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Document Title: Study 185: Protocol for Monitoring Acute and Chronic Toxicity in the San Joaquin River Watershed, Winter 1999-2000.

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STUDY 185: PROTOCOL FOR MONITORING ACUTE AND CHRONIC TOXICITY IN THE SAN JOAQUIN RIVER WATERSHED, WINTER 1999-2000

November 30, 1999

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 part of a five year effort 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). Dormant spray insecticides, at levels acutely toxic to test organisms, were also reported 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 conducted toxicity monitoring at sites along the San Joaquin River and Orestimba Creek (Bennett, 1998). During the first half of winter there was unusually wet weather and 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 samples collected, respectively. The maximum diazinon, carbofuran, and dimethoate concentrations detected were 0.092, 0.238, and 0.082 µg/L, respectively. Twelve percent of water samples from the San Joaquin River near Vernalis were found to have diazinon residues with a maximum concentration of 0.070 µg/L. *Ceriodaphnia dubia* survival ranged from 45 to 100 percent for acute toxicity from Orestimba Creek with only one toxic sample coinciding with a pesticide detection. Chronic toxicity tests conducted on water samples from the San Joaquin River near Vernalis displayed either 90 or 100 percent survival.

The same two locations were sampled again during the winter of 1997-98 for the insecticides. As part of an ongoing effort to gain more information about pesticide residues in state surface waters, additional samples were collected during that study and analyzed for triazine, bromacil and diuron herbicides. The SJR and Orestimba Creek discharge and rainfall was higher than in normal years 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%) collected at the Orestimba Creek site with concentrations ranging from 0.059 to 0.14 ppb. Four of the nine herbicides analyzed were detected in Orestimba creek. Bromacil was detected in three samples of the 18 samples (17%) with concentrations ranging from 0.012 to 0.066 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 had three herbicide detections. After February 4, 1998, there were no detections of any pesticides. For acute toxicity tests three of 18 samples had significantly reduced survival compared to the control. In one of the three samples there were pesticides detected above the detection limit and they were diazinon, diuron and simazine.

Diazinon and methidathion were the two organophosphates detected in the SJR at Vernalis. Diazinon was detected in the SJR in 10 of 30 samples (33%). These detections came in two groupings. The first detection occurred on January 7, 1998 and was very close to the detection limit. There was no detection January 9. On January 12, 14 and 16 diazinon was again detected 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 no *C. dubia* survival in the chronic toxicity test sample collected on February 2 with renewal water collected February 4. The corresponding control had 100% survival. The sample collected February 2 had detectible levels of diazinon, methidathion, diuron and simazine. The other chronic toxicity tests had survival ranging from 80% to 100% with corresponding control survival from 80% to 100%.

Sampling for the insecticides and herbicides continued during the winter of 1998-99 at Orestimba Creek and the SJR at Vernalis. Rainfall was significantly lower than the previous year during the same period. During this study there were no detections of organophosphate or carbamate insecticides in the 20 samples collected at the Orestimba Creek site. However, several herbicides were detected. Diuron was detected in all 20 samples at concentrations ranging from 0.061 to 1.7 µg/L. Bromacil was detected three times (15%) at concentrations ranging from 0.080 to 0.089 µg/L and simazine four times ranging from 0.066 to 0.10 µg/L. Cyanazine and prometryn were each detected once (5%) at 0.37 and 0.076 µg/L, respectively. Five samples (25%) contained more than one herbicide residue. Five herbicides were detected in 1 sample that was collected one day after a rain event on February 22, 1999. There was no significant mortality detected in any acute toxicity sample collected at Orestimba Creek.

Diazinon was detected in 3 of the 30 samples (10%) collected from the SJR at Vernalis. The detections occurred on January 20, 1999 at 0.15 µg/L, January 21 at 0.090 µg/L and on February 10 at 0.053 µg/L. Each detection occurred 1 to 2 days after a rain event. Similar to the Orestimba Creek samples, there were no other organophosphate or carbamate insecticides detected. Diuron was detected in every sample collected at Vernalis ranging in concentration from 0.10 to 1.9 µg/L. Bromacil was detected in one of 30 samples (3.3%) at a concentration of 0.059 µg/L. Cyanazine came in two pulses, and was detected in a total of six samples (20%) ranging in concentrations from 0.097 to 0.42 µg/L. Prometryn was detected in two samples (7%) ranging from 0.053 to 0.071 µg/L and simazine was detected seven times (23%) with concentrations ranging from 0.059 to 0.12 µg/L. Thirteen samples (43%) had more than one triazine detection and three of them (10%) had three detections in one sample. There was no significant mortality in any chronic toxicity sample collected at the SJR.

In this fourth 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. Proposed toxicity criteria have been established by the California Department of Fish and Game (DFG) and the US Environmental Protection Agency (USEPA) for diazinon. The acute criteria are 0.080 and 0.090 µg/L for DFG and USEPA, respectively. The chronic criterion is 0.040 for DFG, and has yet to be determined by USEPA. Due to the lack of water quality criteria with which to compare other detected concentrations, long-term monitoring of acute and chronic toxicity, along with detection concentrations will help scientists at the DPR 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 Marshall Lee, Senior Environmental Research Supervisor. Key personnel are listed below:

Project Leader: DeeAn Jones
Field Coordinator: To be determined
Senior Scientist: Lisa Ross, Ph.D.
Statistician: Terrell Barry, Ph.D.
Contractor (toxicity tests): George Faggella., California Department of Fish & Game
Chemists: Jorge Hernandez, Jean Hsu, Jane White, Duc Tran and Hsiao Feng,
California Department of Food & Agriculture
Agency and Public Contact: Kevin Bennett

Questions concerning this monitoring project should be directed to Kevin Bennett at (916) 324-4100. 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 1999) and continue through the middle of March 2000. 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 17, 2000.

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 1). The herbicides are not expected to reach levels that would contribute to *C. dubia* toxicity 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 sub samples 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 conductivity, 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, and 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 1. 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. Environmental Protection Agency objectives, and California Department of Fish and Game suggested criteria. Pesticide concentrations will also be compared with acute and chronic LC50s 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 preliminary analysis results, further analysis may include logistic regression.

IX. TIME TABLE

Field Sampling - December 1999 and application onset through March 2000
Toxicity Testing and Chemical Analysis - December 1999 through April 2000
Preliminary Memorandum - August 2000
Final Report - January 2001

X. REFERENCES

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Table 1. California Department of Food and Agriculture, Center for Analytical Chemistry organophosphate and carbamate insecticide and multiple 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	0.2
Malathion	0.05			Hexazinone	0.2
Methidathion	0.05			Metribuzin	0.2
Methyl parathion	0.05			Prometon	0.05
Phosmet	0.05			Prometryn	0.05
				Simazine	0.05

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

Table 2. Relative acute 96 hour LC50 of the pesticides in the insecticide and herbicide screens. This table is for reference only and does not represent an exhaustive search of the literature. References cited are all compendiums of the results of numerous studies.

	Organism						
Insecticides	*Ceriodaphnia dubia*	*Daphnia magna*	*Daphnia pulex*	*Pteronarcys californica*	Rainbow Trout	Fathead Minnow	Bluegill
	All concentrations in mg/L (ppm)						
Carbaryl	*0.012c*	*0.0056 - 7.1b*	*0.0064b*	0.0048a	1.2 –4.5b	1.4c – 7.7b	0.76 – 290b
Carbofuran	*0.0026g*	*0.029 – 0.041b*			0.36 – 0.42b	0.88 – 1.99b	0.088 – 3.1b
Chlorpyrifos	0.00008c	*0.0001-0.0017b*		0.01d	0.0071-0.027b	0.12 – 0.20b	0.0013-0.11b
Diazinon	*0.0005c*	*0.0005-0.001b*	*0.0008b*	0.025a	0.0026e – 1.8b	7.8b	0.1 – 0.5b
Dimethoate		**4.7e**		0.043a	6.2d		6a
Fonofos	0.00026c	*0.002b*			0.05 – 2.8b	1.09c	0.0068-0.32b
Malathion		*0.001-0.0022b*	*0.0018b*	0.0011a	0.041 – 0.2a	8.7 – 11.0a	0.02b
Methidathion	0.002c	*0.0072b*			0.01 – 0.014b		0.0022-0.017b
Methyl-parathion	*0.0026f*	*0.00014-0.028b*			2.2 – 161b	7.2 – 9.5b	1.0 – 13.3b
Phosmet		*0.0056-0.011b*			0.11 – 1.56b	7.3 – 9.0b	0.022 – 0.31b
Herbicides							
Atrazine		*6.9 – 115b*			4.5 – 24b	15b	6.7 – 69.0b
Bromacil		*119e - 121b*			32 – 127b		36 – 180b
Cyanazine		*42 – 49b*			9.0b	16.3 – 21.3b	22.5b
Diuron	12.1c	*8.4b – 12e*	*1.4b*	(1.2a)	1.95 – 23.8b	14.2b	2.8 – 300b
Hexazinone		*33.1b – 442e*			146.7 – 420b	274b	100 – 420b
Metribuzin		*4.2 – 98.5b*			42 – 147b		76 – 131b
Prometon		*25.7 – 59.8b*			16 – 20b		15.5b – 40e
Prometryn		*12.7e - 18.6b*			2.9 – 7.2b		10.0b
Simazine		*1.1b - >100e*	*1.0d*	1.9a	10 – 100b	5 – 510b	16 – 100b

NOTES:

- Numbers in *italics* are for 48-hour EC50 toxicity tests.
- Numbers in **bold** are for 24-hour LC50 toxicity tests.
- Numbers in parenthesis are for animals where the species was not indicated.
- Number ranges are for all studies listed in the indicated source and may represent 2-6 individual studies.

SOURCES:

a. Manual of Acute Toxicity, U.S. Fish and Wildlife Service
b. DRAFT Pesticide Ecological Effects Database, U.S. EPA
c. Background Information on 9 Selected Pesticides, CVRWQCB
d. Handbook of Environmental Data on Organic Chemicals, 2nd Ed., Karen Verschueren
e. The Pesticide Manual, 11th Ed., C.D.S. Tomlin, British Crop Protection Council
f. Hazard Assessment of the Insecticide Methyl Parathion to Aquatic Organisms in the Sacramento River System. California Department of Fish and Game, 1992.
g. Hazard Assessment of the Insecticide Carbofuran to Aquatic Organisms in the Sacramento River System. California Department of Fish and Game, 1992.

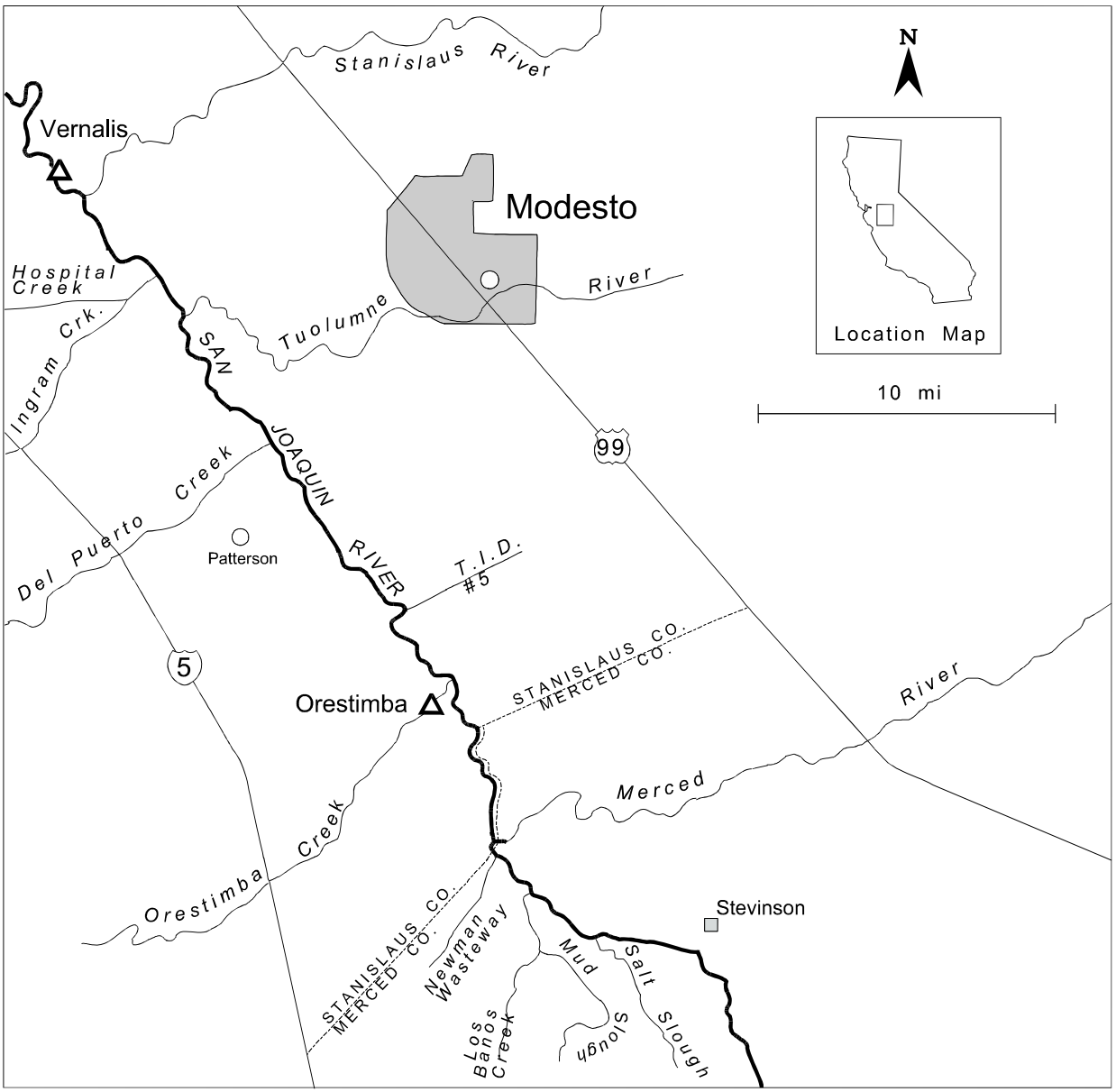

Figure 1. Location of toxicity sampling sites (Δ) and rainfall stations (○) in the San Joaquin River Watershed: Winter 1999-2000.