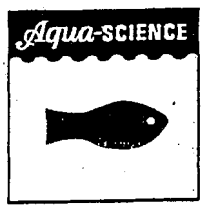


ENVIRONMENTAL TOXICOLOGY SPECIALISTS

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:

DR. CHRIS FOE
CENTRAL VALLEY REGIONAL
WATER QUALITY CONTROL BOARD
3443 ROUTIER ROAD
SACRAMENTO, CA 95827

SUBMITTED BY:

AQUA-SCIENCE
17 ARBORETUM DRIVE
DAVIS, CA 95616

JUNE 26, 1998

TABLE OF CONTENTS

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

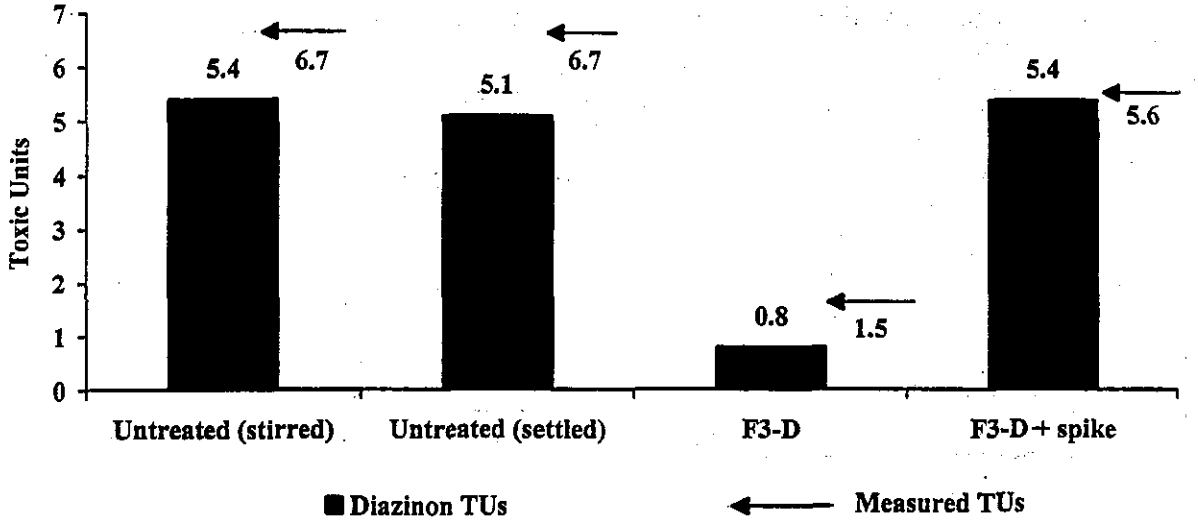
c Predicted EC_{50} = 100/Diazinon TUs

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.

5.0 REFERENCES

1. Kuivila, K., And C. Foe. 1995. Concentrations, Transport and Biological Effects of Dormant Spray Pesticides in the San Francisco Estuary, California. Env. Toxicol. Chem. 14:1141-1150.
2. Foe, C. 1995. Insecticide Concentrations and Invertebrate Bioassay Mortality in Agricultural Return Water from the San Joaquin Basin. Central Valley Regional Water Quality Control Board Staff Report. Sacramento Office.
3. Kratzer, C. 1997. Transport of Diazinon in the San Joaquin River Basin, California. U.S. Geological Survey Open File Report. 97-411.
4. USEPA. 1992. Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluent, Phase I. EPA/600/6-91/005F.
5. USEPA. 1993. Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity. EPA/600/R-92/080.
6. USEPA. 1993. Methods for Aquatic Toxicity Identification Evaluations: Phase III Toxicity Confirmation Procedures for Samples Exhibiting Acute and Chronic Toxicity. EPA/600/R-92/081.
7. USEPA. 1993. Methods for Measuring the Acute Toxicity of Effluent and Receiving Waters to Freshwater and Marine Organisms. 4th Edition. EPA/600/4-90/027F.
8. Miller, J.L., M.J. Miller, V. deVlaming and C. Foe. Selective Removal of Diazinon and Chlorpyrifos from Aqueous Matrices Using Antibody-Mediated Procedures. 18th Annual Meeting, Society of Environmental Toxicology and Chemistry. San Francisco, CA. November 16-20, 1997. Abstract.
9. *AQUA*-Science. 1996. Toxicity Identification Evaluation Using Antibody-Mediated Chemical-Specific Removal Procedures. Report for Dow-Elanco. Indianapolis, IN.

10. *AQUA*-Science. 1997. Toxicity Identification Evaluation Using Antibody-Mediated Chemical-Specific Removal Procedures. Report for Dow-Chemical Co. Midland, MI.

11. MacCoy, D., K.L. Crepeau and K.M. Kuivila. 1995. Dissolved Pesticide Data for the San Joaquin River at Vernalis and the Sacramento River at Sacramento, California, 1991-1994. U.S. Geological Survey Open File Report 95-110.

12. Zangg, S.D., M.W. Sandstdrom, S.G. Smith and K.M. Fehlberg. 1995. Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory - Determination of Pesticides in Water by C-18 Solid Phase Extraction and Capillary-Column Gas Chromatography and Capillary-Column Gas Chromatography/Mass Spectrometry with Selected Ion Monitoring. U.S. Geological Survey Open File Report 95-181.

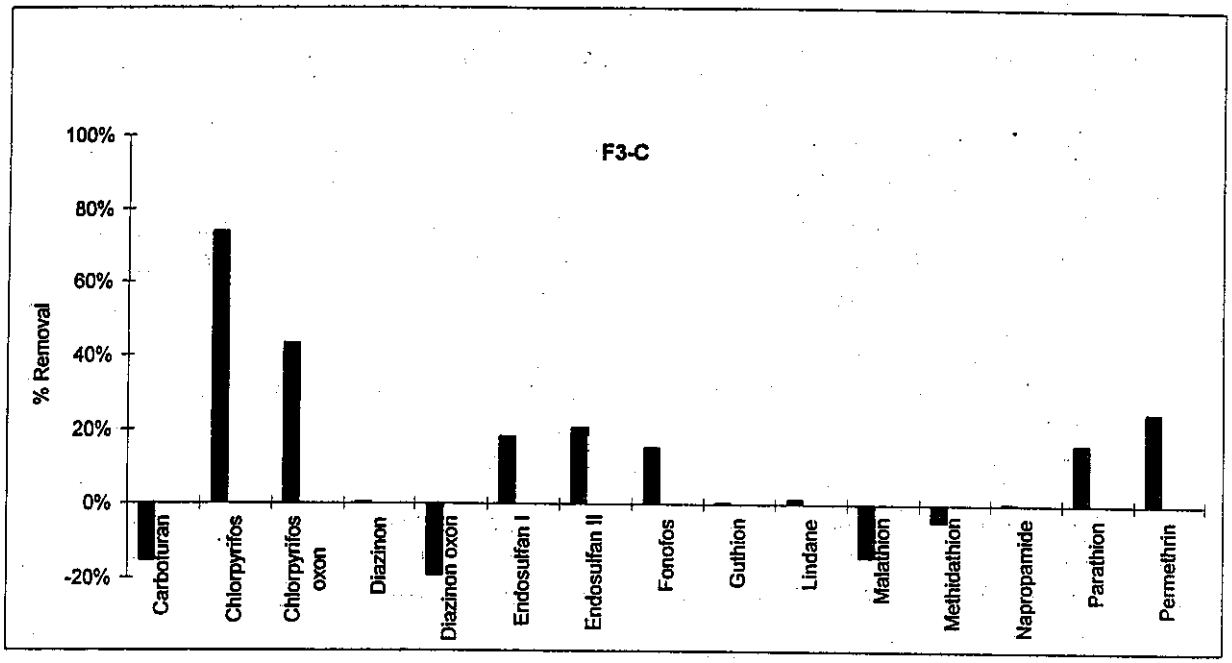
13. Ankley, G.T., J.R. Dierkes, D.A. Jensen and G.S. Peterson. 1991. Piperonyl Butoxide as a Tool in Aquatic Toxicological Research with Organophosphate Insecticides. Ecotoxicol. Environ. Saf. 12:266-274.

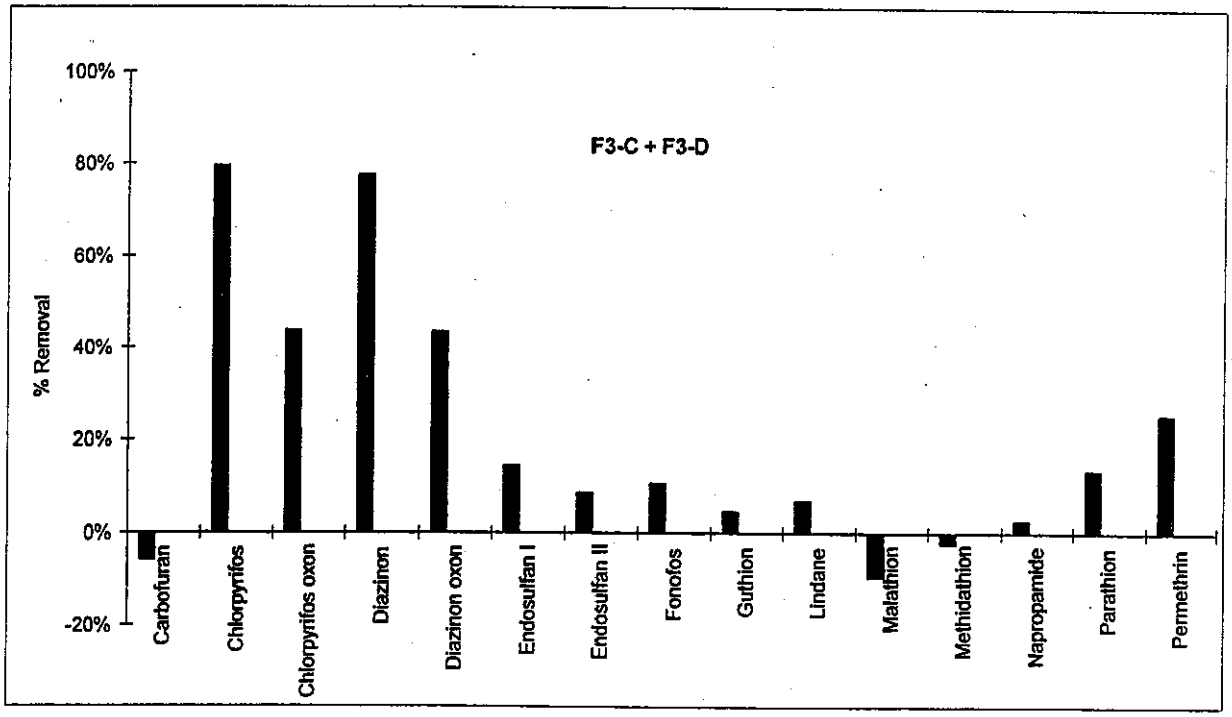
14. Isaac. G. and P. Phillips. 1994. Toxicity of Agricultural Chemicals to Water Fleas and Young Mysid Shrimp. California Department of Fish and Game, Environmental Services Division, Sacramento, CA. Administrative Report 94-02.

15. Bailey, H.C., J.L. Miller, M.J. Miller, L.C. Wiborg, L. Deanovic and T. Shed. 1997. Joint Toxicity of Diazinon and Chlorpyrifos to *Ceriodaphnia dubia*. Environ. Toxicol. Chem. 16: 2304-2308.

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