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CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

REGIONAL WATER QUALITY CONTROL BOARD
CENTRAL VALLEY REGION

TOXICITY IDENTIFICATION EVALUATIONS OF ORCHARD DORMANT SPRAY STORM RUNOFF

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Forward

This project has been funded by the Bay Protection Toxic Clean-up Program under contract number FG 2305 ES with the California Department of Fish and Game to conduct toxicity identification evaluations of orchard dormant spray runoff into the Sacramento San Joaquin Delta Estuary. The contents of this document do not necessarily reflect the views and policies of the California Department of Fish and Game nor of the State Water Resources Control Board nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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EXECUTIVE SUMMARY California is the nation's leading producer of nut and tree fruit. Each winter about half a million pounds of pesticide active ingredient is applied in the Central Valley on stone fruit[1], apple, pear, and almond orchards for boring insect control. Diazinon accounts for about half of the dormant spray market. Orchards are the only major use of diazinon at this time of year.

Studies conducted in 1990, 1992, 1993 and 1994 in the San Joaquin River and in 1993 and 1994 in the Sacramento River detected diazinon in storm runoff samples at toxic concentrations to *Ceriodaphnia* and other sensitive invertebrates. These findings are of regulatory significance as the Central Valley Regional Water Quality Control Board's Basin Plan contains a narrative toxicity objective stating the "all waters shall be maintained free of toxic substances in concentrations that produce detrimental physiological responses...in aquatic life". In 1998 both the Sacramento and San Joaquin Rivers and the downstream Estuary were placed on the Clean Water Act's 303(d) list by the Central Valley Regional Water Quality Control Board as impaired because of invertebrate toxicity from elevated diazinon concentrations during the dormant spray season. Not known was whether other contaminants besides diazinon might be present in storm runoff and also contribute to toxicity as Toxicity Identification Evaluations (TIEs) had not been conducted on orchard dormant spray runoff.

TIEs are procedures developed to identify chemicals responsible for toxicity in bioassays. Esfenvalerate and permethrin, two pyrethroid insecticides, are increasingly being used on orchards. No TIE "finger-print" has been developed for pyrethroids nor have the chemicals been included in pesticide scans by any regulatory agency monitoring dormant spray runoff. TIE procedures and analytical monitoring data are needed to evaluate the potential contribution of pyrethroids to surface water toxicity during winter storm runoff. Also, the sensitivity of the Phase III TIE process has not yet been evaluated for pesticides like diazinon. In particular, it is not known how much unexplained toxicity must be present in a sample before concluding that other unidentified contaminants are also present and contribute to beneficial use impairments.

Objectives of this study were fourfold. First, develop TIE "finger prints" to help identify toxicity from pyrethroid insecticides. Second, evaluate the sensitivity of the Phase III TIE process for organophosphate pesticides. Third, continue monitoring in the Sacramento and San Joaquin Basins to ascertain whether *Ceriodaphnia* toxicity is still present in orchard dormant spray runoff and, finally, use TIE procedures to identify and confirm the chemicals responsible for invertebrate toxicity.

The toxicity of the pyrethrin insecticide esfenvalerate to *Ceriodaphnia* was evaluated in the presence and absence of piperonyl butoxide (PBO) to determine whether PBO could be used as a TIE "finger-print" for distinguishing pyrethrin induced toxicity. PBO enhanced esfenvalerate toxicity in each exposure tested suggesting that PBO might be an effective "finger-print" for

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[1]Apricot, cherries, nectarines, peaches, plums, and prunes.

identification of pyrethrin induced toxicity.

Purpose of a phase III TIE is to ascertain how much of the ambient toxicity in a bioassay sample can be explained by the chemicals identified in phase I and II. This is accomplished by simultaneously comparing the response of *Ceriodaphnia* in a retest of the ambient sample and in laboratory water amended with the identified toxicant(s) at their ambient concentration. There are two main sources of variability in the phase III TIE: differences in animal sensitivity and imprecision in pesticide measurements. Analysis of the variability suggests that the resolution of the phase III TIE process is about one toxic unit. Differences in *Ceriodaphnia* response in the ambient and amended sample which are greater than this value can be assumed to indicate the presence of other unidentified chemicals and should be subjected to greater chemical and TIE analysis to attempt to identify the responsible chemicals.

Thirty-three samples were collected during and immediately after half inch or larger rainfall events in January and February of 1996 and 1997 and screened for toxicity with *Ceriodaphnia* at the U.C. Davis Aquatic Toxicology Laboratory. In 1996 toxicity was measured in four samples from the San Joaquin River at Vernalis. In 1997 toxicity was detected in two samples from Orestimba Creek and in four from Sacramento Slough. TIES were run on all samples. A combination of bioassay, TIE and chemical analysis confirmed that diazinon, within the resolution of the phase III TIE process, was the only contaminant in each sample. No evidence for any other toxic agents, including pyrethroids, was ever obtained. Pyrethroid induced toxicity was discounted as PBO consistently removed all toxicity.

AQUA-Science[2] has developed a proprietary diazinon antibody mediated selective TIE removal process. A toxic sample of water collected on 24 January 1997 from Sacramento Slough was split between the U.C. Davis Aquatic Toxicology Laboratory and AQUA-Science to ascertain whether both laboratories would identify the same toxic agent. TIE analysis at both facilities established that diazinon was the main contaminant and accounted for between four and five toxic units.

Finally, no attempt was made in this study to determine the ecological significance of the diazinon excursions. However, two facts are worth noting. First, the California Department of Fish and Game has a proposed acute diazinon hazard assessment criteria to protect freshwater aquatic life. The Department recommends that their 80 ng/l criteria only be exceeded for one hour once every three years in order not to unduly affect aquatic organisms. Sampling was only conducted after rainfall events. However, one quarter (2/8) and one half (4/8) of all samples collected at Orestimba Creek and at Sacramento Slough exceeded the acute criteria in 1997. These results demonstrate, like in previous years, that exceedances of the acute hazard criteria are common in the basin after storms.

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[2]AQUA-Science, 17 Arboretum Drive, Davis, CA 95616.

Second, Novartis, the Registrant for diazinon, has recently completed a probabilistic risk assessment for their chemical in the Central Valley. In this report Novartis ranked freshwater organisms according to their diazinon sensitivity. The highest average diazinon concentrations measured in this study were in the San Joaquin River at Vernalis on 1-2 February 1996 and in Sacramento Slough on 22-25 January 1997. Average two and four day concentrations were 7,105 and 1,111 ng/l, respectively. If one assumes that the distribution of diazinon species sensitivity is the same in the Central Valley as in the published literature, then the highest diazinon concentration in the San Joaquin River at Vernalis should have exceeded the LC_{50} value of about 50 percent of all arthropod species. Likewise, the highest concentration measured in Sacramento Slough should have exceeded acutely toxic conditions for about 30 percent of all arthropod taxa. Organisms at risk include a variety of daphnid, chironomid, amphipod, copepod, mysid and mayfly species. No fish should have been killed.

Fall run chinook salmon fry[3] are present in the San Joaquin River in January and February. Likewise, spring[4] and fall run salmon fry are present in Sacramento Slough in early spring. Principal food items for young salmon while in freshwater in the Central Valley are cladocerans, chironomids, copepods, and homopterans. As noted above, many of these species are sensitive to diazinon and may be impacted by the dormant spray pulses. Not yet known is the extent to which the in-stream invertebrate community is affected by the diazinon excursions nor whether salmon and other fish fry can switch to different prey when their primary food resource is reduced or eliminated. Follow-up research is needed to address these issues.

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[3]Considered a species of concern by U.S. Fish and Wildlife Service.

[4]Spring run salmon are listed as a State endangered species.

Introduction

California is the nation's leading producer of nut and tree fruit. Each winter about half a million pounds of insecticide active ingredient is applied in the Central Valley on stone fruit[1], apple, pear, and almond orchards for boring insect control. Diazinon accounts for about half the dormant spray market with chlorpyrifos, methidathion, malathion, esfenvalerate, and permethrin making up the remainder of the use. Orchards are the only major use of diazinon in the basin in January and February.

In February 1990 acute[2] *Ceriodaphnia dubia* toxicity was observed in storm runoff in the San Joaquin River using the U.S. EPA three species bioassay test (Foe and Connor, 1991). Follow-up studies conducted in 1992, 1993 and 1994 in the San Joaquin River and in 1993 and 1994 in the Sacramento River confirmed that diazinon was present in these and other storm samples at toxic concentrations to *Ceriodaphnia* and other sensitive invertebrates (Foe and Sheipline, 1993; Ross *et al.* 1996; Foe 1995; Holmes *et al.*, in prep; Kratzer, 1997;1998). In February 1993 pulses of diazinon in the Sacramento River at the City of Sacramento were traced as far seaward in the Estuary as the City of Martinez, 75 miles below the City of Sacramento. *Ceriodaphnia* toxicity was observed as far west in the Estuary as Chipps Island, 60 miles below the City of Sacramento. In the same study, elevated concentrations of diazinon and acute invertebrate toxicity were observed in the San Joaquin River at Vernalis for 12 days and traced as far downstream as the City of Stockton, 45 miles below Vernalis (Kuivila and Foe, 1995).

These findings are of regulatory significance as the Central Valley Regional Water Quality Control Board's Basin Plan contains a narrative toxicity objective stating the "all waters shall be maintained free of toxic substances in concentrations that produce detrimental physiological responses...in aquatic life". In 1985 the U.S. EPA recommended that the EPA three species bioassay procedure be considered one method of assessing compliance with State narrative toxicity objectives (54FR23868). In 1998 both the Sacramento and San Joaquin Rivers and the downstream Estuary were placed on the Clean Water Act's 303(d) list by the Central Valley Regional Water Quality Control Board as impaired because of invertebrate toxicity from elevated diazinon concentrations during the dormant spray season.

Not known was whether other contaminants besides diazinon might be present in dormant spray runoff and also contribute to the observed toxicity. The Central Valley is intensively farmed and roughly 4 million pounds of a variety of pesticides are used during the dormant spray season in the Sacramento Valley alone (as reported in Nordmark *et al.*, 1998). One or more of these insecticides could move off-site in storm runoff and contribute to the observed *Ceriodaphnia* toxicity. No Toxicity Identification Evaluations (TIEs) have been conducted on orchard storm

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[1]Apricot, cherries, nectarines, peaches, plums, and prunes.

[2]Statistically significant mortality within 96 hours.

runoff. TIEs are a recently developed procedure to identify the chemicals responsible for toxicity in bioassays (U.S. EPA 1991, 1993a,b). The procedures were primarily developed for use with sewage treatment plant and industrial effluent but are now being modified for use in determining the cause of toxicity in non-point source agricultural runoff (Bailey *et al.*, 1996; Deanovic *et al.*, 1996;1998). Initial non-point source interest focused on the chemical identification phase of the TIE procedure (Phase I and II). The work has included the development of TIE "finger prints" for pesticides commonly used in the Central Valley and also for all the dormant spray insecticides except esfenvalerate and permethrin (Bailey *et al.*, 1996;Crepeau *et al.* 1997). The latter two insecticides belong to a new class of compounds collectively identified as pyrethroid insecticides. Antidotal evidence suggests that agricultural pyrethroid use is increasing in the Central Valley including on orchards during winter as a dormant spray. Neither insecticide has been included in analytical pesticide scans by any Agency monitoring for dormant spray runoff. TIE procedures and analytical monitoring data are needed to evaluate the potential contribution of pyrethroid and possibly other chemicals to surface water toxicity in winter storm runoff.

Few Phase III TIEs have been conducted on agricultural runoff. The purpose of a Phase III TIE is to determine how much of the ambient bioassay toxicity is explained by the chemical(s) implicated in the Phase I and II process. However, the sensitivity of the Phase III pesticide TIE process has not yet been evaluated. In particular, it is not known how much residual unexplained toxicity must be present in a sample before concluding that other unidentified contaminants are also present and are contributing to the observed impairment.

The Bay Protection Toxic Cleanup Program was created by the California legislature in 1989 (SB 475 Torres and SB 41 Wright) and was reauthorized in 1993 (SB 1084 Calderon). Purpose of the legislation was to insure protection of coastal and estuarine resources by the identification of "toxic hot spots" and by development of control strategies to remediate the worst of these. The definition of a hot spot included pollutants that cause aquatic life impacts. The presence of repeated invertebrate bioassay mortality in dormant spray runoff in the Sacramento and San Joaquin Rivers and downstream in the Estuary was recognized as a potential candidate hot spot. However, the Bay Protection program also requires that the principal chemical(s) responsible for toxicity be conclusively identified through procedures like TIEs so that control strategies may be developed.

Objectives of this study were fourfold. First, develop TIE "finger prints" to facilitate the identification of toxicity from pyrethroid insecticides. Second, evaluate the sensitivity of the Phase III TIE process for organophosphate pesticides. Third, continue monitoring in the Sacramento and San Joaquin Basins to ascertain whether *Ceriodaphnia* toxicity would be present in orchard dormant spray runoff and, finally, use TIE procedures to identify and confirm the chemicals responsible for invertebrate toxicity.

Method and Materials

Sampling Sites and Sample Collection The purpose of monitoring was to collect representative dormant spray runoff samples for bioassay and toxicity identification evaluation (TIEs) analysis. As such, the sample collection was not designed to fully characterize the frequency and duration of dormant spray impairments. All sampling was conducted during and for several days after large winter storms in late January and early February of 1996 and 1997. Rainfall data for the Cities of Sacramento and Stockton was obtained from the Desert Research Institute[3]. Two monitoring sites were chosen in 1996: San Joaquin River at Vernalis and Sacramento River at Greene's Landing (Figure 1). Site locations are described in Table 1. The Sacramento and San Joaquin are the two largest Rivers discharging to the Estuary. Water collected from each site was assumed to be indicative of what each basin exported to the Estuary during storms. *Ceriodaphnia* toxicity was observed in 1996 at Vernalis but not at Greene's Landing. Therefore, in 1997 the sampling site on the San Joaquin River at Vernalis was retained while Greene's Landing on the Sacramento River was discontinued. Two additional upstream sites were selected, one at Orestimba Creek in the San Joaquin Basin and the second at Sacramento Slough in the Sacramento Basin (Figure 1). Both water courses drain watersheds with extensive acreage in orchards and toxicity has been observed at each location in the past. The upstream sites were selected as it was felt that water samples with potentially higher chemical concentrations and a greater amount of unknown toxicity might be obtained closer to agricultural sources. A larger amount of toxicity should facilitate identification of unknown contaminant(s).

Water for bioassay and for TIE analysis was collected in one gallon amber borosillicate glass bottles as subsurface grabs. Samples for pesticide analysis were collected in one liter glass bottles. All samples were immediately placed on ice for transport to the U.C. Davis Aquatic Toxicology Laboratory where they were stored at $<4.0^{0}$C.

Bioassays Water samples were screened at the U.C. Davis Aquatic Toxicology Laboratory for toxicity to *Ceriodaphnia* using the U.S. EPA bioassay procedure. The bioassay screen followed U.S. EPA (1991b) protocols with exceptions noted in Deanovic *et al.* (1998). Briefly, storm runoff samples were accumulated for up to eight days, then a seven day toxicity test was run on each daily sample. U.S. EPA (1991b) suggests that one time subsurface grabs of receiving water may be employed to assess compliance with state narrative toxicity objectives. For bioassay testing, one daphnid, 8 to 24 hours old, was randomly placed in each of ten 20 ml boroscillicate vials containing 15 mls of sample. *Ceriodaphnia* were from an in-house culture. Trout chow and *Selenastrum* were added as food each day. Every 24 hours each *Ceriodaphnia* was pipetted into a new vial containing fresh sample. When neonates were present, they were counted and discarded. Test duration was seven or eight days with reproduction (number of offspring/adult) and mortality being the endpoints of interest.

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[3]Desert Research Institute, University of Nevada System, P.O. Box 60220, Reno , Nevada, 89506

Laboratory control water was obtained from the U.C. Davis Ecology Institute well and was diluted with water from a glass distiller (Corning Mega-Pure System, Model MP-3A distillation unit) to a hardness of 78 mg/l as $CaCO_3$.

Ceriodaphnia toxicity was defined as a statistically significant difference ($P<0.05$) between a sample and the laboratory control. Bartlett's test for homogeneity of variance was run on all reproduction data. If the reproduction data were normally distributed, then organism performance was compared to the control using an analysis of variance and a Dunnett mean separation test. Generally, a 30 percent difference between a control and a sample was required to obtain a statistically significant difference. If variance was non-homogenous, then comparisons were made against the control using Kruskal-Wallis and Dunn non-parametric multiple comparison tests. Daphnid survival was compared to the control with a Fisher's Exact Test. Generally, a 40 percent difference between the sample and the control was required for statistical significance. Finally, U.S. EPA (1991b) recommends that *Ceriodaphnia* bioassay results only be considered acceptable for regulatory purposes if control survival is at least 90 percent in four and 80 percent in seven day tests. Furthermore, the U.S. EPA requires that control organisms produce at least 15 neonates/adult and that 6 of 10 adults have three broods for a test to be acceptable. In this study the bioassay was repeated when a statistically significant mortality rate was observed but the performance of the control organisms was unacceptable.

Toxicity Identification Evaluations All samples testing toxic in screening bioassays were evaluated with a TIE. TIEs are a sequential process combining chemical and bioassay manipulations to identify the responsible contaminants and quantify the amount of toxicity produced by each. The TIE process is in three phases. Procedures used in this study follow those of Bailey *et al.*, 1996;Crepeau *et al.*, 1997; U.S. EPA, 1991a. 1993a,b. Each phase is reviewed briefly below.

The purpose of a Phase I TIE is to determine the general class of chemical responsible for toxicity. Only insecticides have been identified as the cause of toxicity in previous invertebrate TIEs in the San Joaquin basin (Deanovic *et al.* 1996;1998). Therefore, a modified Phase I TIE specific for pesticides was employed first. If the results indicated that pesticides were not responsible, then a conventional U.S. EPA Phase I TIE (U.S. EPA, 1991a) could be performed. However, as in previous years, all TIEs continued to demonstrate that pesticides were the cause of toxicity. The modified Phase I TIE consisted of five bioassay-chemical treatments: reconfirmation of the original toxicity, determination of the number of toxic units in the ambient sample, addition of piperonyl butoxide (PBO), and both a C8 Solid Phase Extraction (SPE) cartridge rinsate and eluate treatment. All toxicity tests associated with the TIE results included four replicates of five animals each in 20 ml of water using procedures outlined in Deanovic *et al.* 1998. Each treatment is briefly described. First, all samples were retested to confirm toxicity. This eliminates any chance of false positives. Second, the number of toxic units in each sample was determined. If statistically significant partial mortality was observed during any day of the test, then the sample was assumed to contain one toxic unit. However, if one

hundred percent mortality was observed on day one, then a serial dilution was performed with laboratory control water. Toxic units were calculated by dividing 100 by the dilution of the original sample which produced a partial kill during any day of the test[4]. Next, PBO was added to both the sample and the control water. PBO inhibits a daphnid's Mixed Function Oxidase (MFO) system preventing the activation and toxicity of organophosphate insecticides[5]. The effect of PBO on pyrethroid induced *Ceriodaphnia* toxicity is not known. Next, the toxic sample was pumped through a C8 SPE cartridge. The water draining from the cartridge is called a "rinsate" treatment in this study. C8 SPE cartridges bind non-polar organics including both organophosphate and pyrethrin insecticides (Crepeau *et al.*, 1997). Therefore, if an organophosphate or a pyrethrin insecticide is the cause of the toxicity, then the column treatment should render the sample non-toxic. Finally, many pesticides can be quantitatively recovered from C8 SPE cartridges by the addition of methanol[6]. In this study when the methanol fraction was amended back to either rinsate or laboratory water then the resulting sample was called an "eluate" treatment. If an organophosphate insecticide is responsible for the toxicity then both the chemical and the associated toxicity should be recovered in the eluate. Bioassays were performed on the rinsate, eluate and both a C8 SPE cartridge blank and a methanol control. Control experiments by Crepeau *et al.* (1997) have determined that methanol is not sufficiently strong a solvent to remove pyrethrins from a C8 SPE cartridge. Therefore, pyrethrin induced toxicity can be expected to be eliminated in the rinsate treatment, but not recovered in the eluate.

The purpose of a Phase II TIE is to identify the precise chemical responsible for toxicity. Only one Phase II TIE was conducted in this study as the Phase I work coupled with chemical analysis always implicated a known dormant spray insecticide as the cause of toxicity. The Phase II TIE protocol for non-polar organics consisted of pumping a toxic sample through a C8 SPE cartridge and eluting the cartridge with increasing concentrations of methanol[7] to sequentially remove non-polar organics of decreasing polarity (U.S. EPA, 1993a). Bioassays were performed on each fraction and on a methanol laboratory control blank to determine whether any of the fractions were still toxic. All the common orchard dormant spray insecticides have been "finger-printed" for use in Phase II TIEs (Crepeau *et al.*, 1997; Bailey *et al.*, 1996). Methidathion elutes in the 70 percent fraction, malathion in the 70 and 75 percent fraction, diazinon in the 75 percent and to a lesser extent the 80 percent fraction while chloryprifos elutes in the 80 and 85 percent fractions.

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[4]This diverges from the toxic unit definition presented by the U.S. EPA (1991b). U.S. EPA defines an acute toxic unit as any concentration which produces a 50 percent kill in 96 hours.

[5]Diazinon, malathion, chlorpyrifos, and methidathion are all organophosphate insecticides and all are detoxified by PBO.

[6]This includes diazinon, chlorpyrifos, malathion and methidathion.

[7]The eight methanol:water fractions were 25:75, 50:50, 75:25, 80:20, 85:15, 90:10, 95:5, and 100:0.

As previously mentioned, pyrethroids are not eluted by methanol from a C8 SPE cartridge.

Phase III TIEs were conducted on each acutely toxic sample. The purpose of the Phase III TIE is to ascertain how much of the overall toxicity can be explained by the chemical(s) implicated in Phase I and II and by chemical analysis. Phase III TIEs consist of a serial dilution of both the ambient sample and of laboratory or rinsate water amended with the suspected toxicant(s) at the same concentration as in the ambient sample. *Ceriodaphnia* bioassays were performed on each and the number of toxic units compared.

AQUA-Science has developed a proprietary antibody mediated selective TIE removal process for diazinon. The procedure differs from the traditional U.S. EPA (1991b) method in that an antibody specific for diazinon is employed, instead of a C8 SPE cartridge, to remove the insecticide. Toxic samples are bioassayed and analyzed chemically before and after the addition of the antibody to determine the amount of toxicity accounted for by the diazinon removal.

In 1997 one toxic sample was evaluated in a TIE by both the U.C. Davis Aquatic Toxicology Laboratory and by AQUA-Science. Purpose of the paired TIE was to ascertain whether both laboratories would agree on the cause of toxicity.

Pesticide Analysis Pesticides were analyzed by both Enzyme Linked Immuno-Sorbent Assays (ELISA) and by a Gas Chromatograph/Mass Spectrometer (GC/MS). In 1996 only samples testing toxic in bioassays were analyzed for pesticides. In 1997 all samples were analyzed by both ELISA and GC/MS.

ELISA, a colorimetric method, uses chemical specific antibodies to detect and quantify chemical concentrations. ELISA kits exist for both diazinon and chlorpyrifos. ELISA analysis was conducted at the U.C Davis Aquatic Toxicology Laboratory within 10 days of water collection using procedures recommended by the manufacturer[8]. The ELISA detection limit for diazinon and chlorpyrifos is 30 and 50 ng/l, respectively.

GC/MS analysis was conducted at the U.S. Geological Survey's Laboratory in Sacramento, California using methods in Zaugg *et al.* (1995). Briefly, samples were filtered through a 0.7 µm filter, extracted through a 6 ml C8 SPE cartridge and submitted to the U.S. Geological Survey for analysis. Pesticides in the U.S. Geological Survey scan, their detection limit and percent recovery are summarized in Table 2 . The detection limit and percent recovery of diazinon was 38 ng/l and 74 percent, respectively. Both methidathion and chlorpyrifos were also in the scan. However, esfenvalerate, pyrethrin and malathion were not.

[8]Strategic Diagnostics, Inc., 128 Sandy Drive, Newark, DE. 19713-1147.

Results and Discussion

Results are presented below in five parts. First, the effect of the addition of PBO on esfenvalerate induced toxicity was evaluated to determine whether PBO could be used as a TIE "finger-print" for pyrethroid insecticides. Second, the results of tests to determine the relative sensitivity of *Ceriodaphnia* to diazinon are presented. Third, the resolution of the Phase III TIE process was evaluated for organophosphate insecticides. Fourth, the results of bioassay and TIE analyses of ambient water samples collected in 1996 and 1997 are presented. The TIE analyses include both the use of conventional U.S. EPA procedures (U.S. EPA 1991, 1993 a,b) and a new antibody mediated process. Finally, observed diazinon concentrations are compared to the reported toxicity of the chemical to other aquatic organisms to help assess the potential ecological risk posed by diazinon excursions.

Piperonyl Butoxide-Esfenvalerate Experiments. The purpose of the testing was twofold. First, was to ascertain the toxicity of esfenvalerate to *Ceriodaphnia* and determine how this response might change upon addition of PBO. Second, was to determine the organism's response to mixtures of esfenvalerate and an organophosphate insecticide in the presence and absence of PBO.

The 96 hr LC_{50} concentration of esfenvalerate to *Ceriodaphnia* was 211 ng/l (Table 3). Addition of 100 mg/l PBO enhanced esfenvalerate toxicity in each exposure tested. Comparison of the LC_{50} concentration of esfenvalerate in the absence and presence of PBO demonstrated that toxicity was potentiated 64 fold by PBO (211.4/3.3=64). The toxicity of another pyrethrin, permethrin, is also reported to be potentiated by PBO (Dr Jeff Miller, personal communication). Similar toxicological patterns have been observed in mammalian systems (reviewed in Andur and Doull, 1994). The reported explanation is that the MFO system is an effective detoxification mechanism for both natural and synethic pyrethroid insecticides. Addition of PBO inhibits the system resulting in smaller doses of esfenvalerate being necessary to cause toxicity.

The second experiment was to ascertain whether PBO would potentiate esfenvalerate toxicity in mixtures with an organophosphate insecticide. The results are important as both organophosphate and pyrethroid insecticides are applied as dormant sprays and both might be expected to be present together in runoff. Also, as previously noted, MFO systems metabolize non-toxic organophosphate insecticides to their more toxic oxon form while detoxifying pyrethrins. Not known was how *Ceriodaphnia* would respond to the simultaneous presence of both a metabolically activated organophosphate insecticide and deactivated pyrethroid insecticide.
The experimental protocol consisted of exposing *Ceriodaphnia* to mixtures of chlorpyrifos and esfenvalerate in the presence and absence of PBO (Table 4). The results showed, as expected, that in single exposures PBO ameliorated the toxicity of chlorpyrifos while potentiating that of esfenvalerate. The addition of PBO potentiated the toxicity of all mixtures which contained esfenvalerate.

These results demonstrate that PBO potentiates esfenvalerate toxicity in either single exposures or in mixtures with an organophosphate insecticide. The results are important as they suggest a unique "finger-print" to distinguish pyrethroid induced toxicity. There are three main classes of insecticides in use in California today: organophosphate, carbamate, and synthetic and natural pyrethroids. PBO has been shown to ameliorate organophosphate toxicity, have no effect on carbamate induced toxicity (Bailey *et al.*, 1996) and, now, potentiate pyrethrin toxicity. This unique pyrethrin-PBO "fingerprint" will be employed later to argue that pyrethrins were not responsible for any of the toxicity observed in dormant spray runoff as PBO always eliminated all ambient toxicity.

Sensitivity of *Ceriodaphnia* to Diazinon Diazinon has been reported to be the primary toxicant in dormant spray runoff (Foe and Sheipline, 1993; Foe, 1995). The toxicity of diazinon to *Ceriodaphnia* was ascertained by measuring the response of the organism in a seven-day serial dilution test (Table 5). The 96 hr LC_{50} concentration was 477 ng/l. This value is consistent with the reported toxicity of diazinon in other studies (Bailey *et al*, 1996; Fujimura, personal communication). Values range between 410-510.

In this study the number of toxic units in a sample was defined as 100 divided by the dilution causing a statistically significant, but partial, mortality rate during the seven-day test. Results in Table 5 demonstrate that 800 ng/l was equivalent to two *Ceriodaphnia* toxic units while concentrations between 400 and 750 ng/l were comparable to a single unit of toxicity. Obviously, the concentration of diazinon producing toxicity is variable and depends upon both animal sensitivity and the accuracy of the pesticide analytical method.

Precision of the Phase III TIE process. The U.C. Davis Aquatic Toxicology Laboratory maintains a diazinon ELISA control chart (Figure 2). The purpose of the chart is to ascertain the repeatability of the ELISA measurements. The chart was produced by analyzing a subsample of the same diazinon stock with each set of field samples. The running average or best estimate of the "true" value of the stock solution was about 300 ng/l or about half a *Ceriodaphnia* toxic unit.

The results of 59 ELISA control chart measurements[9] are summarized in Figure 2. The results demonstrate that the running average of the two standard deviation value was about 50 percent of the mean. This implies that for any single measurement, there was a 95 percent probability that the reported concentration was within +/- 50 percent of the running average. Unreported data from the U.C. Davis Aquatic Toxicology Laboratory demonstrate that the average "within-test" precision for a single ELISA run is about the same as the "between-test" results reported in the control chart.

In a Phase III TIE, a comparison is made between the toxicological response of *Ceriodaphnia* in an ambient sample and in laboratory water amended with the same amount of toxicant as the

[9]Conducted between June 1995 and July 1997.

ambient one. Differences in *Ceriodaphnia* response are used to determine how much of the toxicity in the ambient sample may be explained by the chemicals identified in the Phase I and II TIE. There are two main sources of variability in the Phase III TIE which may confound this analysis: differential variability in animal sensitivity and imprecision in pesticide measurement. Both sources of error are additive. Animal variability is minimized by randomly selecting neonates for all treatments from the same pool of animals and conducting the bioassays simultaneously. While no measurement of "within-test" animal variance is available for the U.C Davis Laboratory, it is thought to be quite small, certainly much less than the ELISA measurement. No similar technique is available for minimizing ELISA variance. Therefore, the ELISA kit is assumed to be the major source of error in the Phase III TIE. The precision of the ELISA measurement was estimated from laboratory control charts. Typically, at the start of a Phase III TIE both the ambient and amended samples are reanalyzed by ELISA to ensure that the two concentrations are as comparable as possible to each other. As noted above, each analytical value has a 95 percent probability of being within 50 percent of its "true" concentration. By extrapolation, the 99 percent confidence limits[10] around the difference between any two measurements is still about 100 percent of the mean or about a *Ceriodaphnia* toxic unit. Therefore, it is argued that the resolution of the Phase III TIE process is about one toxic unit. Differences in *Ceriodaphnia* response in the ambient and amended sample which are greater than this value can be assumed to indicate the presence of other unidentified chemical(s) and may warrant additional TIE and chemical analysis.

Comparison of ELISA and GC/MS Measurements Twenty-two samples were analyzed by both ELISA and GC/MS (Figure 3;R^2=0.99;Appendix A). ELISA and GC/MS measurements also compared favorably in a correlation analysis of a much larger data set (n=155, R^2 = 0.79) collected during the 1994 dormant spray season (Holmes *et al.*, in prep). Both data sets demonstrate that ELISA and GC/MS procedures produce similar diazinon analytical values.

Biossays Water samples were collected in January and February of 1996 and 1997 after rainstorms for invertebrate bioassays. Purpose of testing was threefold. First, ascertain whether *Ceriodaphnia* toxicity would be present in storm runoff as in previous years. Second, determine through TIE procedures how much of the toxicity was due to diazinon and whether other unidentified contaminant(s) might be present. Third, identify, if possible, any other toxic agents present. Results are presented for 1996 and 1997 below.

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[10]The size of the 99 percent confidence limit was estimated in a two step process. First, the frequency of a single event which co-occurs in two samples with a probability of 0.01 percent was estimated. This frequency was determined to be 10% (1 - (0.1 x 0.1) = 0.99). Next, a t-table was used to estimate the size of the confidence limits in toxic units (1.65/1.96 x 1.0 = 0.85) where 1.65 and 1.96 are the t values for probabilities of 0.1 and 0.05 percent, respectively. Finally, 0.85 was rounded up to 1.0 toxic unit.

1996 Bioassay Results Water year 1996 was classified in both the Sacramento and San Joaquin Basins as wet[11]. Sporadic rain occurred between 15 January and 5 February 1996 (Figures 4 and 5). The flow of the Sacramento River began to rise on 16 January and peaked on 5 February. The San Joaquin River was more constant at about 4,000 cfs. Water samples were taken for three days from the Sacramento River at Greene's Landing (27-29 January) and for ten days from the San Joaquin River at Vernalis (27 January-5 February).

The samples were split into two groups for *Ceriodaphnia* bioassay screening. No toxicity was observed in water collected from the Sacramento River at Greene's Landing (Table 6). In contrast, 100 percent mortality was observed in water samples collected on 28 January and on 1 February from the San Joaquin River at Vernalis. The mortality was statistically significant when compared against the controls. However, control mortality was 33 percent invalidating the results for bioassay acceptability. In the second screening, water samples collected on 2 and 3 February from the San Joaquin River at Vernalis had 100 percent mortality (Table 7). Laboratory controls met all criteria for test acceptability.

All toxic samples from the San Joaquin River were tested with and without PBO in both screening studies (Table 6 and 7). PBO removed all the toxicity from three of the four samples suggesting that mortality was caused by a metabolically organophosphate compound like diazinon. The PBO results rule out the possibility of a pyrethrin contributing to any of the *Ceriodaphnia* toxicity. PBO did not alter the toxicological response of the sample collected from the San Joaquin River at Vernalis on 1 February 1996.

Water samples collected from the San Joaquin River at Vernalis on 28 January and on 2 and 3 February produced no *Ceriodaphnia* mortality within the first 24 hours suggesting that they contained only one toxic unit of contamination (Tables 6 and 7). In contrast, complete *Ceriodaphnia* mortality occurred within 24 hours in the 1 February sample suggesting the possibility of multiple toxic units. Therefore, a dilution series was conducted by mixing this sample with laboratory control water. Complete *Ceriodaphnia* mortality was observed within 1 day at all dilutions down to 2.5 percent (Table 8). No impairment was noted at a dilution of 1.25 percent. The results indicate that the sample contained approximately 40 toxic units (100/2.5=40).

Diazinon analyses of the 1 February Vernalis sample was consistent with the number of toxic units estimated from the bioassay dilution series. Analysis by ELISA estimated that the sample contained 16,840 ng/l diazinon (Table 8). GC/MS analysis by the U.S. Geological Survey, the Department of Pesticide Regulation and APPL confirmed that the sample contained between 13,900 and 16,900 ng/l diazinon (Appendix A, Table 1). No other chemical was observed in the

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[11]Water year 1996 is defined as the time period between 1 October 1995 and 30 September 1996. Water year types are classified in California according to the natural water production of the major basins.

sample at a toxic concentration. The lower range of a *Ceriodaphnia* diazinon toxic unit is about 400 ng/l (Table 5). At a concentration of 400 ng/l, the Vernalis 1 February 1996 sample would be expected to exhibit between 35-42 toxic units, close to 40 toxic units measured. The PBO results were also consistent with the large number of diazinon toxic units. PBO at a concentration of 100 mg/l is reported to only eliminate about 5 toxic units of organophosphate toxicity (Bailey *et al.*,1996). Forty toxic units would be predicted to overwhelm the PBO amendment and result in no change in the time to death, as was observed (Table 6). In conclusion, all the evidence obtained suggested that diazinon was the primary toxicant in the sample collected at Vernalis on 1 February 1996. No further TIE work was conducted.

Diazinon was also detected in the 2 and 3 February samples from Vernalis (Table 9). Analyses of both samples revealed diazinon at 401 and 433 ng/l and 311 and 135 ng/l by ELISA and GC/MS, respectively. It is not known why the diazinon ELISA and GC/MS results were so different for the 3 February sample. Usually, there was greater agreement between both methods (Figure 3).

A Phase I and III TIE were conducted on both the 2 and 3 February 1996 Vernalis samples. First, both samples were retested and *Ceriodaphnia* toxicity reconfirmed (Table 9). Next, water from each sample was passed through a C8 SPE cartridge and the rinsate and eluate fractions evaluated. No toxicity was observed within 7 days in the rinsate while mortality was recovered in both eluate[12] samples. These results coupled with the chemical analysis were consistent with just diazinon induced toxicity so a phase III TIE was initiated. Diazinon was added back to both rinsates at a concentration as equivalent possible to the respective ambient samples and one unit of *Ceriodaphnia* toxicity measured in all four waters (Table 9). The results confirm that diazinon was the main contaminant in each sample.

The faster mortality rate in both ambient samples, as compared to their rinsate amendments, suggested the possibility of additional contaminant(s) although the Phase III TIE responses were within the one toxic unit resolution of the process. So, a phase II TIE was conducted on each sample. Each methanol/water fraction was added back at three times the ambient concentration to amplify the toxicity of any other chemicals which might be present (Table 10). Toxicity was recovered in the 75 percent methanol/water fraction in the 2 February Vernalis sample and in the 75 and 80 percent fractions in the 3 February one. No toxicity was observed in any other fraction within seven days. Diazinon is reported to eluate primarily in the 75 percent fraction and to a lesser extent in the 80 percent one (Bailey *et al.*, 1996;Crepeau *et al.*, 1997). Therefore, the results of both phase II TIEs are consistent with the conclusions of the earlier Phase I and II results and of the chemical analysis. All the data suggest that diazinon was the only contaminant present at toxic concentrations in either sample. Why the mortality rate of *Ceriodaphnia* in the Phase III ambient samples was greater than in rinsate water is not known, however, the difference is well within the one toxic unit resolution ability of the Phase III TIE process and so is ascribed to experimental error.

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[12]Eluate was added back to laboratory water at three times the original concentration.

In summary, three water samples from the Sacramento and ten from the San Joaquin River were collected in storm runoff and screened with bioassays for toxicity. Toxicity was detected in four samples from the San Joaquin River. TIEs were run on three of these. A combination of bioassay, TIE and chemical analysis confirmed that diazinon was the main contaminant in each sample. No evidence for any other chemical at a toxic concentration was obtained.

1997 Bioassay Results The primary objective of the 1997 work was to attempt, if possible, to collect storm runoff samples with other contaminants besides diazinon. Therefore, sampling was conducted on the San Joaquin River at Vernalis, but additional samples were collected upstream on Orestimba Creek. No sampling was conducted at Greene's Landing on the Sacramento River as no toxicity was seen there the previous year. Instead, sampling was moved upstream to Sacramento Slough at HWY 113. Both Orestimba Creek and Sacramento Slough drain orchard areas where elevated diazinon concentrations and *Ceriodaphnia* toxicity has been observed in previous years (Holmes *et al.* in prep; Foe, 1995). The strategy was to emphasize collection of samples closer to the source of contamination enhancing the possibility of collecting water with a greater contribution of toxicity from previously unidentified chemicals. Higher concentrations of unknown toxicity should help facilitate identification of such chemicals.

Water year 1997 was classified as wet in both the Sacramento and San Joaquin Basins. Both the Sacramento and San Joaquin Rivers and many of the smaller tributaries were at flood stage for most of the winter (Figures 4 and 5). The Sacramento River was allowed to discharge into the Sutter Bypass. This produced a mix of Sacramento and Butte Creek water in Sacramento Slough. Most of our sampling was conducted on the eastside of the slough. However, on two occasions water was collected from the westside off the HWY 113 bridge for chemical analysis. This sampling was done to ascertain whether the Sutter Bypass was well-mixed. The westside may contain a larger quantity of Sacramento River water while the eastside should be dominated more by agricultural inputs from the Chico and Yuba City areas.

Twenty samples were collected and screened for toxicity with *Ceriodaphnia*. Four were from the San Joaquin River at Vernalis (20-23 January), eight from Orestimba Creek (20-26 January) and eight from Sacramento Slough (20-26 January). Samples were screened in two trials. In the first, toxicity was detected on 23 January from both Orestimba Creek and from Sacramento Slough (Table 11). One hundred percent mortality occurred in both samples on test day one. In the second trial, statistically significant mortality, as compared to the control, was observed in water samples collected on 25 January from Orestimba Creek and on 24, 25, and 26 January from Sacramento Slough (Table 12). Complete mortality occurred in all Sacramento Slough samples within 24 hours. However, only 70 percent of the controls had a third brood invalidating the results for bioassay test acceptability. Finally, each toxic sample was tested with and without PBO. PBO removed all toxicity from the 25 January Orestimba Creek sample and from the 25 and 26 January Sacramento Slough samples but only delayed the mortality rate in the 24 January sample from one to eight days (Tables 11 and 12). The PBO results implicated a metabolically activated compound like diazinon and eliminated pyrethrins as responsible for any of the mortality.

Diazinon was measured and concentrations between 340 and 1944 ng/l were detected in all toxic samples by both ELISA and GC/MS (Appendix A, Table 2). Diazinon was also measured in all non-toxic samples. Diazinon was never observed at a toxic concentration in any sample testing non-toxic in a bioassay. Measured diazinon concentrations in toxic samples represented between one and four toxic units implying that the insecticide was the dominant contaminant. A Phase I TIE was not conducted. Instead, two Phase III TIEs were done. The strategy was to determine the diazinon range representing a toxic unit in the ambient samples and then amending laboratory water with a similar amount of chemical to ascertain whether it would also produce a comparable amount of *Ceriodaphnia* toxicity. Samples with more than one unexplained toxic unit could then be selected for further evaluation.

In the first Phase III TIE, samples collected from Sacramento Slough and from Orestimba Creek on 23 January were serially diluted and determined to contain two toxic units while the sample collected on 24 January from Sacramento Slough had 4 units (Table 13). Each sample was diluted to one toxic unit and the diazinon concentration measured by ELISA. The concentrations ranged between 350-555 ng/l. Similarly, diazinon was amended to laboratory water and one toxic unit of insecticide was found to range between 440-660 ng/l. The concentration of diazinon producing a unit of toxicity in laboratory and field water appear consistent and suggest that the insecticide was the major toxicant. Insufficient residual toxicity appeared in any of the samples to warrant further follow-up. Chemical analysis detected methidation, another potential toxicant in two of the samples. Methidathion was measured in the 23 and 24 January Sacramento Slough samples at 438 and 578 ng/l or at about a quarter of a toxic unit[13] (Appendix A, Table 2). Methidation and diazinon are both organophosphate pesticides and their toxicity is additive to aquatic invertebrates (Huang *et al.*, 1994). However, the quarter of a toxic unit represented by the addition of methidation is not readily discernable within the one toxic unit resolution ability of the present Phase III TIE procedure in this laboratory. Independent chemical analysis is needed to determine its presence.

In the second Phase III TIE, water was collected on 25 and 26 January from Sacramento Slough and on 25 January from Orestimba and was serially diluted to determine the number of toxic units present (Table 14). Both Sacramento Slough samples were found to contain two toxic units while Orestimba Creek had one unit of toxicity. Diazinon concentration in the one toxic unit serial dilution of all field samples was between 482-524 ng/l. Similarly, one toxic unit of diazinon in laboratory water amended with the insecticide ranged between 491-768 ng/l. Again, the field and laboratory results appear consistent and implicate diazinon as the dominant contaminant. Insufficient unexplained toxicity appears to be present in any of the samples to warrant further follow up. Also, no pesticides, other than diazinon, were measured at toxic concentrations in any of the chemical analyses (Appendix A, table 2).

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[13]The 96 hr LC_{50} methidathion concentration for *Ceriodaphnia* has been reported at 1,980 ng/l (Issac and Phillips, 1994).

On two occasions water was collected from both the east and westside of the Sacramento Slough for pesticide analysis. The paired sampling was done to attempt to determine whether the slough was well mixed during flood conditions and whether the pesticide results which were obtained for the eastside applied to the whole water body. Eastside diazinon concentrations on 21 and 25 January were 36 and 1,286 ng/l (Table 2 Appendix A) while westside concentrations were <30 and 53 ng/l. The large difference for 25 January suggests that the Slough was not well mixed during flood flows and that the elevated pesticide concentrations observed on the eastside were probably restricted to that half of the waterway.

In summary, four samples from the San Joaquin River, eight from Orestimba Creek and eight from Sacramento Slough were collected in storm runoff and screened with *Ceriodaphnia* bioassays for toxicity. Toxicity was detected in two samples from Orestimba Creek and four from the eastside of Sacramento Slough. A combination of bioassay, TIE, and chemical analyses demonstrated that diazinon was the main contaminant. As in 1996, no evidence for other chemicals, including pyrethrins, was obtained. Pyrethrin toxicity was discounted as PBO consistently removed all mortality effects.

Antibody Mediated TIE Process AQUA-Science has developed a proprietary diazinon antibody mediated selective TIE removal process. A subsample of water collected on 24 January from Sacramento Slough was submitted to AQUA-Science for evaluation (Appendix B). The analysis concluded that diazinon was the principal contaminant and accounted for about five toxic units.

The U.C Davis Aquatic Toxicology Laboratory also conducted a TIE on the same sample and concluded that diazinon was the main contaminant (Table 13). U.C. Davis estimated that the sample contained about four units of diazinon toxicity (Table 13). The discrepancy in the number of toxic units between the two laboratories probably results from interlaboratory differences in animal sensitivity.

Department of Pesticide Regulation Dormant Spray Monitoring Program The Department of Pesticide Regulation (DPR) also conducted a dormant spray monitoring program in 1997 (Nordmark *et al.*, 1998;Bennett *et al.*, 1998). The program consisted of the collection of grab samples for bioassay and chemical analysis. Three of the waterways were the same as this study: lower San Joaquin River, Orestimba Creek, and Sacramento Slough. Water samples for DPR were collected three times per week between the first of December and March on a fixed schedule. Pesticide analysis was performed by the California Department of Food and Agriculture's Laboratory in Sacramento California while bioassays were done by the California Department of Fish and Game (DFG) at Elk Grove, California.

Comparison of paired bioassay and chemical data suggest reasonable agreement between the two programs. No direct comparison of field bioassay results was attempted as the two programs used slightly different procedures. However, on three occasions water was collected by DPR and split between UC Davis and DFG (Nordmark *et al.*, 1988). Both laboratories used the same

procedure for the bioassay analysis. One acute and two chronic tests were conducted. All three water samples tested non-toxic at both facilities.

Six grab samples were collected by both programs on the same date for pesticide analysis (Table 15). All results appear comparable except for the diazinon concentration measured at Sacramento Slough on 24 January 1997. The difference is attributed to the fact that DPR sampled on the westside off Kirkville Road while UC Davis collected water from the eastside. The difference in concentration imply, as was concluded earlier, that Sacramento Slough was not thoroughly mixed during flood conditions. The westside was likely dominated by Sacramento River water while the eastside was more influenced by runoff from local orchards in the Yuba City area.

Overall, conclusions of the two programs appear markedly different though, in spite of the similarity of the paired analytical results. DPR only collected one sample (1/45) which tested toxic to *Ceriodaphnia*. Similarly, only 2.5 percent of their samples (2/80) exceeded DFG proposed acute Hazard Assessment Criteria of 80 ng/l (Menconi and Cox, 1994). In contrast, the Regional Board measured toxicity in 33 percent (6/18) of storm samples, with the acute Hazard Assessment Criteria being exceeded in 60 percent (10/16) of these samples. The differences mainly result from the fact that DPR maintained a fixed sampling schedule which mostly consisted of monitoring non-storm runoff periods while the Regional Board only collected water during and immediately after half inch or larger rainfall events. Off site movement of orchard dormant sprays is well documented to be a rain induced runoff phenomena (Foe and Sheipline, 1993; Kuivila and Foe, 1995; Kratzer 1997,1998) and can probably only be evaluated accurately by intensive sampling during storm runoff periods.

Ecological Significance No attempt was made in this study to determine the ecological significance of diazinon excursions on the entire aquatic community. However, two facts are worth noting. First, as mentioned previously, DFG has a proposed acute hazard assessment criteria for diazinon to protect freshwater aquatic life (Menconi and Cox, 1994). DFG recommends that their 80 ng/l criteria only be exceeded for one hour once every three years in order not to unduly affect aquatic life. Sampling was only conducted in this study after rainfall events. However, one quarter (2/8) and one half (4/8) of all samples collected at Orestimba Creek and at Sacramento Slough exceeded the acute criteria in 1997. These results demonstrate, like in previous years, that exceedance of the acute hazard criteria is common in the basin after storms.

Second, Novartis, the Registrant for diazinon, has recently completed a probabilistic risk assessment for their chemical in the Central Valley (Novatis, 1997). In their report Novartis

ranked freshwater organisms according to their diazinon sensitivity[14]. The highest average diazinon concentrations measured in this study were in the San Joaquin River at Vernalis on 1-2 February 1996 and in Sacramento Slough on 22-25 January 1997 (Table 16). Average two and four day concentrations were 7,105 and 1,111 ng/l, respectively. If one assumes that diazinon species sensitivity is the same for the aquatic community in the Central Valley as for all organisms tested in the published literature, then the highest diazinon concentrations in the San Joaquin River at Vernalis should have exceeded the LC_{50} value of about 50 percent of all arthropod species (Table 15). Likewise, the highest concentration measured in Sacramento Slough should have exceeded acutely toxic conditions for about 30 percent of all arthropods. Taxa at risk include a variety of daphnid, chironomid, amphipod, copepod, mysid and mayfly species. No fish should have been killed.

Fall run chinook salmon[15] fry are present in the San Joaquin River between January and June (Reynolds *et al.*, 1993). Likewise, both spring[16] and fall run salmon fry are present in Sacramento Slough between December and June (Reynolds *et al.*, 1993). Principal food items for young salmon in freshwater in the Central Valley are cladocerans, chironomids, copepods, and homopterans (Kjelson *et al.*, 1981). As noted previously, many of these invertebrate species are sensitive to diazinon and may be impacted by the pesticide pulses. Not yet known is the extent to which the in-stream invertebrates community is affected by the diazinon pulses nor whether salmon and other fish fry can switch to different prey when their primary food resource is reduced or eliminated. Follow-up research is needed to address these issues.

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[14]Sensitivity is defined in terms of 48 and 96 hr LC_{50} concentrations reported in the peer reviewed literature. An LC_{50} concentration is the amount of chemical required to kill half the test organisms in laboratory water during the exposure period.

[15]Considered a "species of concern" by the U.S. Fish and Wildlife Service.

[16]Spring run salmon are listed as a state endangered species.

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Table 1. Description of site locations used in the 1996 and 1997 Bay Protection dormant spray runoff project.

Site	Location
Vernalis	San Joaquin River sampled from Airport Way Bridge (County Rd J3).
Orestimba Creek	Sample collected from River Road Bridge at high flow and from bank under bridge at lower flows.
Greene's Landing	Sacramento River sampled from end of U.S. Bureau of Reclamation water quality pier off Randall Island Road. Site is about 5 miles downstream of the Freeport gauging station.
Sacramento Slough	Slough was sampled from north side of HWY 113 bridge. Water on this side is predominately from Butte Creek and from orchards in the Yuba City area. On two occasions water was collected from the south side of HWY 113. Water here is mostly Sacramento River flood water diverted through the Sutter Bypass.

Table 2. Mean percent recovery of pesticides amended at 100 ng/l into organic free Sacramento River water at the U.S. Geological Survey Laboratory at Sacramento, California (from Crepeau *et al.*, 1994)

Compound	observed concentration (ug/l)	mean recovery percent	estimated MDL (ug/l)
Carbofuran	0.082	82	0.044
Diazinon	0.074	74	0.038
Methidation	0.075	75	0.031
Molinate	0.089	89	0.11
Simazine	0.074	74	0.06
Metochlor			
Chlorpyrifos			
Dacthal			
Napropamide			

ug/l, microgram per liter
MDL, method detection limit

Table 3. Toxicological response of *Ceriodaphnia* to esfenvalerate in the presence and absence of peperonyl butoxide (PBO).

Treatment	% Mortality by Day							Conclusions
	1	2	3	4	5	6	7	
Lab water	0	0	0	5	5	5	5	Controls O.K.
Lab water + PBO	0	0	0	0	0	0	0	
640 ng/L Esfenvalerate	15	90	100	100	100	100	100	96hrLC_{50}=211.4 ng/L
320 ng/L Esfenvalerate	0	50	95	95	100	100	100	
160 ng/L Esfenvalerate[1/]	0	0	5	15	25	25	25	
80 ng/L Esfenvalerate	0	0	0	0	0	0	0	
40 ng/L Esfenvalerate	0	0	0	0	0	0	0	
40 ng/L Esfenvalerate + PBO	25	100	100	100	100	100	100	96 hr LC_{50}=3.3 ng/L Potentiation: 211.4/3.3=64
20 ng/L Esfenvalerate + PBO	20	100	100	100	100	100	100	
10 ng/L Esfenvalerate + PBO	0	71	100	100	100	100	100	
5 ng/L Esfenvalerate + PBO	0	0	90	90	95	95	95	
2.5 ng/L Esfenvalerate +PBO	0	0	15	15	25	40	40	
1.25 ng/L Esfenvalerate + PBO	0	0	0	0	5	5	5	

[1/]130 ng/l esfenvalerate by GC/MS analysis. LC_{50} concentration was based on the nominal concentration.

Table 4. Response of *Ceriodaphnia* to mixtures of esfenvalerate and chlorpyrifos in the presence and absence of peperonly butoxide (PBO). A toxic unit of chlorpyrifos and esfenvalerate was assumed to be 70 and 280 ng/l, respectively.

Treatment	% Mortality by Day							Conclusions
	1	2	3	4	5	6	7	
Lab water	0	0	5	5	5	5	5	Controls OK
Lab water + 100 ppb PBO	0	0	5	5	20	20	20	
1 TU Chlor (70 ng/L)	0	20	80	95	95	100	100	PBO Ameliorates Organophosphate Toxicity
1 TU Chlor + PBO	0	0	0	0	0	0	0	
0.5 TU Chlor (35 ng/L)	0	0	0	0	0	0	0	
0.5 TU Chlor + PBO	0	0	0	0	0	0	0	
1 TU Esfen (280 ng/L)	0	20	45	85	100	100	100	PBO Potentiates Esfenvalerate Toxicity
1.0 TU Esfen + PBO	100	100	100	100	100	100	100	
0.5 TU Esfen (140 ng/L)	0	0	0	20	30	40	55	
0.5 TU Esfen + PBO	100	100	100	100	100	100	100	
1.0 TU Chlor+0.5 TU Esfen	0	60	100	100	100	100	100	PBO Potentiates Toxicity In Mixtures Of Esfenvalerate And Chlorpyrifos
1.0 TU Chlor+0.5 TU Esfen+PBO	80	100	100	100	100	100	100	
0.5 TU Chlor+0.5 TU Esfen	10	10	20	40	60	75	75	
0.5 TU Chlor+0.5 TU Esfen+PBO	90	100	100	100	100	100	100	
0.5 TU Chlor+1 TU Esfen	5	25	70	90	95	100	100	
0.5 TU Chlor+1 TU Esfen+PBO	95	100	100	100	100	100	100	

Table 5. *Ceriodaphnia* mortality by day in laboratory water amended with diazinon. Diazinon concentrations of 800 ng/l are said to contain two toxic units while concentrations between 400-750 ng/l contained a single unit of toxicity.

Treatment Diazinon (ng/L)	Percent Mortality by Day							Comments
	1	2	3	4	5	6	7	
Lab control	0	0	0	0	0	0	0	Controls O.K.
300	0	0	0	0	0	0	0	
350	0	0	0	0	0	0	5	
400	0	0	0	0	0	68	74	
450[1/]	0	5	5	35	90	100	100	96 hr LC_{50} = 460
500	0	20	50	80	100	100	100	
550	0	50	100	100	100	100	100	
600	0	75	100	100	100	100	100	
650	20	100	100	100	100	100	100	
700	45	100	100	100	100	100	100	
750	80	100	100	100	100	100	100	
800	100	100	100	100	100	100	100	

1/ ELISA measurement suggested actual concentration was 477 ng/L diazinon.

Table 6. Summary of *Ceriodaphnia* bioassay screening results for water samples collected from the San Joaquin River at Vernalis between 27 January and 1 February and from the Sacramento River at Greene's Landing between 27 and 29 January 1996. Toxicity was detected in San Joaquin River samples collected on 28 January and 1 February 1996.

Treatment	Reproduction (neonates/adult)		Mortality[1,3] (%)	Comments
	X	SE		
lab control	40.1	2.5	33.3[2]	Controls unaccepable
lab control + 100 ppb PBO	35.5	2.0	0	
Vernalis 1/27	45.9	1.5	0	
Vernalis 1/27 + 100 ppb PBO	49.6	1.2	0	
Vernalis 1/28	4/		100(8)	toxic sample
Vernalis 1/28 + 100 ppb PBO	43.9	1.4	0	toxicity removed
Vernalis 1/29	44.3	1.3	0	
Vernalis 1/29 + 100 ppb PBO	48.6	1.4	0	
Vernalis 1/30	42.4	3.9	40	
Vernalis 1/30 + 100 ppb PBO	49.1	1.6	0	
Vernalis 1/31	45.4	1.9	33.3	
Vernalis 1/31 + 100 ppb PBO	51.0	1.7	0	
Vernalis 2/1	4/		100(1)	toxic sample
Vernalis 2/1 + 100 ppb PBO	4/		100(1)	toxicity not removed
Greene's Landing 1/27	46.0	2.1	0	
Greens's Landing 1/28	40.7	1.7	0	
Greene's Landing 1/29	40.9	2.1	11.1	

1/Highlighted area indicates a significant increase in mortality relative to the laboratory control ($P<0.05$).
2/The test did not meet all EPA criteria for test acceptability as control mortality was greater than 20% .
3/Number in parenthesis indicates days to 100% mortality.
4/Reproduction was not measured because of high mortality.

Table 7. Summary of *Ceriodaphnia* bioassay screening results for water samples collected from the San Joaquin River at Vernalis on 2-5 February 1996. Toxicity was detected in water samples collected on 2 and 3 February 1996.

Treatment	Reproduction (neonates/adult)		Mortality[1,3] (%)	Comments
	X	SE		
lab control [2/]	26.6	1.3	0	controls O.K.
lab control + 100 ppb PBO	23.8	1.1	0	
Vernalis 2/2[1]			100(5)	toxic sample
Vernalis 2/2 + 100 ppb PBO	26.7	2.6	10	toxicity removed
Vernalis 2/3			100(4)	toxic sample
Vernalis 2/3 + 100 ppb PBO	33.4	1.5	0	toxicity removed
Vernalis 2/4	28.9	0.6	0	
Vernalis 2/4 + 100 ppb PBO	31.0	1.2	0	
Vernalis 2/5	27.7	0.8	0	
Vernalis 2/5 + 100 ppb PBO	29.7	0.9	0	

1/Highlighted area indicates a significant increase in mortality relative to the laboratory control ($P<0.05$).
2/Lab control met all EPA criteria for test acceptability.
3/Number in parenthesis indicates days to 100% mortality.

Table 8. Seven day *Ceriodaphnia* dilution series of a water sample collected from the San Joaquin River at Vernalis on 1 February 1996. The dilution series demonstrated that the sample contained 40 toxic units of contamination. The diazinon concentration in the undiluted sample ranged between 13,900 and 16,840 ng/l by GC/MS and ELISA, respectively (Appendix A).

Treatment[1]	Percent Mortality by day[2]							Diazinon (ng/L)		Comments
	1	2	3	4	5	6	7	ELISA	GC/MS	
Lab control	0	0	0	0	0	0.0	0.0			Controls O.K.
10% Vernalis 2/1	100	100	100	100	100	100	100	1,684[3/]	1390	
5% Vernalis 2/1	100	100	100	100	100	100	100			
2.5% Vernalis 2/1	0	90	100	100	100	100	100			40 toxic units
1.25% Vernalis 2/1	0	0	5	5	5	5	5			

1/ Ambient sample diluted with laboratory control water.
2/ Shaded areas indicate a statiscially significant mortality rate ($P<0.05$)
3/ Undiluted sample contained ten times this amount or 16,840 ng/l.

Table 9. Phase I and III toxicity identification evaluations for samples collected at Vernalis on 2 and 3 February 1996. Testing demonstrated that diazinon was the principal contaminant responsible for *Ceriodaphnia* mortality in both samples.

Treatment	Percent Mortality by day[1]							Diazinon (ng/L)		Comments
	1	2	3	4	5	6	7	ELISA	GC/MS	
Lab control	0	0	0	0	0	0	0			Controls OK
Lab control + 0.5% MEOH	0	0	0	0	0	0	0			
Lab control C8 blank	0	0	0	0	0	0	0			
Vernalis 2/2	0	100	100	100	100	100	100			toxicity reconfirmed
Vernalis 2/2 Rinsate	0	0	0	0	0	0	0			toxicity removed
Vernalis 2/2 Eluate (3X)[1]	100	100	100	100	100	100	100	1028		toxicity recovered
Vernalis 2/3	0	100	100	100	100	100	100			toxicity reconfirmed
Vernalis 2/3 Rinsate	0	0	0	0	0	0	0			toxicity removed
Vernalis 2/3 Eluate (3X)[1]	100	100	100	100	100	100	100	1061		toxicity recovered
Vernalis 2/2	0	100	100	100	100	100	100	401	311	1 toxic unit
Vernalis 2/2 Rinsate + Diazinon @ 0.92X[2]	0	0	0	0	25	80	95	369		1 toxic unit
Vernalis 2/2 Rinsate + Diazinon @ 0.85X	0	0	0	0	0	5	74	342		1 toxic unit
Vernalis 2/2 Rinsate + Diazinon @ 0.68X	0	0	0	0	0	0	0	276		
Vernalis 2/3	0	100	100	100	100	100	100	433	135	1 toxic unit
Vernalis 2/3 Rinsate + Diazinon @ 1.08X[2]	0	0	5	5	45	100	100	466		1 toxic unit
Vernalis 2/3 Rinsate + Diazinon @ 0.90X	0	0	10	10	10	30	40	385		1 toxic unit
Vernalis 2/3 Rinsate + Diazinon @ 0.69X	0	0	0	0	0	0	0	298		

1/ Eluate added back at three times the orignial concentration. 2/ Concentration in ambient sample

Table 10. Phase II TIE of water collected at Vernalis on 2 and 3 February 1996. Mortality was recovered in the 75 and 80 percent fractions consistent with diazinon induced toxicity.

Treatment	Percent Mortality by day[1,2]							Comments
	1	2	3	4	5	6	7	
Lab control	0	0	0	0	0	0	0	Controls OK
Lab control+MEOH	0	0	0	0	0	0	0	
Vernalis 2/2	0	0	100	100	100	100	100	Toxicity confirmed
50% fraction	0	0	0	0	0	0	0	
70% fraction	0	0	0	0	0	0	0	
75% fraction	0	93	100	100	100	100	100	Toxicity detected
80% fraction	0	0	0	0	0	0	0	
85% fraction	7	7	7	7	8	8	8	
90% fraction	0	0	0	0	0	0	0	
95% fraction	0	0	0	0	7	7	7	
100% fraction	0	0	0	0	0	0	0	
Vernalis 2/3	0	53	100	0	0	0	100	Toxicity confirmed
50% fraction	0	0	0	0	0	0	0	
70% fraction	0	7	0	0	0	0	7	
75% fraction	0	86	100	100	100	100	100	Toxicity detected
80% fraction	0	0	13	13	47	73	87	Toxicity detected
85% fraction	0	0	0	0	0	0	0	
90% fraction	0	0	0	0	0	0	0	
95% fraction	0	0	0	0	0	0	0	
100% fraction	0	0	0	0	0	0	0	

2/ 1800 mls of sample were run through a C8 SPE column at 10 ml/min. The column was eluted with 3 mls of MEOH: water fractions and then added back to laboratory water at 3 times the ambient concentration.

Table 11. Summary of *Ceriodaphnia* bioassay screening results for water samples collected on 20-23 January 1997 from the San Joaquin River at Vernalis, from Orestimba Creek at River Road, and from Sacramento Slough at HWY 113. Toxicity was detected in samples collected on 23 January from both Orestimba Creek and from Sacramento Slough.

Treatment	Reproduction (neonates/adult)		Mortality[1,4/] (%)	Comments
	X	SE		
lab control[2/]	22.9	1.2	0	Controls O.K.
Vernalis 1/20	25.7	1.8	10.0	
Vernalis 1/21	27.0	1.1	0	
Vernalis 1/22	29.2	0.6	0	
Vernalis 1/23	25.1	1.1	0	
Orestimba 1/20	13.8	0.9	0	
Orestimba 1/21	19.0	0.8	0	
Orestimba 1/22	19.1	2.2	10.0	
Orestimba 1/23	3/		100(1)	Toxicity detected
Sacramento Slough 1/20	13.6	0.8	0	
Sacramento Slough 1/21	13.6	0.8	0	
Sacramento Slough 1/22	13.9	0.8	0	
Sacramento Slough 1/23	3/		100(1)	Toxicity detected

1/Highlighted area indicates significant mortality relative to laboratory control water ($P<0.05$).
2/The laboratory control met all EPA criteria for test acceptability.
3/Reproduction was not analyzed because of significant mortality.
4/Number in parenthesis represents days to 100% mortality.

Table 12. Summary of *Ceriodaphnia* bioassay screening results for water samples collected from Orestimba Creek at River Road and from Sacramento Slough at HWY 113 on 24-26 January 1997. Toxicity was detected at Orestimba Creek on 25 January and at Sacramento Slough on 26, 27, and 28 January 1997.

Treatment	Reproduction (neonates/adult)		Mortality[1,4/] (%)	Comments
	X	SE		
lab control[2/]	10.1	1.8	20.0	Control unacceptable
lab control + 100 ppb PBO	20.4	1.0	0	
Orestimba 1/24	10.0	1.4	10.0	
Orestimba 1/24 + 100 ppb PBO	10.0	1.5	10.0	
Orestimba 1/25	3/		100(3)	Toxicity detected
Orestimba 1/25 + 100 ppb PBO	13.6	0.8	0	Toxicity removed
Orestimba 1/26	9.6	1.2	10.0	
Orestimba 1/26 + 100 ppb PBO	13.3	0.7	0	
Sacramento Sl. 1/24	3/		100(1)	Toxicity detected
Sacto Sl. 1/24 + 100 ppb PBO	14.0	2.6	100(8)	Toxicity reduced
Sacramento Sl. 1/25	3/		100(1)	Toxicity detected
Sacto Sl. 1/25 + 100 ppb PBO	22.3	1.0	0	Toxicity removed
Sacramento Sl. 1/26	3/		100(1)	Toxicity detected
Sacto Sl. 1/26 + 100 ppb PBO			10.0	Toxicity removed

1/Highlighted area indicates a significant increase in mortality relative to the laboratory control water ($P<0.05$).
2/The laboratory did not meet all EPA criteria for test acceptability. Only 70% of daphnids had a third brood..
3/Reproduction was not analyzed because of significant mortality.
4/Number in parenthesis represents days to 100% mortality.

Table 13. Seven day *Ceriodaphnia* dilution series in water collected at Orestimba Creek on 23 January 1997 and at Sacramento Slough on 23 and 24 January to establish the number of toxic units and to compare with mortality rates of laboratory water amended with similar amounts of diazinon. Diazinon was determined to be the primary toxicant in each of the three samples.

Treatment	Percent Mortality by day							diazinon (ng/L)		Comments
	1	2	3	4	5	6	7	ELISA	GC/MS	
Lab control	0	0	0	0	0	0	0			Controls O.K.
Orestimba 1/23@100%	100	100	100	100	100	100	100	706	720	Toxicity Reconfirmed
Orestimba 1/23@50%	0	0	100	100	100	100	100	350		2 Toxic Units
Orestimba 1/23@25%	0	0	10	10	10	10	10			
Sacramento Sl 1/23@100%	100	100	100	100	100	100	100	1111	1250	Toxicity Reconfirmed
Sacramento Sl 1/23@50%	0	100	100	100	100	100	100	555		2 Toxic Units
Sacramento Sl 1/23@25%	0	0	0	0	0	0	0			
Sacramento Sl 1/24@100%	100	100	100	100	100	100	100	1944	1696	Toxicity Reconfirmed
Sacramento Sl 1/24@50%	100	100	100	100	100	100	100	972		4 Toxic Units
Sacramento Sl 1/24@25%	0	100	100	100	100	100	100	486		
Lab water+900 ng/L diaz	100	100	100	100	100	100	100	880		2 Toxic Units
Lab water+675 ng/L diaz	0	100	100	100	100	100	100	660		1 toxic Unit
Lab water+450 ng/L diaz	0	0	0	0	20	100	100	440		1 Toxic Unit

ble 14. Four day *Ceriodaphnia* dilution series of water collected at Sacramento Slough on 25 and 26 January 1997 and at Orestimba Creek on 25 January to establish the number of toxic units and to compare with mortality rates of laboratory water amended with similar amounts of diazinon.

Treatment	Percent Mortality by day				Diazinon (ng/L)		Comments
	1	2	3	4	ELISA	GC/MS	
Lab control	0	0	0	0			Control OK
Sacramento Sl 1/25 @100%	100	100	100	100	1048	1282	Toxicity reconfirmed--2 toxic unit
Sacramento Sl 1/25 @50%	0	100	100	100	524		
Sacramento Sl 1/25 @25%	0	0	0	0			
Sacramento Sl 1/26 @100%	100	100	100	100	975	1029	Toxicity reconfirmed--2 toxic unit
Sacramento Sl 1/26 @50%	40	100	100	100	482		
Sacramento Sl 1/26 @25%	0	0	0	0			
Orestimba Ck 1/25 @100%	0	100	100	100	516	340	Toxicity reconfirmed--1 toxic unit
Orestimba Ck 1/25 @ 50%	0	0	0	0			
Lab water + 800 ng/L Diaz	75	100	100	100	768		1 Toxic Unit
Lab waterl + 700 ng/L Diaz	60	100	100	100	687		1 Toxic Unit
Lab water + 600 ng/L Diaz	5	100	100	100	589		1 Toxic Unit
Lab water + 500 ng/L Diaz	0	90	100	100	491		1 Toxic Unit
Lab water + 400 ng/L Diaz	0	10	15	15	393		

Table 15. Comparison of diazinon concentrations in grab samples collected in 1997 by the Department of Pesticide Regulation and by the Central Valley Regional Water Quality Control Board. All analytical results appear comparable except for Sacramento Slough on 24 January 1997.

		Diazinon (ng/l)	
Location	Date	DPR	Regional Board
Sacramento Slough	20 January 1997	<40	-
	24 January 1997	61	1,696
Orestimba Creek	20 January	<40	56
	24 January	<40	140
San Joaquin River	20 January	<40	34
at Vernalis	22 January	<40	37
	24 January	70	98

Table 16. Highest mean diazinon concentrations (ng/l) observed during the 1996-1997 dormant spray monitoring program. Also presented is an estimate of the percentage of local aquatic species whose LC_{50} concentration[1] was likely exceeded by the two diazinon excursions (toxicity data from Novartis, 1997).

Location	San Joaquin River @ Vernalis	Sacramento Slough @ HWY 113
Date	1-2 February 1996	22-25 January 1997
Averaging Period	2 days	4 days
Mean Diaz Conc	7,105 ng/l	1,111 ng/l
Fish Species Affected (%)	0	0
Arthropod Species Affected (%)	50	30

[1/]Chemical concentration required to kill half of test organisms in laboratory water.

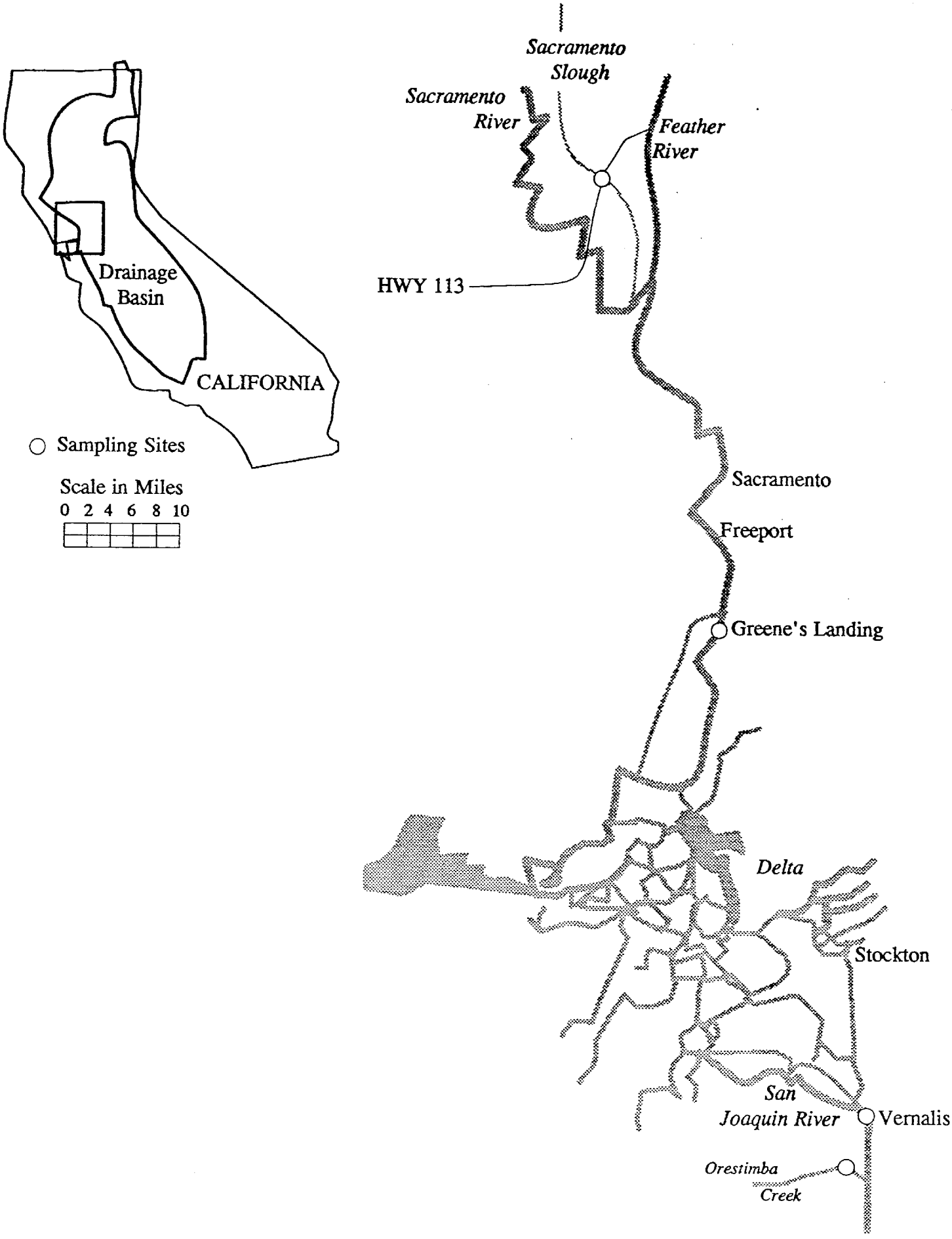

Figure 1. Map of sampling sites for the 1996-97 Bay Protection orchard dormant spray sampling program

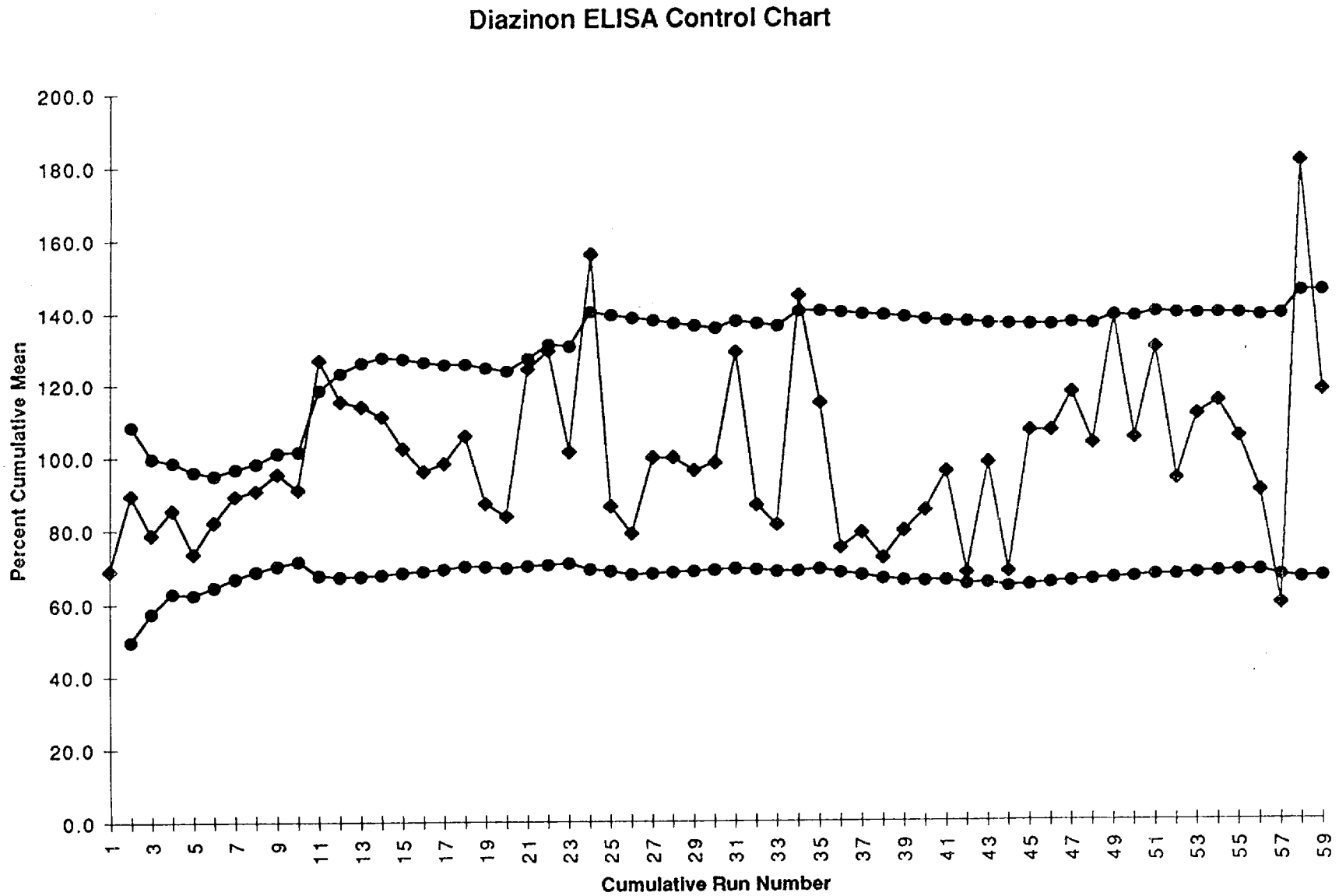

Figure 2. Diazinon ELISA control chart for the time period of June 1995 through July 1997. Diamonds represent the measured value as a percent of the long-term mean (300 ng/l). Circles are +/- 2 standard deviations of the mean

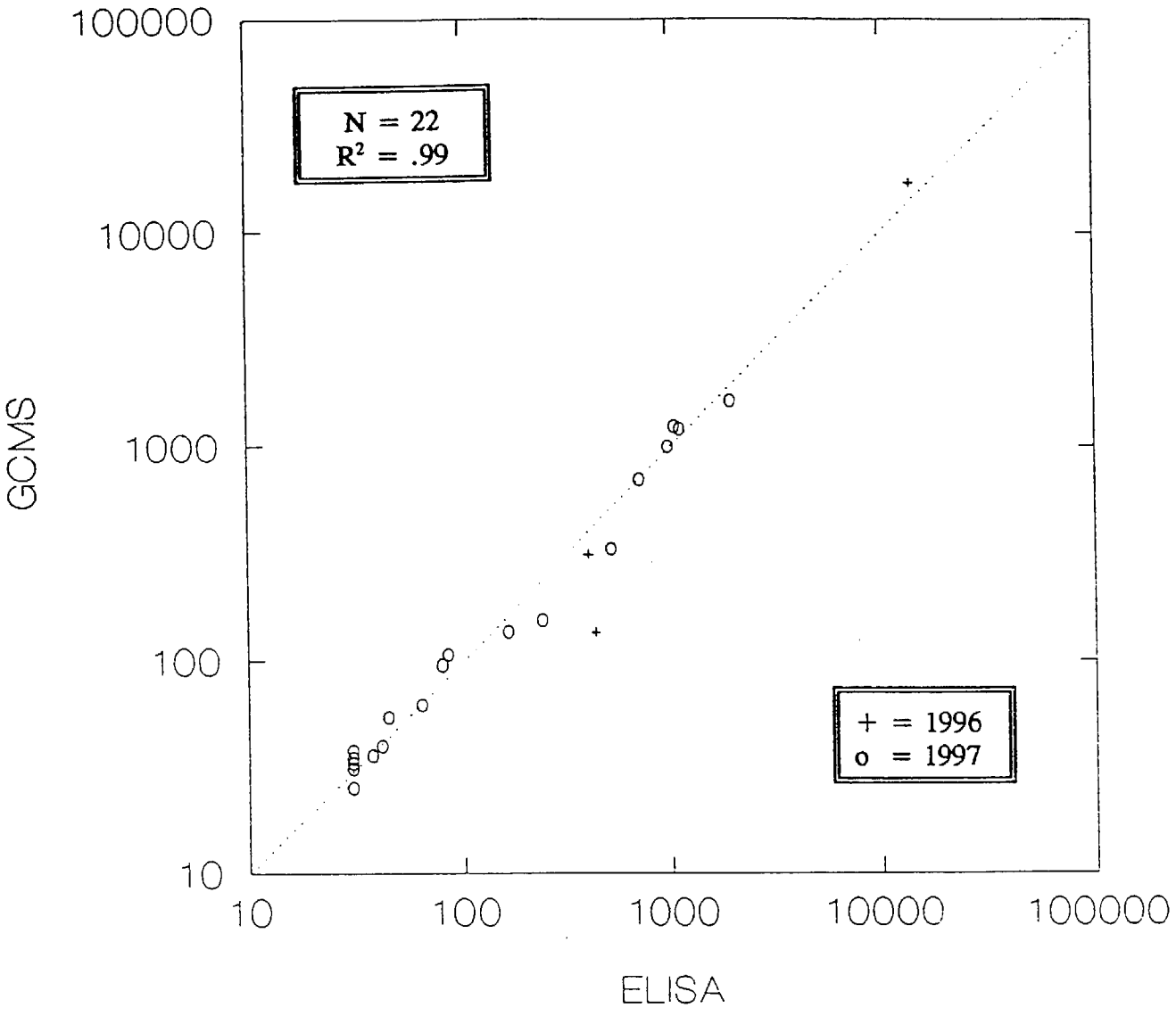

Figure 3. Correlation of diazinon concentrations measured by ELISA at the U.C. Davis Aquatic Toxicology Center and by GC/MS at the U.S. Geological Survey at Sacramento California.

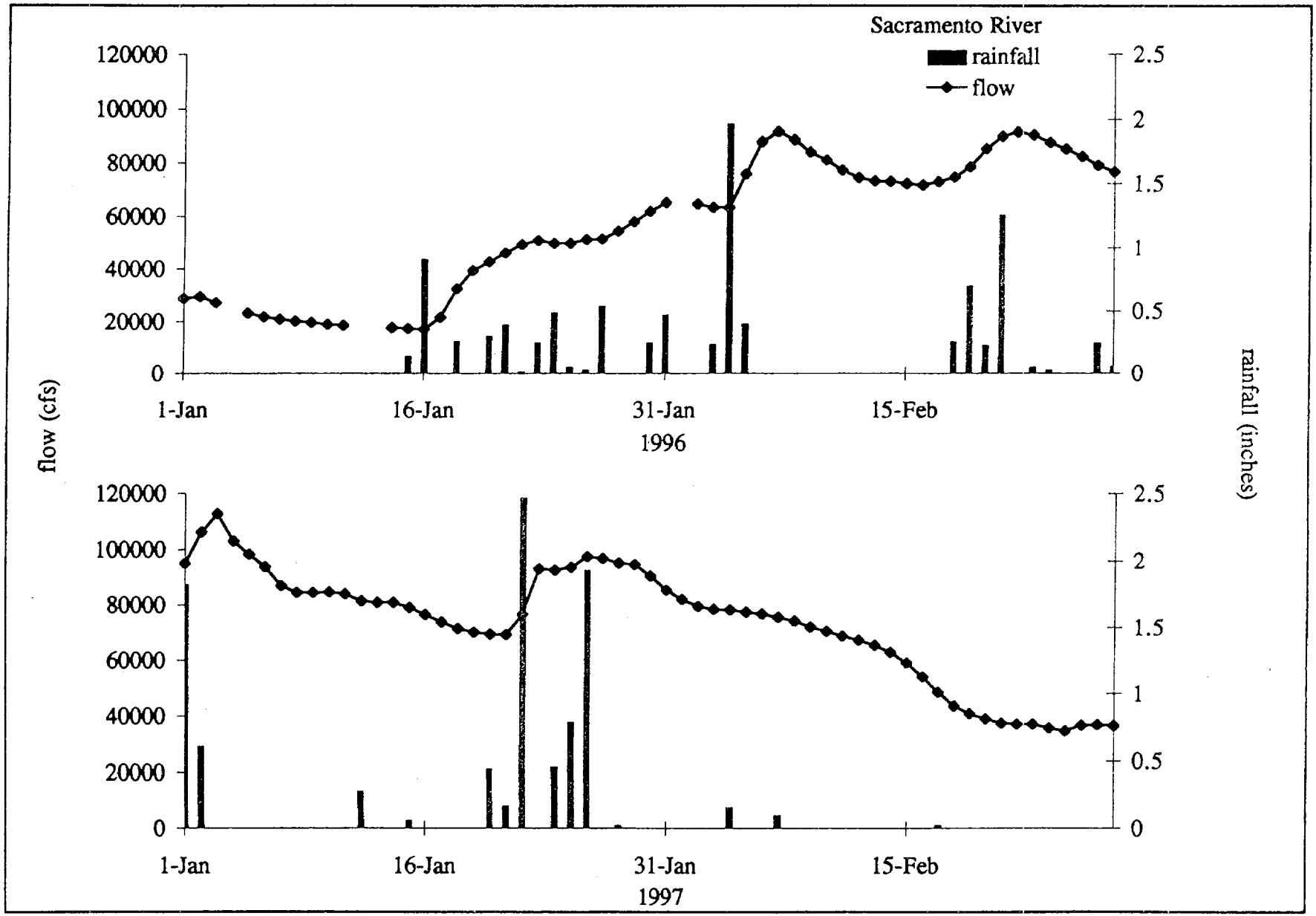

Figure 4. Flow and rainfall pattern for the Sacramento River at Freeport for January and February 1996 and 1997.

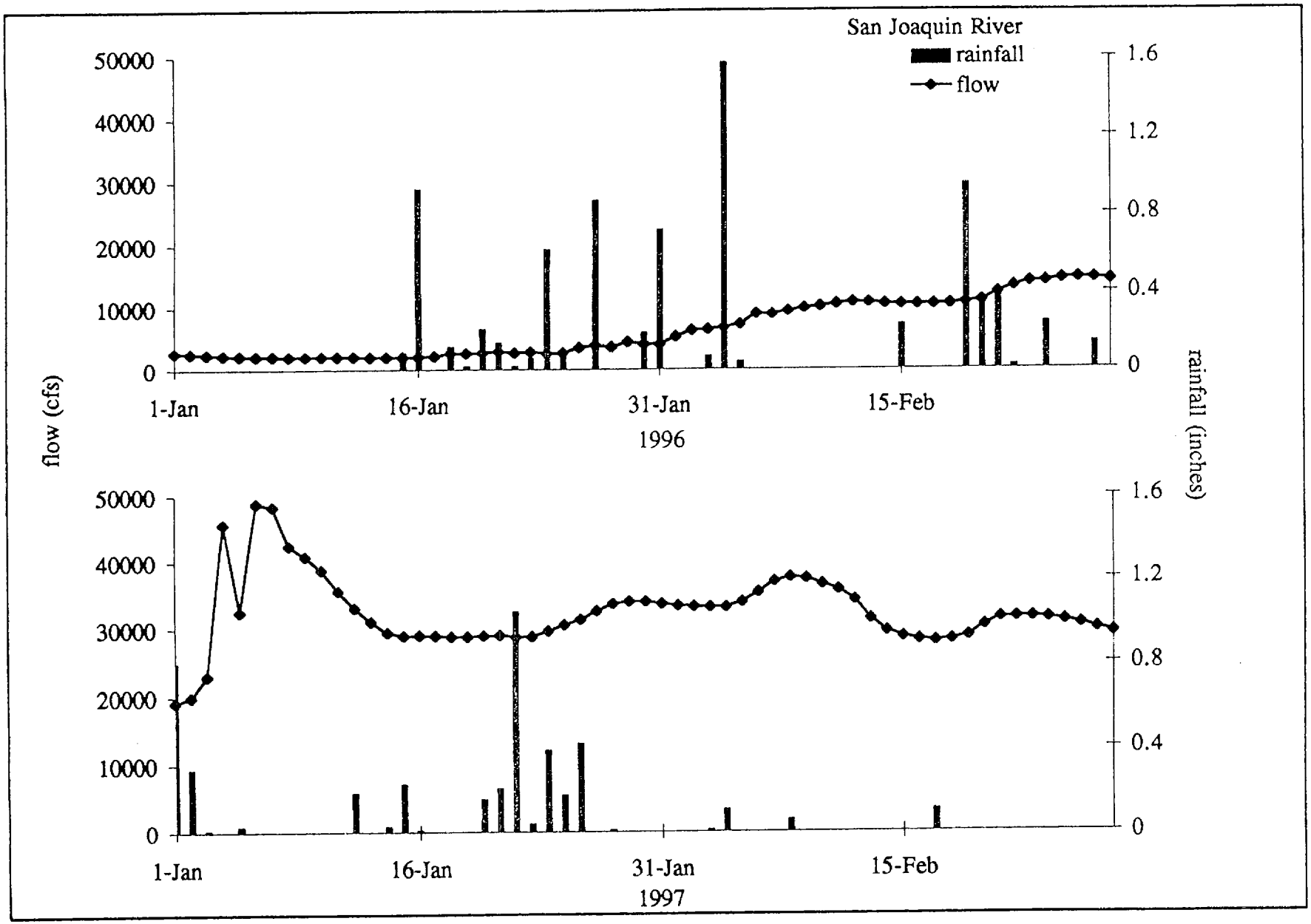

Figure 5. Flow and rainfall pattern for the San Joaquin River at Freeport for January and February 1996 and 1997.

APPENDIX A: SUMMARY OF CG/MS PESTICIDE ANALYTICAL DATA

Table 1. Summary of pesticides detected in samples testing toxic in *Ceriodaphnia* bioassays in 1996.

		Pesticide (ng/L)					
Location	Date	diazinon	simazine	diazinon oxon	metolachlor	methidathion	napropamide
Vernalis	2/1	13,900[1/]	224	52	24	75	34
Vernalis	2/2	311	308			130	243
Vernalis	2/3	135	130			96	31

1/ Sample also analyzed by the Department of Pesticide Regulation and APPL in Fresno California. Diazinon was reported at 16,940 and 16,000 ng/L, respectively.

Table 2. Summary of pesticide detections (ng/L) in dormant spray bioassay samples collected in 1997. Dashes indicate below detection limit.

Location	date	diazinon	simazine	carbofuran	metolachlor	chlorpyrifos	dacthal	methidathion	napropamide
Vernalis	1/20	34	57	-	4	5	-	-	-
Vernalis	1/21	32	52	-	2	-	-	-	-
Vernalis	1/22	37	65	-	5	-	2	29	-
Vernalis	1/23	64	113	-	6	6	-	31	-
Vernalis	1/24	98	134	-	17	5	3	43	15
Orestimba	1/20	56	146	-	-	6	3	-	-
Orestimba	1/21	110	131	-	11	-	4	-	-
Orestimba	1/22	140	84	-	19	-	3	-	-
Orestimba	1/23	720	406	-	6	-	-	-	-
Orestimba	1/24	26	161	-	3	-	-	-	-
Orestimba	1/25	340	38	-	5	-	-	-	-
Orestimba	1/26	no data							
Sacramento SI	1/20	no data							
Sacramento SI	1/21	36	16	-	12	-	-	-	-
Sacramento SI	1/22	213	147	-	-	-	-	201	-
Sacramento SI	1/23	1,250	351	-	17	-	2	438	29
Sacramento SI	1/24	1,696	252	-	13	-	-	578	18

Table 2. cont.

Location	date	diazinon	simazine	carbofuran	metolachlor	chlorpyrifos	dacthal	methidathion	napropamide
Sacramento Sl	1/25	1,286	142	-	10	-	-	319	-
Sacramento Sl	1/26	213	147	-	-	-	-	201	-
Detection Limit		9	22	6	4	6	25	5	60

APPENDIX B: ANTIBODY MEDIATED TIE PROCESS

Aqua-SCIENCE

ENVIRONMENTAL TOXICOLOGY SPECIALISTS

IDENTIFICATION OF DIAZINON TOXICITY TO *CERIODAPHNIA* IN DORMANT SPRAY RUN-OFF USING ANTIBODY-MEDIATED SELECTIVE REMOVAL PROCESSES

SACRAMENTO SLOUGH SAMPLE 1/24/97

SUBMITTED TO:

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CENTRAL VALLEY REGIONAL
WATER QUALITY CONTROL BOARD
3443 ROUTIER ROAD
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JUNE 26, 1998

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IDENTIFICATION OF DIAZINON TOXICITY TO *CERIODAPHNIA* IN DORMANT SPRAY RUN-OFF USING ANTIBODY-MEDIATED SELECTIVE REMOVAL PROCESSES

1.0 INTRODUCTION

Monitoring studies conducted by the Central Valley Regional Water Quality Control Board (CVRWQCB) and others[1-3] have identified organophosphate insecticides (OPs), including diazinon, in California dormant spray run-off at concentrations which cause toxicity to *Ceriodaphnia*. However, no Toxicity Identification Evaluation (TIE) studies were conducted on these samples to determine whether the contaminants might also contribute to the toxicity. Therefore, TIE studies were undertaken by the CVRWQCB to determine whether diazinon was the principle toxicant in dormant spray run-off. To supplement these studies, which used published TIE procedures[4-6], *AQUA*-Science used a proprietary process ("F3") to identify and confirm the role of diazinon in a sample of dormant spray run-off which caused acute lethality to *Ceriodaphnia*.

2.0 MATERIALS AND METHODS

2.1 Sample Collection

Subsurface grab samples of run-off were collected in one-gallon glass amber bottles from Sacramento Slough at Highway 113 by CVRWQCB staff. Samples were transported to the University of California, Davis, Aquatic Toxicology Laboratory (UCDATL) in ice chests containing wet ice for initial screening for *Ceriodaphnia* toxicity. A split sample was sent to *AQUA*-Science for confirmation of toxicity and treatment with F3. At *AQUA*-Science, the samples were stored in the dark at 4 °C until screening toxicity tests were conducted within 24 hours of sample delivery.

2.2 Ceriodaphnia Toxicity Tests

Acute 72-hour toxicity tests were conducted using procedures described in the EPA 4th Edition[7] as guidance. *Ceriodaphnia* (<24 hours old) from in-house cultures were tested in 20 mL glass scintillation vials containing 10 mL of solution. Five to seven dilutions bracketing the expected toxicity were used for each treatment. Four replicates containing five *Ceriodaphnia* were tested for each dilution. The dilution water was spring water (Sierra Spring Water Co.) amended with dry salts to EPA moderately hard specifications (EPAMH). Mortality was monitored daily for the 72-hour test period. Solutions were not renewed and animals were not fed during the test.

2.3 Sample Treatments

Acute 72-hour *Ceriodaphnia* toxicity tests were conducted on the following treatments:

- Untreated sample which was shaken prior to dilution (untreated-shaken).

- Untreated sample which was settled overnight prior to dilution (untreated-settled).
- F3-D treated sample. The F3 process is explained in Section 2.4.
- F3-D treated and spiked with diazinon at the initial sample concentration (F3-D+spike).

Note that both settled and treated samples were tested to ascertain the role of diazinon bound to settlable particles in the overall toxicity of the sample.

2.4 F3 Treatment Process

2.4.1 Theory of the F3 Process

F3 is an antibody-mediated chemical-specific process which uses highly purified antibodies, which have a high binding affinity for specific 'target' chemicals. The F3 is comprised of purified rabbit polyclonal antibody that is covalently bound to inert spherical particles which can be readily recovered from the aqueous sample matrices. F3 selectively removes the target chemicals from aqueous matrices by antibody-antigen bonding mechanisms. Previous studies with storm water, surface water, and municipal effluent samples have demonstrated that the F3 process provides high removal of the target chemical with low removal of non-target chemicals[8-10]. F3 is currently available for diazinon (F3-D) and chlorpyrifos (F3-C). The F3-D and F3-C treatments can be conducted singly or in combination to determine the toxicity due to both diazinon and/or chlorpyrifos when both chemicals are present in the sample.

2.4.2 Application of the F3 Process

The F3 process consists of three steps. First, the initial toxicity of the sample is determined by toxicity test and the toxic units (TUs) in the sample is calculated. Second, the target chemical (either diazinon or chlorpyrifos) is selectively removed from the sample matrix using the F3 process. Finally, toxicity tests are conducted on the F3-treated sample to determine the remaining, or 'residual', toxicity (TUs), if any. The difference between the TUs determined in Steps 1 and 3 is the toxicity due to the target chemical alone.

2.4.3 Confirmation of Toxicity Due to Diazinon

To confirm the role of the diazinon in the sample's toxicity, technical-grade diazinon was spiked back into the F3-D-treated solution at the level present in the sample prior to F3 treatment (F3-D + spike). If the F3 treatment has removed only the target chemical from the sample matrix, then the TUs of the F3-D+spike sample and the TUs of the untreated sample should be similar.

2.5 Confirmation of F3 Selectivity

A study was conducted to confirm that the F3 process provides highly selective removal of the target chemical in the presence of other OP insecticides. EPAMH lab water was spiked with environmental concentrations (1-3 ppb) of fifteen of commonly detected pesticides in California surface waters[11]. The sample was treated sequentially with F3-C and then F3-D and analyzed for the pesticides by GC using procedures described in Section 2.9. The results (Appendix I) showed that the F3-C and F3-D treatments removed approximately 80% of the target chemical (diazinon and chlorpyrifos, respectively), and about 40-50% of the respective oxone metabolite. Removal of the other pesticides was generally less than 20%, which is believed to be the approximate limit of the GC/MS analytical procedure.

2.6 Water Quality Measurements

Water quality parameters, including temperature, pH, dissolved oxygen (DO), conductivity, hardness and alkalinity were measured at test initiation in the untreated sample from which F3 aliquots are prepared. At test termination, pH and DO were measured in all solutions.

2.7 Enzyme-Linked Immunosorbant Assay (ELISA)

Concentrations of diazinon in the untreated and F3-treated samples were determined using ELISA kits from Insite™ (Beacon Analytical, Portland, ME). Analyses were conducted according to manufacturers instructions. The reported limit of detection for diazinon was 30 µg/L.

2.8 Gas Chromatography Analyses

The levels of diazinon in all four sample treatments were measured by capillary-column gas-chromatography/mass spectrometry (GC/MS)[12] to confirm ELISA results. Briefly, water samples were filtered and extracted with solid phase extraction (SPE) columns. The SPE columns were dried and eluted with hexane-isopropanol (3:1) and analyzed by capillary column GC/MS with selected ion monitoring of three characteristic ions. Single-operator method detection limits in reagent-water samples ranged from 0.001 to 0.018 µg/L. Recoveries in reagent-water samples ranged form 37 to 126 percent for most pesticides.

2.9 Piperonyl Butoxide Treatment

Piperonyl butoxide (PBO) at 100 µg/L in methanol was added to an aliquot of the sample to assess the role of metabolically-activated OP insecticides in the sample's toxicity. PBO is a biochemical reagent that prevents the metabolic activation and subsequent toxicity of certain OP insecticides, such as diazinon[13]. A PBO control (100 µg/L PBO in laboratory dilution water) was tested concurrently with each PBO treatment.

2.10 Endpoint Definitions and Calculations

The EC_{50} was calculated from the mortality data from each treatment using a computer program (ToxCalc™ 5.0). The EC_{50} value is the calculated concentration that is associated with 50% mortality.

2.10.1 Predicted Diazinon TUs

The predicted diazinon TUs is the amount of toxicity that diazinon would be predicted to contribute to a sample. This calculation is based on the concentration of diazinon in the sample and on the toxicity of diazinon in laboratory dilution water, as shown below:

Predicted TUs = ng/L diazinon in sample/EC_{50} of diazinon in lab water (358 ng/L)[a]

2.10.2 Measured TUs

Measured TUs are determined from the EC_{50} values calculated by ToxCalc. The measured TUs were calculated as follows:

Measured TUs = 100/EC_{50} of the sample (%)

2.10.3 Residual TUs

Residual TUs are the toxicity remaining in the sample after treatment with F3 and are calculated as follows:

Total Residual TUs = Total Measured TUs - Total Predicted TUs

3.0 RESULTS

3.1 Predicted and Measured TUs Using ELISA Measurements of Diazinon

Table 1 shows the diazinon concentrations measured by ELISA along with the predicted and measured *Ceriodaphnia* toxicity (EC_{50} and TUs) for the four sample treatments. Figure 1 shows the predicted TUs due to diazinon (shown as the horizontal bars) and the measured TUs (shown as the arrow) for each of the four sample treatments. The 24-, 48-, and 72-hour toxicity test data associated with these samples are shown in Appendix III.

[a] The LC_{50} of diazinon in laboratory dilution water is the mean of nine acute toxicity studies which had exposure concentrations confirmed by ELISA (Appendix II).

Table 1 **Diazinon Concentrations Measured by ELISA and *Ceriodaphnia* Acute (72-Hour) Toxicity of Sacramento Slough Dormant Spray Run-Off Sample[a]**

Sample Treatment	*Predicted Diazinon Toxicity*			*Measured Toxicity*		*Residual Toxicity (TUs)[f]*
	ng/L[b]	*Pred. EC_{50}[c] (%)*	*Pred. TUs[d]*	*EC_{50} (%)*	*TUs[e]*	
Untreated (shaken)	1941	18.5	5.4	15	*6.7*	*1.3*
Untreated (settled)	1824	19.6	5.1	15	*6.7*	*1.6*
F3-D	277	>100	0.8	66.3	*1.5*	*0.7*
F3-D + Spike	1922	18.3	5.9	17.7	*5.6*	*0.2*

a Sacramento Slough sample collected on 1/24/97

b Diazinon concentrations were determined by ELISA

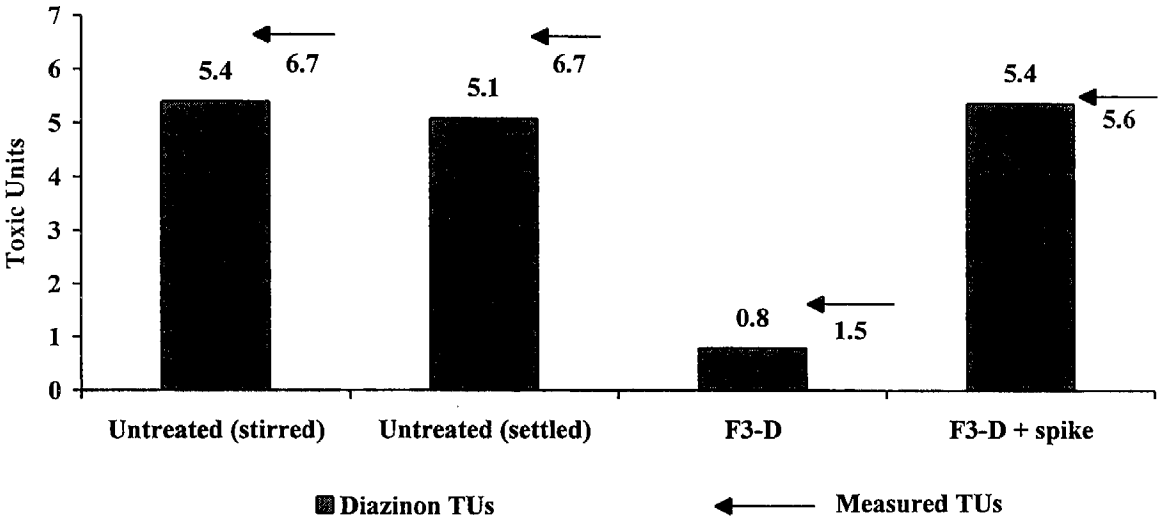
c Predicted EC_{50} = 100/Diazinon TUs

d Predicted TUs = Diazinon Concentration in Sample (ng/L)/Diazinon EC_{50} (351ng/L)

e Measured TUs = 100/Observed EC_{50} (see Appendix II for mortality data)

f Residual TUs = Measured TUs - Predicted TUs

Figure 1 **Effect of F3-D on the Acute 72-Hour Toxicity of Sacramento Slough Dormant Spray Run-Off to *Ceriodaphnia***



3.1.1 Untreated Samples

The settled sample contained 1,834 ng/L (5.1 TUs) of diazinon, which was only slightly less than the diazinon measured in the unsettled sample (1,941 ng/L, 5.4 TUs). This result indicates that essentially none of the diazinon measured by

ELISA was associated with the settleable particles. Bioassay of the shaken and settled samples produced identical EC_{50} values of 15% (6.7 TUs). The residual TUs (measured TUs - predicted TUs) for these samples ranged from 1.3-1.6 TUs, indicating that there was more toxicity present in the samples than was predicted from the diazinon concentrations. The residual toxicity suggests there may have been one or more additional toxicants present in the sample. Furthermore, since the toxicity of the sample was prevented by PBO, the unidentified toxicity was likely due to one or more OP insecticides.

3.1.2 F3-D-Treated Sample

After treatment of the settled sample with F3-D, there was 15% of the diazinon remaining in the sample (277 ng/L, 0.8 TUs). Bioassay of this sample produced an EC_{50} of 66.3% (1.5 TUs). The residual toxicity in this sample was 0.7 TUs.

3.1.3 F3-D-Treated Plus Diazinon Spike

This sample contained 105% of the diazinon present in the original sample (1,922 ng/L, 5.4 TUs). Bioassay of this sample produced an EC_{50} of 17.7% (5.6 TUs). The residual toxicity in this sample was 0.2 TUs.

3.1.4 PBO Treatment

PBO treatment (100 µg/L) of the four samples, at the highest concentration tested, resulted in no detectable toxicity in any of the samples.

3.2 Comparison of ELISA and GC Measurements

Table 2 shows the comparison of ELISA and GC analyses for diazinon in the four sample treatments.

Table 2 Comparison of ELISA and GC Measurements in Sacramento Slough Dormant Spray Run-Off Sample Treatments

Sample Type	*ELISA (ng/L)*[a]	*GC (ng/L)*[b]	
	Diazinon	*Diazinon*	*Methidithion*
Untreated (shaken)	1941	1716	662
Untreated (settled)	1824	1717	705
F3-D	227	318	753
F3-D + Spike	1922	1407	689

a = ELISA assays were conducted as described in Section 2.7
b = The GC analyses were conducted as described in Section 2.8

Diazinon concentrations detected by the two procedures varied by less than 13% in the untreated settled and shaken samples, and by 29% and 37% in the F3-D and F3-D+spike samples, respectively. Overall, the agreement between the two procedures was acceptable for low level (< 1 ppb) analysis in an ambient sample matrix. The GC analysis also detected 662-753 ng/L of methidithion (Supracide™; O,O-dimethylphosphorodithioate, S-ester-4-[mercaptomethyl]-2-methoxy-1,3,4-thiadiazolin-5-one) in all of the four sample treatments. It was noteworthy that methidithion concentrations were similar in both untreated samples and in the F3-D-treated sample. This demonstrates the selectivity of the F3 process since none of the methidithion was removed by the F3-D treatment, which removed over 85% of the diazinon in the sample.

4.0 DISCUSSION

4.1 General Characteristics of the Sample Toxicity

The Sacramento Slough orchard run-off sample was highly toxic to *Ceriodaphnia*. The EC_{50} of the untreated samples (settled and shaken) were both 15% (6.7 TUs). Treatment of the sample with PBO completely eliminated the toxicity of the sample. This result indicates that all of the toxicity in the sample was due to one or more metabolically-activated OP insecticides.

The similarity of the TUs in the settled and shaken samples indicates that very little of the diazinon that was measured by ELISA was associated with the settleable particulates. Moreover, since the measured toxicity of the two samples was identical, none of the diazinon associated with the settleable particles contributed measurable toxicity to the sample. These results are similar to other samples of ambient waters containing diazinon and/or chlorpyrifos that we have tested[9-10]. Collectively, the results suggest that little or none of the particulate-bound residues of these two OPs are bioavailable to *Ceriodaphnia*.

4.2 Role of Diazinon in the Sample Toxicity

ELISA analysis of the settled and shaken samples detected 1,824 and 1,941 ng/L, respectively, of diazinon, which corresponds to 5.1-5.4 predicted TUs. These TU calculations assume that the diazinon in the sample matrix has the same bioavailability as in laboratory dilution water. Treatment of the sample with F3-D removed 85% of the diazinon and reduced the observed sample toxicity by 78%. This treatment confirmed that diazinon was the principal toxicant. The F3-D+spike treatment closely matched the diazinon concentration present in the original sample. This treatment produced 5.6 TUs, 84% of the sample's original level of toxicity, further confirming that diazinon was the principal toxicant in the sample.

4.3 Role of Unidentified OP Insecticides

The level of toxicity predicted by the diazinon concentration in the untreated and F3-D-treated samples was less than the measured toxicity in these samples by 0.2-1.6 TUs. Since the PBO treatment prevented all measurable toxicity in the sample, this residual toxicity was likely due to one or more OP insecticides. The GC analysis identified methidithion in the sample at concentrations of approximately 700 ng/L, which, based on this chemical's toxicity to *Ceriodaphnia* (EC_{50} = 2,000 ng/L)[14] and assumed direct additivity to diazinon toxicity[15], would add approximately 0.3 TUs to the sample. The available information to date suggests that the resolution of the F3 process to identify residual toxicity is approximately 0.5 TUs. The relatively small amount of residual toxicity that is unaccounted for by application of the F3 process to this sample has not been identified. HPLC/MS and GC/MS analysis of C-8 SPE column eluates of the settled sample to identify other OPs which may be present in the sample are on-going.

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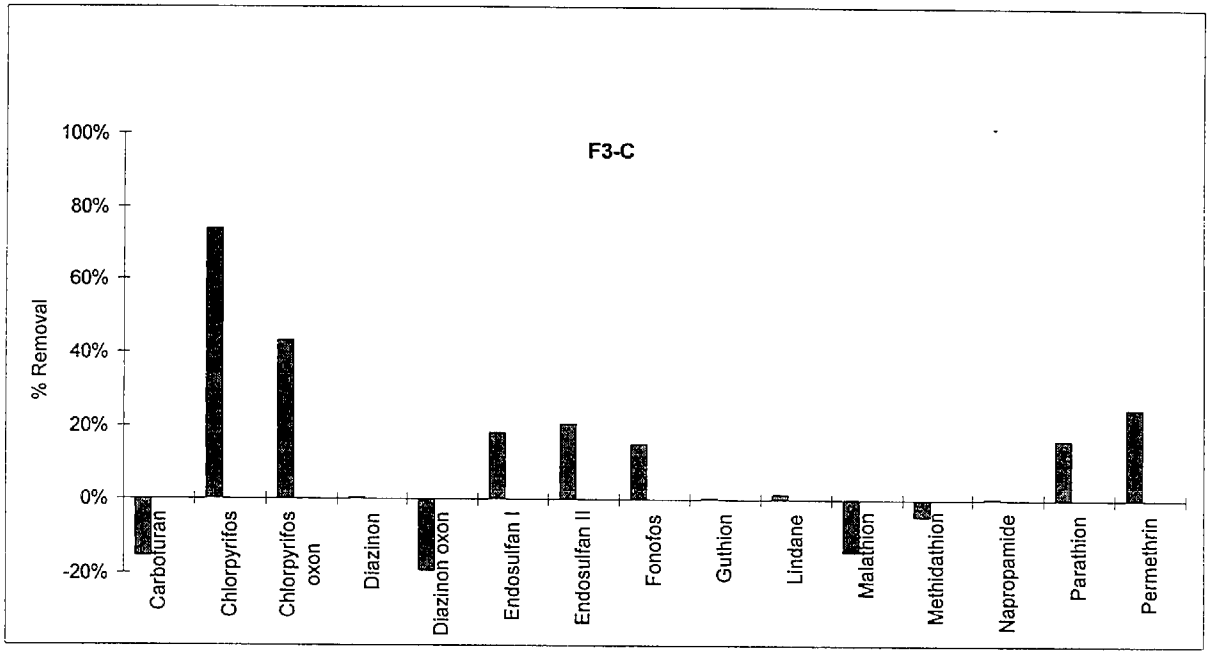
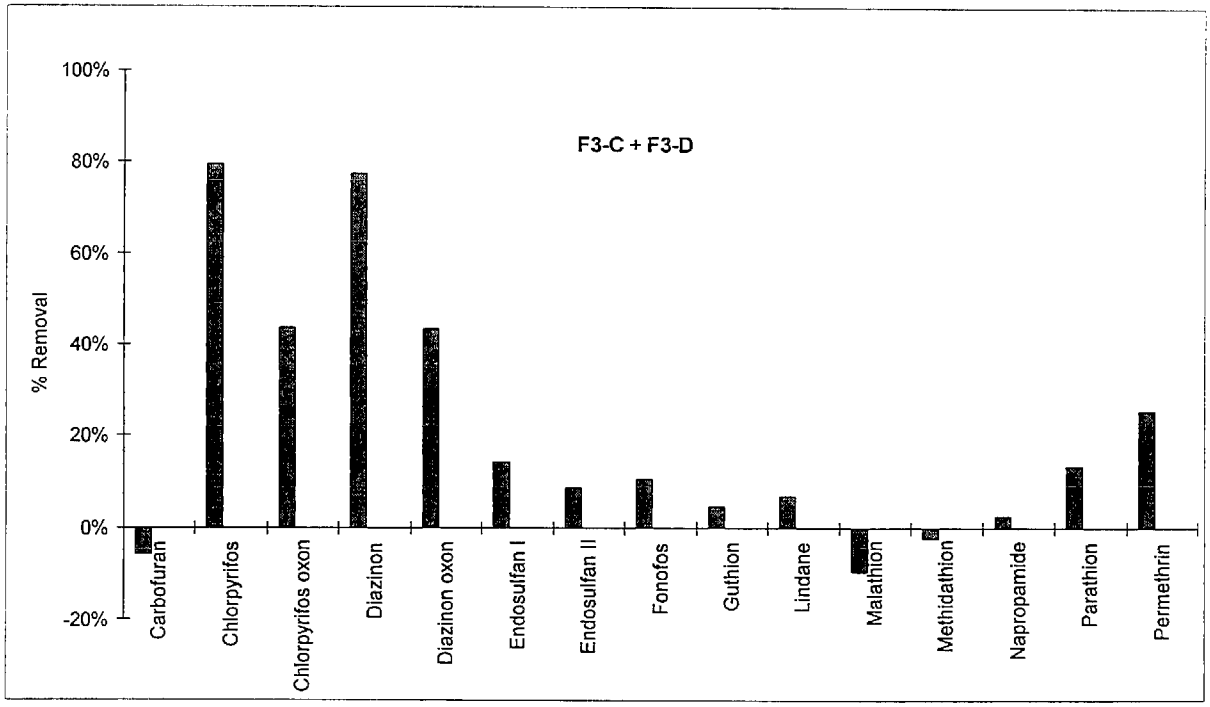
APPENDIX I

Selectivity of F3 Process





APPENDIX II

Toxicity of Sacramento Slough Dormant Spray Run-Off to *Ceriodaphnia*

Treatment	*Concentration* (%)	*Cumulative Mortality*[a]			EC_{50} (TUs)
		24-Hr	*48-Hr*	*72-Hr*	
Untreated (shaken)	0	0	0	*5*	24 hr: 21.7 (4.6)
	10	0	0	*5*	
	20	40	100	*100*	48-hr: 15.0 (6.7)
	30	100	100	*100*	
	40	100	100	*100*	*72-hr: 15.0 (6.7)*
	50	100	100	*100*	
	50% + PBO	0	0	*0*	
Untreated (settled)	0	0	0	*15*	24 hr: 23.3 (4.3)
	10	0	0	*15*	
	20	20	100	*100*	48-hr: 15.0 (6.7)
	30	100	100	*100*	
	40	100	100	*100*	*72-hr: 15.0 (6.7)*
	50	100	100	*100*	
	50% + PBO	0	0	*0*	
F3-D	0	0	5	*5*	24 hr: >100
	60	0	0	*0*	
	70	0	0	*80*	48-hr: >100
	80	0	0	*100*	
	90	0	0	*100*	*72-hr: 66.3 (1.5)*
	100	0	0	*100*	
	100% + PBO	0	0	*0*	
F3-D + Spike	0	0	0	*0*	24 hr: 26.0 (3.8)
	10	0	0	*0*	
	20	5	5	*65*	48-hr: 24.7 (4.0)
	30	80	100	*100*	
	40	100	100	*100*	*72-hr: 17.7 (5.6)*
	50	100	100	*100*	
	50% + PBO	0	0	*0*	

a = Mortality shown is the combined mortality of 4 replicates with 5 *Ceriodaphnia* per concentration. 96-hour mortality is not presented because mortality in the controls exceeded 20% during this interval.

APPENDIX III

Acute Toxicity of Diazinon to *Ceriodaphnia*

Test Date	*72-Hour EC_{50} (ng/L)*	*Mean ± SD (ng/L)*	*Comments*
8/12/94	273	358 ± 61	Exposure concentrations confirmed by ELISA in all toxicity tests at test initiation
8/23/94	274		
8/30/94	327		
9/29/94	389		
10/13/94	447		
10/14/94	337		
10/27/94	414		
8/19/97	353		
8/27/97	407		

Note: The EC_{50}, mean and standard deviation of 25 72-hour acute *Ceriodaphnia* toxicity tests conducted since 10/91 at *AQUA*-Science is 371 ± 133.0