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Document Title: STUDY 199: PROTOCOL FOR MONITORING ACUTE AND CHRONIC TOXICITY IN THE SACRAMENTO RIVER WATERSHED: WINTER 2000-01

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STUDY 199: PROTOCOL FOR MONITORING ACUTE AND CHRONIC TOXICITY IN THE SACRAMENTO RIVER WATERSHED: WINTER 2000-2001

December 1, 2000

I. INTRODUCTION

The Department of Pesticide Regulation (DPR) has a responsibility to ensure that pesticides are distributed and used in a safe manner. California law requires DPR to consider and encourage the use of pest control products and procedures that reduce human and environmental health hazards. DPR has developed a Pest Management Strategy to increase the adoption of reduced-risk pest management practices. The reduced-risk management practices involve economically viable techniques, that either currently exist or can be developed through research and education, which will lower the health and environmental risks of controlling pests. DPR is charged with evaluating the effectiveness of efforts to facilitate the adoption of these practices. One measure of success of this strategy would be decreasing incidences of pesticide-related toxicity in the rivers and waterways of California.

The Sacramento River is the largest river in California both in flow and in drainage area (Figure 1). From Mount Shasta in the north to the Sacramento-San Joaquin Delta in the south, the river flows for 327 miles and drains approximately 27,000 square miles including agricultural, urban, and undeveloped land (Domalgalski and Brown, 1994). The Sacramento River provides 35% of the State's water supply, both drinking and agricultural, and is also an important resource for recreation and wildlife (Reynolds, et al., 1993). The primary source of water entering the system is surface runoff from the Sierra Nevada Mountains to the east and the Cascade Range to the north (CSLC, 1993). Runoff from rain events occurring in the Sacramento Valley provides significant short term increases in river flow. Seasonal rains occur from October to March with little significant rain from June to September. River flow during the summer is composed mostly of dam releases of snow-melt water for urban, agricultural, recreational, and wildlife purposes.

In the Sacramento Valley, the organophosphorus (OP) insecticides diazinon and methidathion are the primary dormant season insecticides used on stone fruit and nut crops (DPR 1993; DPR 1994; DPR 1995, DPR 1996, DPR 1997). The dormant season application period coincides with the bulk of the seasonal rainfall, providing the potential for these pesticides to wash off target areas and into the Sacramento River. Pesticide use reports (DPR 1993-1997) indicate the majority of dormant spray insecticides are applied along the Feather River north of the Bear River and along the Sacramento River in northern Butte and Glenn counties and southern Tehama County. The primary dormant spray OP insecticides, diazinon and methidathion, are applied in nearly identical areas (Figures 2 and 3) and these areas remain fairly stable from year to year. Winter runoff from orchard areas north of Stoney and Big Chico Creeks flows into the Sacramento River via individual small stream watersheds (Figures 2 and 3). Runoff from orchard areas west of the Sacramento River and south of Stoney Creek chiefly flows into the Colusa Basin Drain that enters the Sacramento River at Knights Landing (Figure 4). Runoff from dormant spray areas east of the Sacramento River and south of Big Chico Creek principally flows into Butte Creek, which has been engineered to drain into the Sutter Bypass via the Butte Slough. Runoff from areas east of the Feather River drains into the Feather River above Nicolaus via individual small watersheds.

Runoff from the west side of the Feather River drains into the Sutter Bypass. During periods of normal flow, the Sutter Bypass enters the Sacramento River via the Sacramento Slough at Karnak. During periods of high flow, the Sutter Bypass channel fills completely with runoff from this area plus water diverted from the Sacramento River. This flow merges with the Feather River eight miles prior to entering the Sacramento River, forming a two-mile wide channel that inundates the Sacramento Slough. During floods, a large portion of the flows for the Sacramento River and the Sutter Bypass/Feather River will be diverted into the Yolo Bypass.

A one-year DPR study and a three-year U.S. Geological Survey (USGS) study of the Sacramento River have shown that most diazinon and methidathion detections were observed during the dormant spray season (MacCoy et al. 1995; Ganapathy et al. 1998). No other organophosphate (OP) or carbamate (CB) insecticides analyzed in those studies were detected. The triazine (TR) herbicides, atrazine and simazine have also been detected during winter monitoring by the USGS. These detections occurred almost exclusively in conjunction with rain events indicating that rain runoff was the primary source of the pesticides entering the rivers. Toxicity has been found at Gilsizer Slough, which drains some of the area west of the Feather River and flows into the Sutter Bypass. In bioassays conducted with the planktonic crustacean *Ceriodaphnia dubia,* acute mortality was 100% in five of the seven consecutive weekly samples. This toxicity appeared related to levels of pesticides detected in four of the samples with an indeterminate cause of toxicity in the fifth sample (Foe and Sheipline, 1993).

DPR began a 5-year dormant season monitoring program in the Sacramento Basin in 1996, which is scheduled to conclude this year. This program currently monitors dormant spray pesticide concentrations at three sites and toxicity at two sites within the basin. Acute toxicity is measured in a sub-watershed and chronic toxicity is measured on the main stem of the Sacramento River. During the winter of 1996-97 DPR conducted monitoring at sites along the Sacramento River and Sutter Bypass (Nordmark et. al. 1998a). Extensive flooding occurred in January 1997 which greatly affected river discharges and modified the sampling schedule. No acute toxicity was found at the Sutter Bypass site and no chronic toxicity or reproductive impairment was found at the Sacramento River at Bryte site. Diazinon was detected in 44% of the Sutter Bypass samples, with a maximum concentration of 0.086 μg/L and in 17% of the Sacramento River samples, with the highest level reported at 0.065 μg/L. Methidathion was detected in one sample from the Sutter Bypass and one from the Sacramento River.

The winter of 1997-98, the second year of the DPR study, was another wetter than average year (Nordmark 1998b). The chronic toxicity sampling site was moved upstream to Alamar Marina due to sampler snagging problems at Bryte. The acute toxicity monitoring site remained the same. No acute toxicity was found at the Sutter Bypass site and no chronic toxicity or reproductive impairment was found at the Sacramento River at Alamar Marina site. DPR detected diazinon in 30% of the Sutter Bypass samples, with a maximum concentration of 0.088 µg/L, and in 40% of the Sacramento River samples, with the highest level reported at 0.17 µg/L. Methidathion was detected once at the Alamar Marina site. DPR added a chemical analysis screen for nine soil applied herbicides to the tests performed at each site during 1997-98. Diuron and simazine were detected at both the Sutter Bypass and Sacramento River sites while bromacil was detected only at the Sutter Bypass site.

The winter of 1998-99 was the third year of the DPR study and was a near average rainfall season in the Sacramento River Basin (Nordmark, 1999). The acute toxicity monitoring site was moved to Wadsworth Canal, as the Sutter Bypass did not truly represent a small watershed for most of the previous two seasons. The Sutter Bypass site was significantly diluted by Sacramento River water for much of the dormant season via diversions at Butte Slough and Tisdale Weir. Samples were collected from the Sutter Bypass for study continuity purposes, however, only chemical analyses were performed. Eight of the 20 samples (40%) collected at Wadsworth Canal were toxic to *C. dubia*. Diazinon was detected in all of the samples that demonstrated significant mortality but was also detected in nine samples that did not show significant mortality. A diazinon concentration of roughly 0.2 µg/L appeared to correspond to a threshold where toxic effects occurred. One chronic toxicity test of Sacramento River water taken at Alamar Marina showed toxicity but no pesticides were detected in this sample. Diazinon was detected in 85% of the samples collected from Wadsworth Canal with the highest level reported at 1.6 µg/L. Methidathion was detected once at this site. Forty-five percent of the samples from the Sutter Bypass contained diazinon with the highest level reported at 0.11 µg/L. No insecticides were detected in any Sacramento River samples. Diuron was the most commonly detected herbicide and it was detected at all three sites. Bromacil, hexazinone and simazine were also detected at the Wadsworth Canal site.

The winter of 1999-2000 was the fourth year of the DPR study and, although dry through the middle of January, was an overall average precipitation season (Nordmark, 2000). Nine of the 22 samples collected at Wadsworth canal were acutely toxic to *C. dubia.* Diazinon was detected in all of the samples that demonstrated significant mortality but was also detected in 4 samples that did not show significant mortality. Once again this year, a diazinon concentration of roughly 0.2 µg/L appeared to correspond to a threshold where toxic effects occurred. Diazinon was detected in 13 (59%) of the 22 samples collected from the Wadsworth Canal with the highest level reported at 2.7 µg/L Methidathion was detected 7 (32%) times, always in conjunction with diazinon, with a maximum concentration of 1.21 µg/L. Carbaryl was detected once on February 14 at 0.092 µg/L. This is the first time that carbaryl has been detected in the Sacramento watershed during the 4 years of dormant season monitoring. Diazinon was detected in 4 of the 22 samples (18%) collected in the Sutter Bypass with a maximum concentration of 0.09µg/L and was detected in 2 of the 33 samples (6%) collected from the Sacramento River at Alamar Marina with a maximum concentration of 0.06 µg/L Diuron was the most commonly detected herbicide and it was detected at all three sites. Bromacil, hexazinone and simazine were also detected at the Wadsworth Canal site, along with bromacil and simazine detected once in the Sutter Bypass.

This study is the last year of a five-year effort to monitor dormant spray runoff in the Sacramento River watershed. In this study we will continue to look at acute toxicity to *C. dubia* in a small watershed where the discharging waters do not contain major inputs from municipal or industrial sources. We will also investigate the potential for chronic toxicity in a section of the Sacramento River downstream of major dormant spray insecticide inputs in the watershed. Selected herbicides will also be monitored as recommended in the memo "Category and recommendation of currently registered pesticides for surface water monitoring during FY97-98" (Goh, 1997). Long-term monitoring of acute and chronic toxicity will help scientists at DPR evaluate the effectiveness of programs designed to decrease the runoff of dormant spray insecticides and selected herbicides.

II. OBJECTIVES

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

III. PERSONNEL

The Environmental Hazards Assessment Program (EHAP) will conduct this project under the general direction of Patricia Dunn, Senior Environmental Research Scientist (Supervisor). Key personnel are listed below:

Project Leader: DeeAn Jones
Field Coordinator: Sheryl Gill
Senior Scientist: Frank Spurlock, Ph.D.
Study Design/Data Analysis: Terrell Barry, Ph.D.
Toxicity Tests: Donald Guy, California Dept. of Fish and Game
Chemist: Jane White, Jorge Hernandez, Duc Tran, California Dept. of Food and Agriculture
Agency and Public Contact: Kevin Bennett

Questions concerning this project should be directed to Kevin Bennett at: (916) 324-4100
Fax: (916) 324-4088

IV. STUDY PLAN

Data from the first three years of the study showed that, for much of the dormant spray season, we can expect high flows in the Sutter Bypass that are composed largely of Sacramento River water entering the via the Tisdale Weir. This scenario does not represent a small watershed, which is our intent when sampling for acute toxicity. Therefore, sampling for acute toxicity will be conducted from the South Butte Road Bridge across the Wadsworth Canal. The Wadsworth Canal site receives predominantly agricultural water from a small watershed, it avoids the backflow from the Sutter Bypass when bypass levels are high, and it discharges into the Sutter Bypass just above the Sutter National Wildlife Refuge (Figure 4). For continuity purposes, we will continue to do a chemical analysis of samples from the bridge across the east channel of the Sutter Bypass at the Karnak pumping station, or from the levee at Kirkville Road in the event of flooding at Karnak. Sampling for chronic toxicity will be conducted on the Sacramento River from the Alamar Marina dock as this site receives discharge from all the major agricultural tributaries (Figure 4) but is above the discharge of the largely non-agricultural American River and the urban runoff of the City of Sacramento. Discharge records are available for Karnak, and Alamar sites from nearby gauging stations. Only stage height is available for the Wadsworth Canal site so it will be hand gauged each time it is sampled. 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.

One week of background samples will be collected in early December 2000, prior to dormant spray applications. Monitoring will resume in early January 2001 once applications have begun and continue until no later than March 16, 2000. Additional data collection will include *in-situ* measurements of water pH, temperature, dissolved oxygen, and specific conductance.

V. SAMPLING METHODS

Acute toxicity, pesticide concentrations and discharge will be monitored twice per week at Wadsworth Canal at South Butte Road. We will continue to collect samples at Karnak twice per week for chemical analysis only. Chronic toxicity and pesticide concentrations will be monitored weekly on the Sacramento River at Alamar. One chronic sample constitutes the collection of samples on days zero, two and four of each week (e.g. Monday, Wednesday and Friday). Water collected on those days will be delivered the same day to the California Department of Fish and Game-Aquatic Toxicology Laboratory (CDFG-ATL) for testing and sample renewal. Chemical analysis will be performed on each sample collected for both acute and chronic tests. Selected OP and CB pesticides will be analyzed in three analyses. Selected herbicides will also be analyzed in a fourth analysis (Table 2). The herbicides are not expected to reach levels where they would contribute to *C. dubia* toxicity, but will be monitored to look for possible effects on other aquatic life (Table 3).

At each sampling site, water will be collected using a depth-integrating sampler (D-77) with a 3-liter Teflon® bottle and nozzle at a single transect as close to the center channel as possible. As river levels rise, the Karnak site is flooded by water from the Sacramento and Feather rivers. When that occurs, samples will be drawn at Kirkville Road, approximately 10 miles upstream. Sampling at the Kirkville Road site may be done with the D-77 sampler from the bridge if conditions permit, or by wading into the stream with a grab pole consisting of a new 1-liter amber glass bottle at the end of a 3-meter pole. Surface water subsamples will be composited temporarily in a stainless steel container until the appropriate volume of water has been collected. The composited sample will be stored on wet ice until delivered to the processing facility at West Sacramento. Immediately upon arrival at the processing facility, the composite sample will be split into 1-liter amber glass bottles, using a Geotech® 10-port splitter, then sealed with Teflon®-lined caps. The organophosphate and carbamate samples will be preserved by acidification with 3N hydrochloric acid to a pH between 3.0 and 3.5. At this pH, most OP and CB pesticides are sufficiently preserved with the exception of diazinon. Therefore, diazinon and the herbicides will be analyzed from separate, unacidified, split samples. Samples submitted for toxicity tests will not be acidified. Sufficient water will be collected at each sampling event to provide approximately four liters for chemical analysis, two liters for toxicity testing, and any additional water required for quality control (QC) and backup samples.

Split samples for chemical analysis will be transported on wet ice to the California Department of Food and Agriculture (CDFA) Center for Analytical Chemistry within three days of collection. Split samples for toxicity testing will be delivered on wet ice to the CDFG-ATL within 24 hours of collection. CDFG will measure and record other parameters of the split samples including totals of ammonia, alkalinity, hardness, and specific conductivity as part of their toxicity testing.

VI. TOXICITY TESTING AND CHEMICAL ANALYSIS

Toxicity testing conducted by the CDFG-ATL will follow current USEPA procedures using the cladoceran *Ceriodaphnia dubia* (U.S. EPA, 1993). The California Department of Health Services Laboratory Accreditation Program has accredited the CDFG-ATL. Acute toxicity will be determined using a 96-hour, static renewal bioassay in undiluted sample water. Chronic toxicity will be determined using a 7-day bioassay of undiluted sample water with *C. dubia* and will follow current USEPA guidelines (U.S. EPA, 1994). For example, test organisms used in chronic testing will be subjected to sample water collected day zero, on the same day (day 0). Sample water collected on days two and four will then replace test water on the same day it is collected. All bioassays must commence within 36 hours of sample collection. 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 CDFA-Center for Analytical Chemistry. The reporting limit will be the lowest concentrations of analyte that the method can detect reliably in a matrix blank (DPR, 1996). The total number of samples is presented in Table 1. The reporting limits for this study are listed in Table 2. Chemical analytical methods will be provided in the final report.

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 rinse blanks, matrix blanks, and blind matrix spikes.

VIII. DATA ANALYSIS

Toxicity data will be used to establish baseline information on the occurrence of acute and chronic events at these sites. A correlation matrix will be established to identify potential relationships between measured environmental parameters, discharge, toxic events, and chemical concentrations. Further analysis may include multivariate analysis, depending on preliminary analysis results.

IX. TIMETABLE

Site Survey and Selection	August 2000
Field Sampling	December 4 through 8, 2000 and January 2 through March 9, 2001
Preliminary Memorandum	September 2001
Final Report	September 2002

X. REFERENCES

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Table 1. Samples collected for toxicity testing and chemical analysis

Number of Toxicity Tests	
2 acute tests/week x 11 weeks of study	22
1 chronic test per week x 11 weeks of study	11
Total number of toxicity tests	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 event at the Sutter Bypass site: 4 analyses x 2 sampling events/week x 11 weeks	88
4 (OP, CB, diazinon and herbicides) per chronic toxicity sampling event: 4 analyses x 3 sampling events (=1 chronic sample)/week x 11 weeks	132
Subtotal	308
Quality Control	
Continuing QC (approx. 10% of total chemical analyses)	30
Total number of chemical analytical samples	338

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

Organophosphate Pesticides in Surface Water by GC		**N-Methyl Carbamate in Surface Water by HPLC**		**Herbicides in Surface Water by HPLC**	
Method: GC/FPD		**Method: HPLC/Post Column-fluorescence**		**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 3. 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:

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c. Mayer F.L. and M.R. Ellersieck. 1986.
d. Menconi, M., and Gray, S., 1992
e. Verschueren, K., 1983.
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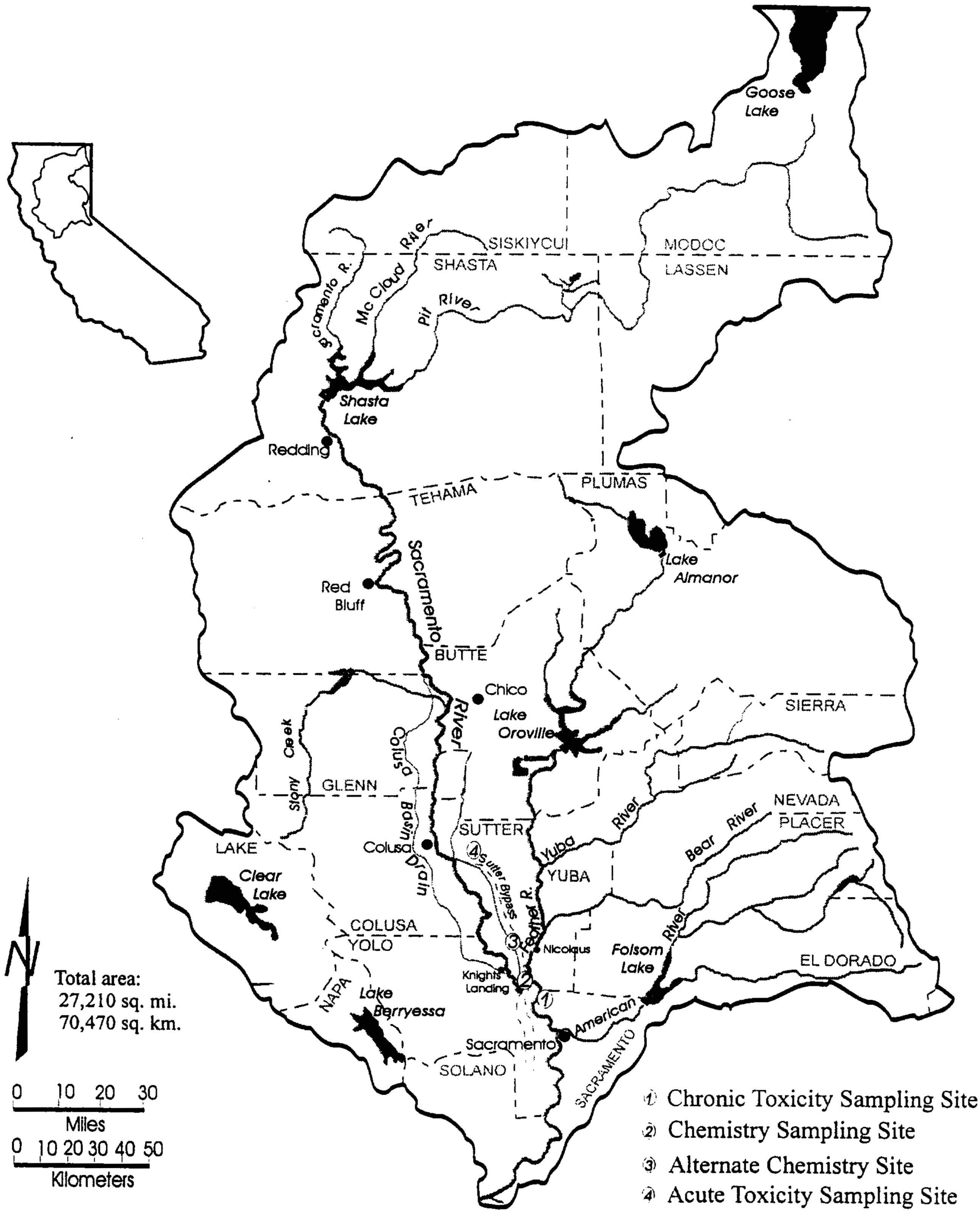

Figure 1. Map of the Sacramento River Hydrologic Basin.

Figure 2. Average Dormant Season Diazinon Use in the Sacramento Watershed, November through February, 1993-97

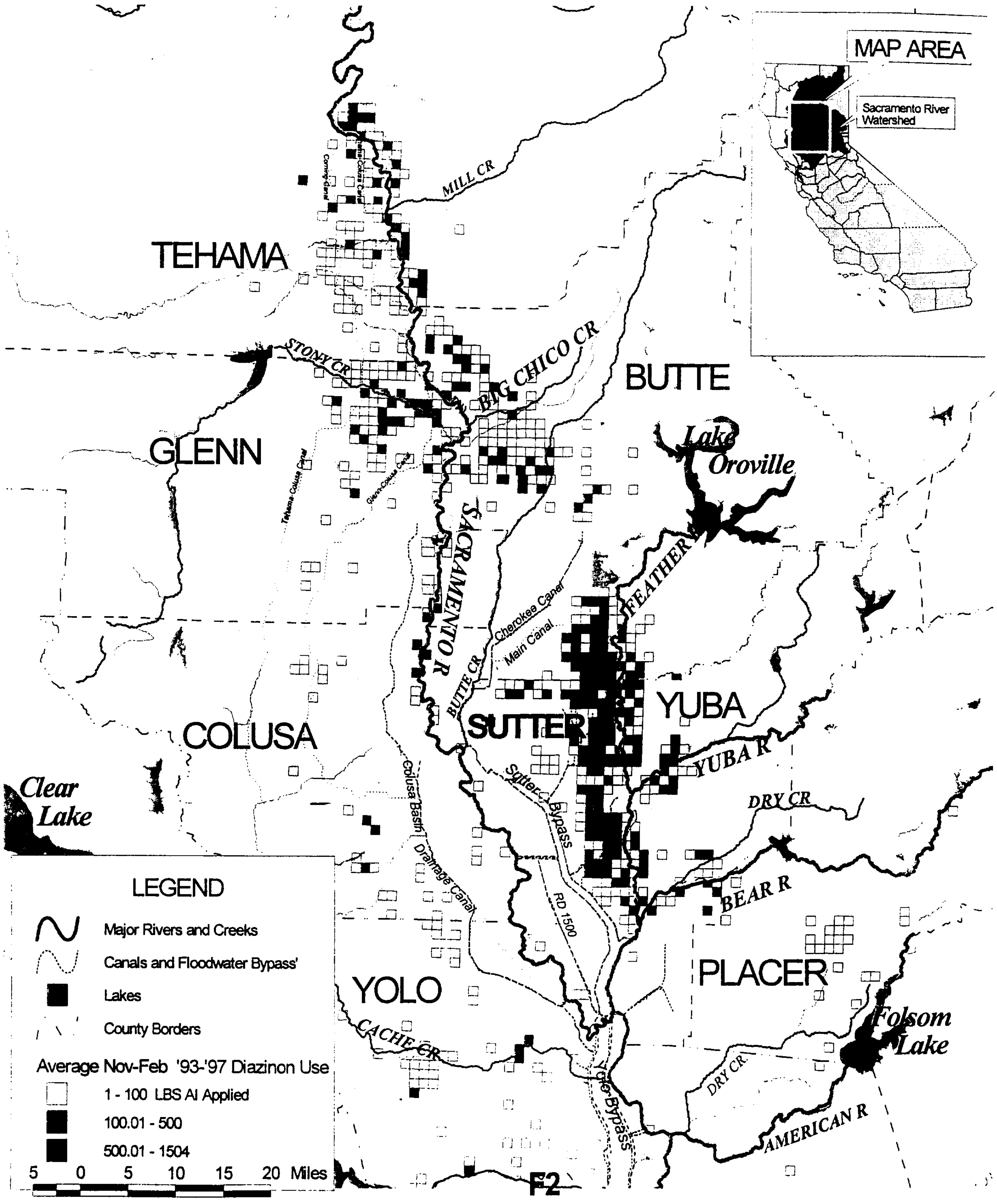

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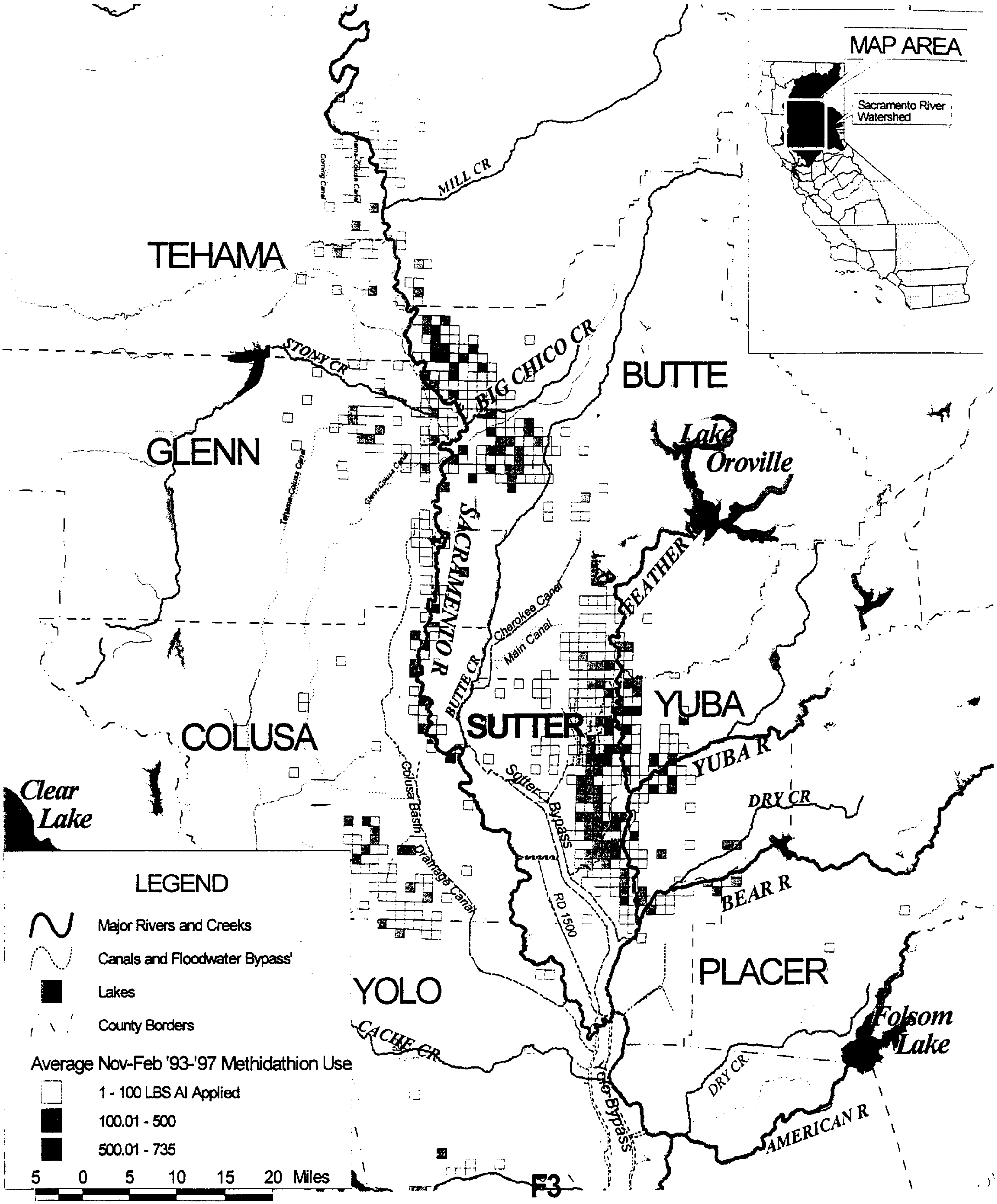
Figure 3. Average Dormant Season Methidathion Use in the Sacramento Watershed, November through February, 1993-97



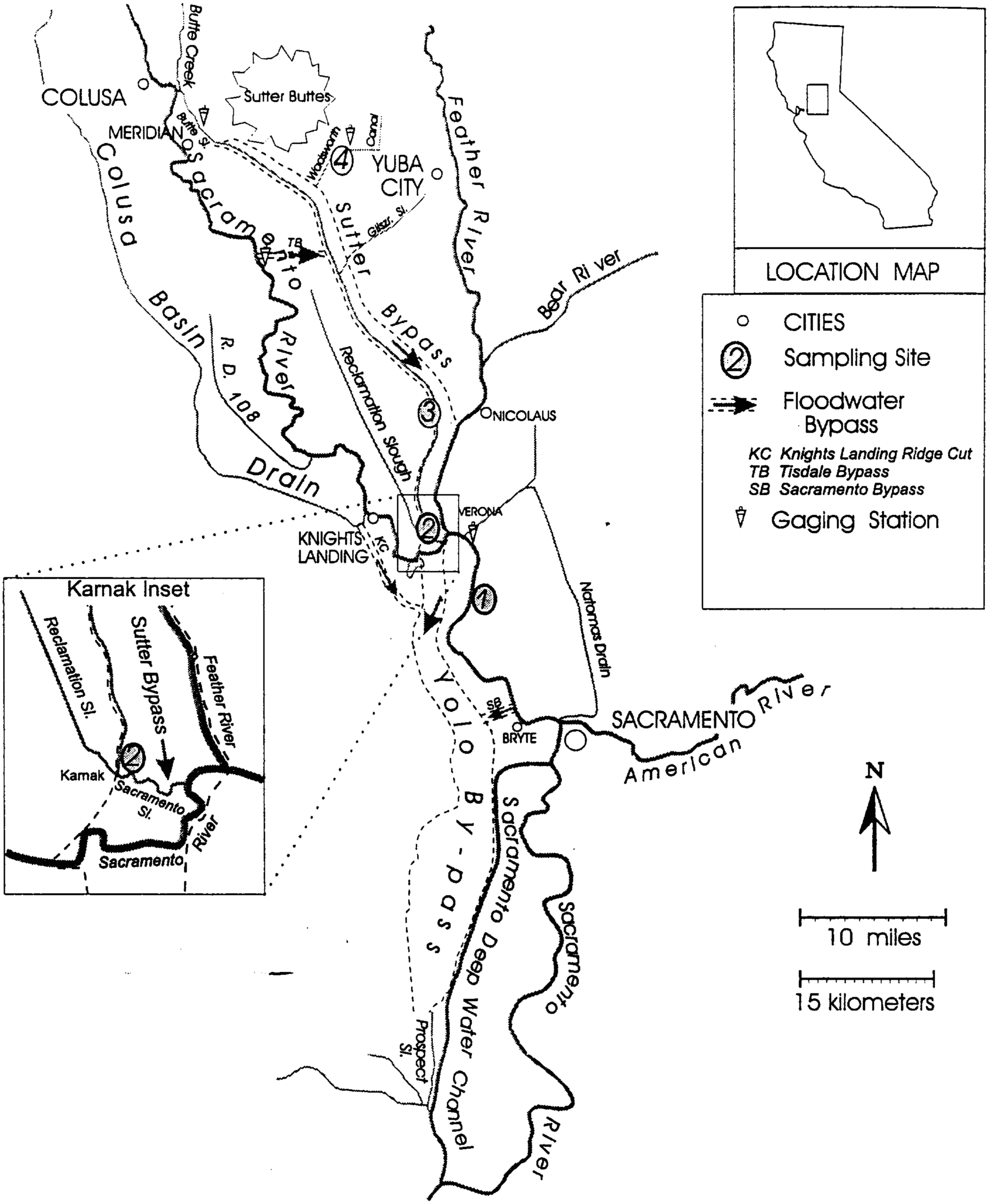


Figure 4: Sampling sites in the Sacramento River watershed.
Site 1 = Alamar Marina, Sacramento River Chronic Toxicity Site.
Site 2 = Sutter Bypass at Karnak Pumping Station, Water Chemistry Site.
Site 3 = Sutter Bypass at Kirkville Road, Alternate Water Chemistry Site.
Site 4 = Wadsworth Canal, Acute Toxicity Monitoring Site.