6-11c*	*(85)a*		230a	7,300d	700a
Herbicides							
Atrazine		*49,000c*	(6,900)a		8,800a	15,000d	16,000a
Bromacil		**121,000d**	*(119,000)*a		*75,000a*		*71,000a*
Cyanazine		*49,000c*	*(42,000-106,000)a*		9,000b	18,000b	20,000b
Diuron	12,100e	*8,000c*	*1,400b*	1,200b	5,600a	14,200d	5,900a
Hexazinone		*152,000c*			320,000-420,000a	274,000a	370,000-420,000a
Metribuzin		42b	*(4,500-35,000)a*		64,000a		80,000a
Prometon		**59,800d**			12,000a		40,000a
Prometryn		*18,900c*	*(12660)a*		2,500a		10,000a
Simazine		*100c*	*(>100,000)a*	1,900b	>100,000a	>10,000b	90,000a

NOTES:
Numbers in parenthesis are for Daphnids where the species was not indicated.
Bold numbers are reported as EC50 for 48 hour tests.
SOURCES:
a. Tomlin, C. 1994. The Pesticide Manual, Tenth Edition
b. Mayer and Ellersieck. 1986. Manual of Acute Toxicity, U.S. Fish and Wildlife Service
c. Department of Pesticide Regulation Aquatic and Wildlife Toxicity compilation.
d. Draft Pesticide Ecological Effects Database, U.S. EPA
e. Sheipline, R. 1993 Draft. Background Information on Nine Selected Pesticides. CVRWQCB.

Table 2. California Department of Food and Agriculture, Center for Analytical Chemistry organophosphate and carbamate insecticide and triazine herbicide screens for the San Joaquin River toxicity monitoring study.

Organophosphate Pesticides in Surface Water by GC Method: GC/FPD		**N-Methyl Carbamate in Surface Water by HPLC Method: HPLC/Post Column-fluorescence**		**Herbicides in Surface Water by HPLC Method: HPLC/Post Column-fluorescence**	
Compound	**Reporting Limit (µg/L)**	**Compound**	**Reporting Limit (µg/L)**	**Compound**	**Reporting Limit (µg/L)**
Chlorpyrifos	0.04	Carbaryl	0.05	Atrazine	0.05
Diazinon[1]	0.04	Carbofuran	0.05	Bromacil	0.05
Dimethoate (Cygon)	0.05			Diuron	0.05
Fonofos	0.05			Cyanazine	0.05
Malathion	0.05			Hexazinone	0.05
Methidathion	0.05			Metribuzin	0.05
Methyl parathion	0.05			Prometon	0.05
Phosmet	0.05			Prometryn	0.05
				Simazine	0.05

[1] Diazinon was analyzed from a separate, unpreserved, split sample. Other OP and CB chemical samples were preserved with 3N HCl to a pH of 3-3.5 to retard analyte degradation. See text.

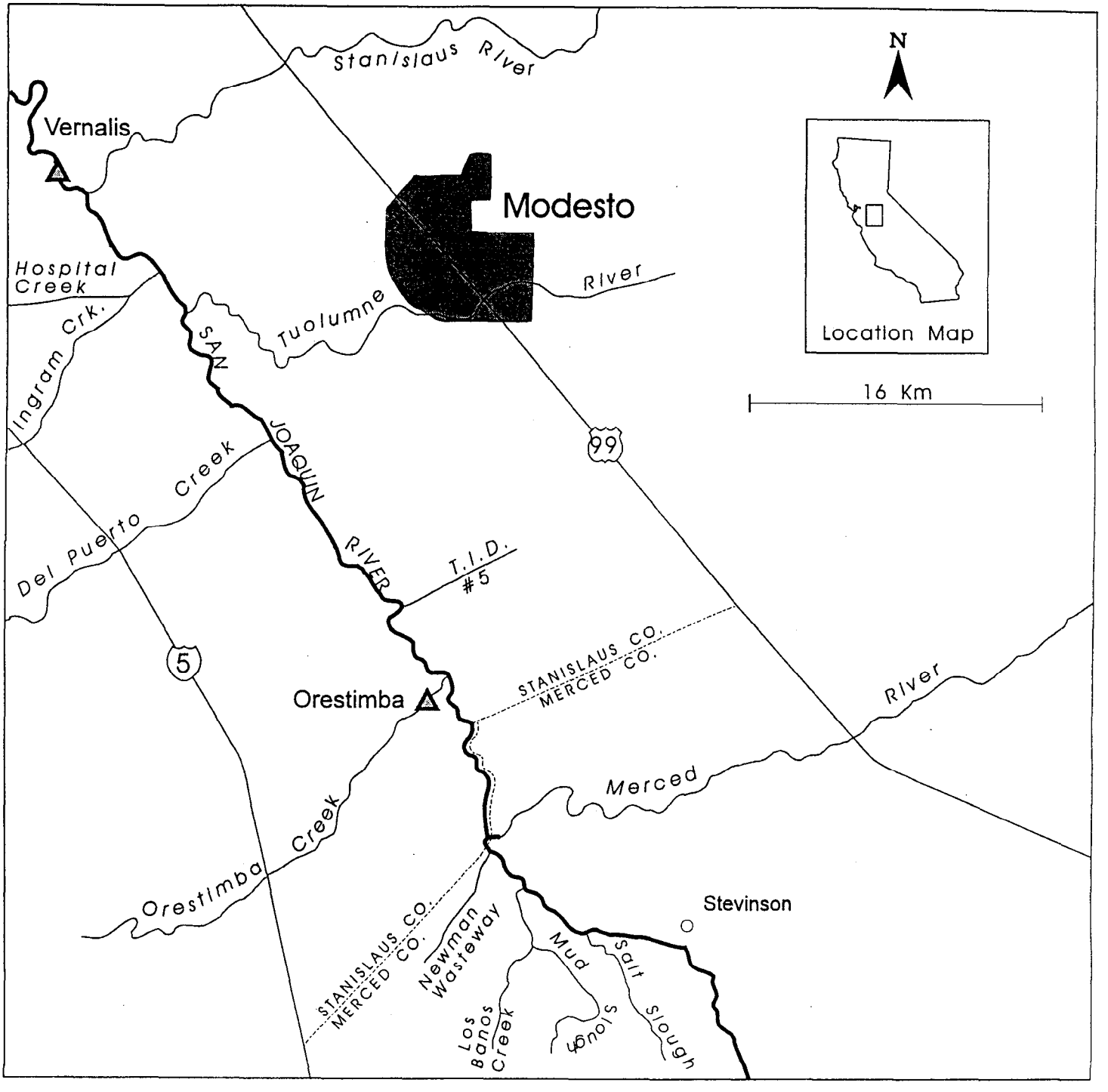

Figure 1. Location of toxicity sampling sites (Δ) in the San Joaquin River watershed: winter 1997-98.

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Document Title: Study 179: Protocol for Monitoring Acute and Chronic Toxicity in the San Joaquin River Watershed, Winter 1998-99.

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Document Date: November 16, 1998

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