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U.S. Department of the Interior
U.S. Geological Survey

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Charles G. Groat, Director

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ABBREVIATIONS

cc	cubic centimeter
L	liter
µL	microliter
mL	milliliter
mL/min	milliliter per minute
ng/L	nanogram per liter

ACRONYMS

EPA	U.S. Environmental Protection Agency
GC/MS	gas chromatography-mass spectrometry
SPE	solid-phase extraction
TIE	toxicity identification evaluation
USGS	U.S. Geological Survey

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By Kathryn M. Kuivila *and* Kathryn L. Crepeau

ABSTRACT

A laboratory study was used to evaluate the response of select insecticides to toxicity identification evaluation procedures. Fourteen insecticides, one degradation product, and one synergist were spiked into organic-grade water and carried through toxicity identification evaluation procedures. Concentrations of each compound were analyzed by gas chromatography/mass spectrometry.

During Phase I, the water sample was pumped through a C-8 solid-phase extraction cartridge and then eluted with methanol. Dimethoate was not removed by the extraction, but remained in the rinsate. In contrast, permethrin was removed by the extraction, but was not recovered by the methanol elution, and 80 percent of the permethrin remained on the cartridge, teflon tubing, and glassware. Chlorpyrifos also was not recovered completely with the methanol elution (only 62 percent was recovered). The other insecticides were extracted by C-8 solid-phase extraction cartridge and recovered by elution with methanol (80 percent or greater).

During Phase II, a new spiked water sample was extracted by C-8 solid-phase extraction cartridge and then eluted with varying concentrations of methanol and water into different fractions. Each methanol:water fraction was analyzed for the added compounds. Most of the insecticides eluted in two fractions, with concentrations of 10 percent or greater. The largest number of insecticides eluted in the 75 percent methanol:water fraction.

INTRODUCTION

U.S. Environmental Protection Agency (EPA) toxicity identification evaluation (TIE) procedures are designed to identify the chemical(s) causing toxicity in water samples (U.S. Environmental Protection Agency, 1991, 1993). The first step (Phase I) is used to characterize the physical and chemical properties of the toxic compound(s). If toxicity is removed by passing the water sample through a solid-phase extraction (SPE) cartridge, the mixture is further separated (Phase II) into different fractions by eluting with varying concentrations of methanol and water. Bioassays are used to determine toxicity throughout the TIE procedures.

Previous bioassay results frequently have shown water samples from the Sacramento and San Joaquin rivers in California in the winter and spring to be toxic to Ceriodaphnia dubia (California Regional Water Quality Control Board, 1991, 1993, 1995; Kuivila and Foe, 1995). A large number of insecticides are applied in the watershed and it is not always obvious from a chemical analysis of the whole water sample which compound(s) are the cause of the observed toxicity. This is because the presence of an insecticide does not necessarily mean that it is bioavailable and causing toxicity and methods are not available for all insecticides applied in the Central Valley at biologically relevant detection limits. Therefore, knowledge of the response of select insecticides is valuable for narrowing the identification of the toxic compound(s) during TIE procedures.

The U.S. Geological Survey (USGS), in cooperation with the California Regional Water-Quality Control Board, is utilizing TIEs and chemical analyses to identify pesticides causing toxicity in ambient waters.

This report summarizes the results of a study to determine the response of select insecticides to TIE

procedures under controlled laboratory conditions. The insecticides studied included a degradation product and a synergist, a compound applied with insecticides to increase the toxicity. Known concentrations of insecticides were spiked into organic-grade water, the water samples processed through the TIE, and the concentrations of the insecticides were measured by gas chromatography/mass spectrometry (GC/MS) during each step.

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STANDARD TOXICITY IDENTIFICATION EVALUATION PROCEDURE

Water samples are tested for toxicity using Ceriodaphnia dubia neonates in 96-hour, static bioassays. If toxic, the water sample is subject to various manipulations to characterize the physical and chemical properties of the toxic compound(s). TIE treatments can include an addition of chelating agents or metabolic inhibitors, pH adjustments, extraction on SPE cartridges, and elution of SPE cartridges with methanol or a methanol:water gradient (Bailey and others, 1996). The focus of this study was the response of insecticides to extraction onto C-8 SPE cartridges followed by elution with methanol.

During Phase I (EPA, 1991), the toxic water sample is pumped through a C-8 SPE cartridge and the rinsate is retested for toxicity. If the toxicity has been removed, the cartridge then is eluted with methanol. The methanol eluate is diluted with organic-grade water and retested for toxicity. All bioassays include controls under similar conditions.

If the toxic compound(s) are removed by extraction and added back by elution, then the TIE proceeds to the identification step (Phase II) (EPA, 1993). A new aliquot of the water sample is extracted onto a C-8 SPE cartridge and the cartridge is consecutively eluted into separate fractions with solutions of increasing concentration of methanol:water. The exact volumes and ratios of methanol:water are not standardized by EPA and typically are determined by the laboratory doing the TIE. Each fraction is tested for toxicity after diluting with organic-grade water in varying ratios to maintain a constant final methanol:water concentration.

METHODS

Phase I Procedure and Mass Balance

During this study, the select insecticides were added to a water sample and then tested to determine if they were quantitatively removed by the C-8 cartridge and quantitatively recovered by the methanol elution. To determine a mass balance for each compound, the rinsate, original eluate, C-8 SPE cartridge after methanol extraction, glassware, and tubing were analyzed for insecticide content.

Three sets of organic-grade water samples (1,800 mL) were spiked with 16 compounds (14 insecticides, 1 degradation product, and 1 synergist; table 1), to a concentration of 528 ng/L at the organic-chemistry laboratory at the California District Office of the USGS. The Aquatic Toxicology Laboratory at the University of California, Davis (U.C. Davis), preconditioned three 6-cc C-8 SPE cartridges with 6 mL methanol followed by 6 mL of organic-grade water. The samples were extracted at a flow rate of 20 mL/min, and the rinsate was collected. The tubing and glassware used were washed further with methanol, and the wash solution was collected (10 mL). The cartridges were eluted with 3 mL of 100 percent methanol at 1 mL/min. The cartridges were eluted a second time with 3 mL hexane:ether (1:1) to remove any insecticide residue on the cartridges.

The methanol and hexane:ether eluates, rinsates, and tubing/glassware washes were extracted with methylene chloride and analyzed by the California District organic-chemistry laboratory. The hexane:ether eluates were transferred to a test tube, and concentrated with nitrogen. Internal standards were added, and the sample further concentrated to approximately 100 µL. The concentrated samples were analyzed by GC/MS with ion-trap detection as detailed by Crepeau and others (1994). The same concentration and analysis procedures were used for the extracts of the other samples.

The methanol eluate (3 mL) and the tubing/ glassware washes (10 mL) were added to organic-grade water for a final volume of 100 mL. This water:methanol mixture was extracted three times for 2 minutes each with 10 mL methylene chloride in a 250 mL separatory funnel. The three extracts for each sample were combined in a round bottom flask and roto-evaporated to a final volume of approximately

Table 1. Removal and recoveries of insecticides in organic-grade water during Phase I of the toxicity identification evaluation procedures.

[Values shown in percent. na, not analyzed because dimethoate did not remain on the cartridge]

Insecticide	Quantitatively removed by C-8[1]	Quantitatively recovered by C-8[2]	Residue remaining on cartridge, tubing, or glassware[3]
Dimethoate	No[4]	na	No
Carbofuran	Yes	Yes	No
Carbaryl	Yes	Yes	No
Methidathion	Yes	Yes	No
Malathion	Yes	Yes	No
Lindane	Yes	Yes	No
Chlorpyrifos Oxon[5]	Yes	Yes	No
Phosalone	Yes	Yes	No
Fonofos	Yes	Yes	No
Sulfotep	Yes	Yes	No
Disulfoton	Yes	Yes	No
Diazinon	Yes	Yes	No
Piperonyl Butoxide [6]	Yes	Yes	No
Ethion	Yes	Yes	No
Chlorpyrifos	Yes	No (62)	Yes (34)[7]
Permethrin	Yes	No (12)	Yes (80)[8]

[1]Residue in rinsate was less than 5 percent.
[2]Recovery was 80 percent or greater.
[3]Residue remaining was 10 percent or greater.
[4]Residue in rinsate was 100 percent.
[5]Degradation product.
[6]Synergist.
[7]Residue remaining on the C-8 cartridge was 15 percent; residue remaining on the tubing and glassware was 19 percent.
[8]Residue remaining on the C-8 cartridge was 72 percent; residue remaining on the tubing and glassware was 8 percent.

2 mL. The samples were further concentrated and analyzed as described in the previous paragraph.

The rinsate was extracted in two aliquots (900 mL each) three times for 2 minutes each with 100 mL methylene chloride in a 2-L separatory funnel. The extracts were roto-evaporated to a final volume of approximately 2 mL, passed through sodium sulfate to remove any residual water, and further concentrated and analyzed as described previously.

Phase II Procedure and Recovery in Different Fractions

During Phase II, the SPE cartridge is eluted with a methanol:water gradient into separate fractions and the concentrations of insecticides are measured in each

fraction. Three new sets of organic-grade water samples were spiked with 14 compounds (12 insecticides, 1 degradation product, and 1 synergist) at the same concentration as was used previously and extracted onto SPE cartridges. During Phase I, permethrin and dimethoate were not quantitatively removed and recovered from the SPE cartridge. Thus, these two insecticides were not tested in Phase II. At the U.C. Davis Aquatic Toxicology Laboratory, each cartridge was consecutively eluted with nine 3-mL methanol:water solutions containing increasing proportions of methanol (50, 70, 75, 80, 85, 90, 95, 100 percent, and a second 100 percent). At the California District organic-chemistry laboratory, varying amounts of water and methanol were added to bring each fraction to a final volume of 100 mL and the same percent methanol (3 percent). Each methanol:water fraction then was extracted with methylene chloride and analyzed as in Phase I.
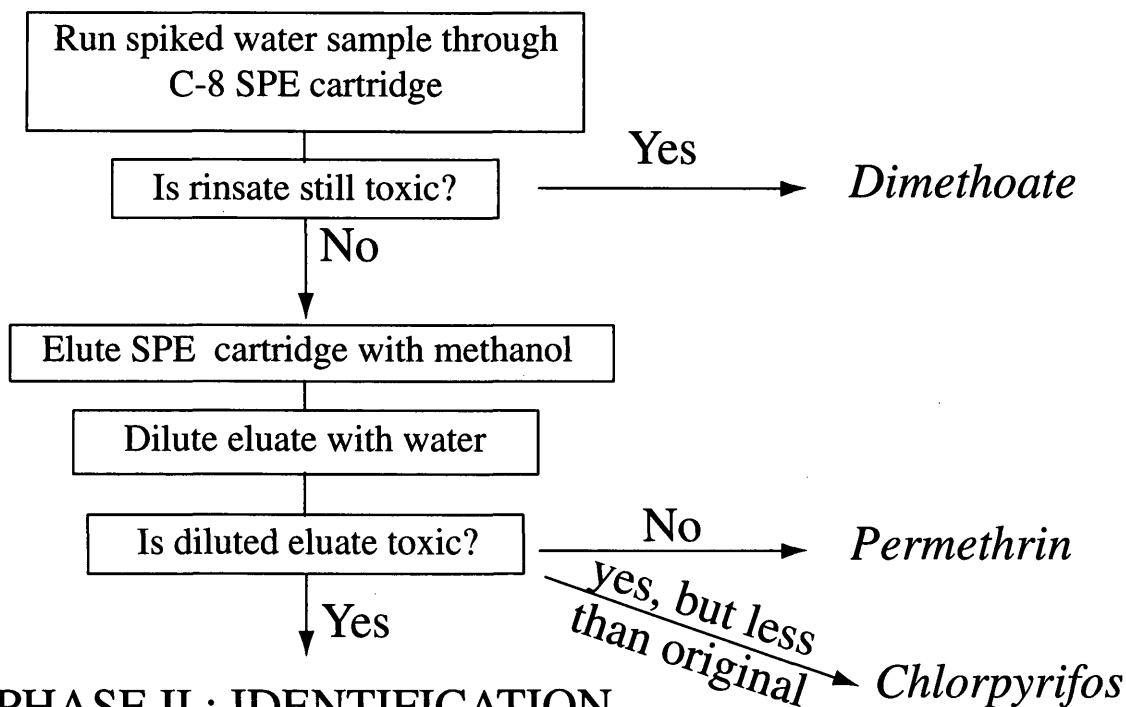
Liquid-Liquid Extraction Quality Assurance

Liquid-liquid extraction with methylene chloride is a standard method of extraction (Eaton and others, 1995). A method detection limit was not determined because this study involved only lab experiments where concentrations of insecticides were well above GC/MS detection limits (Crepeau and others, 1994). Quality assurance included a recovery experiment to verify the quantitative recovery of the 16 insecticides used in this study. Three sets of organic-grade water samples (1,800 mL) were spiked with the 16 insecticides at 950 ng/L and extracted with methylene chloride and analyzed by GC/MS with ion-trap detection. The recoveries of all 16 insecticides were between 83-128 percent, with standard deviations of 16 percent or less.

RESULTS

Results of Phase I and the mass balance are summarized in table 1 and figure 1. The majority of the insecticides were quantitatively removed by the C-8 cartridge (residue in rinsate less than 5 percent) and quantitatively recovered with the methanol elution (80 percent or greater). Chlorpyrifos was quantitatively removed by the cartridge extraction, but only 62 percent was recovered with the methanol elution. The remainder was on the cartridge (15 percent) and on the tubing and glass bottle (18 percent). Similarly,

PHASE I : CHARACTERIZATION

Run spiked water sample through
C-8 SPE cartridge

↓

Is rinsate still toxic? —— **Yes** ——→ *Dimethoate*

No
↓

Elute SPE cartridge with methanol

↓

Dilute eluate with water

↓

Is diluted eluate toxic? —— **No** ——→ *Permethrin*

Yes, but less than original ↘ *Chlorpyrifos*

Yes
↓

PHASE II : IDENTIFICATION

Run spiked water sample through
C-8 SPE cartridge

↓

Elute SPE cartridge with
consecutive methanol:water mixtures

↓

Which methanol:water fraction
contains majority of toxicity?
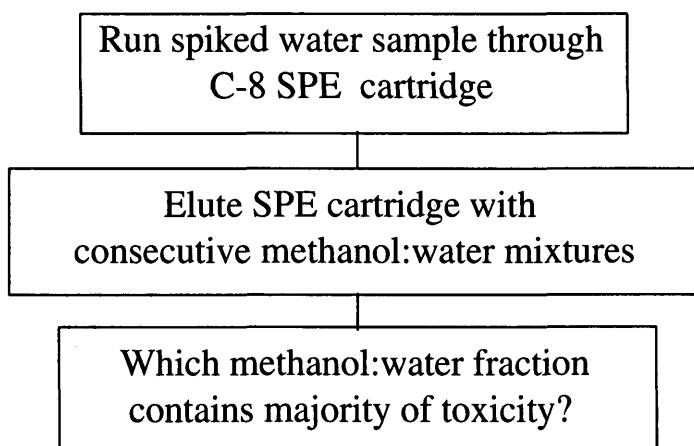
50 PERCENT METHANOL   *Carbofuran, Carbaryl*

70 PERCENT METHANOL   *Methidathion, Malathion*

75 PERCENT METHANOL   *Lindane, Chlorpyrifos Oxon, Phosalone,*
                             Fonofos, Sulfotep, Disulfoton, Diazinon

80 PERCENT METHANOL   *Piperonyl Butoxide, Ethion, Chlorpyrifos*

Figure 1. Summary of the expected response of select insecticides in an ambient water sample to the toxicity identification evaluation procedures. SPE, solid-phase extraction.

Table 2. Percentage of recoveries of insecticides in successive methanol:water fractions, sum of recoveries, weighted mean of methanol, and log K_{ow} during Phase II of the toxicity identification evaluation procedures.

[Shading indicates amounts greater than or equal to 10 percent; <, less than value shown; —, no data]

| Insecticide | Recoveries of insecticides in methanol (percent) | | | | | | | | | Sum | Weighted mean of methanol (percent) | Log K_{ow}[1,2] |
	50	70	75	80	85	90	95	100	Second 100			
Carbofuran	63	17	<1	<1	<1	<1	<1	<1	<1	80	54	1.52
Carbaryl	52	32	<1	<1	<1	<1	<1	<1	<1	84	58	1.59
Methidathion	<1	72	<1	<1	<1	<1	<1	<1	<1	72	70	2.2
Malathion	<1	45	22	<1	<1	<1	<1	<1	<1	67	72	2.75
Lindane	<1	24	41	2	<1	<1	<1	<1	<1	67	73	3.55
Chlorpyrifos Oxon	<1	26	68	<1	<1	<1	<1	<1	<1	94	74	—
Phosalone	<1	5	71	<1	<1	<1	<1	<1	<1	76	75	4.3
Fonofos	<1	12	58	16	<1	<1	<1	<1	<1	86	75	3.94
Sulfotep	<1	9	74	23	2	<1	<1	<1	<1	108	76	4.00
Disulfoton	<1	<1	59	33	<1	<1	<1	<1	<1	92	77	3.95
Diazinon	<1	5	64	31	7	4	1	2	1	115	77	3.3
Piperonyl Butoxide	<1	<1	16	95	16	5	<1	<1	<1	132	80	4.75
Ethion	<1	<1	8	70	31	6	<1	<1	<1	115	82	5.07
Chlorpyrifos	<1	<1	8	56	25	8	7	7	<1	111	82	4.7

[1]Tomlin (1994).
[2]Montgomery (1993).

permethrin was quantitatively removed by the cartridge but not recovered, and a larger fraction (72 percent) remained on the cartridge. In contrast, dimethoate was not removed by the cartridge extraction, and 100 percent of the compound was detected in the rinsate.

Results of Phase II and recovery in the different fractions are given in table 2 and figure 1. The recoveries in the individual fractions are listed in each column followed by the sum of the individual fractions. The weighted mean of methanol (in percent) is calculated because some insecticides were detected in two or more fractions. These results also are plotted in figure 2 to show the separation and overlap of the select insecticides during the Phase II TIE process. The majority of the insecticides were detected in fractions ranging from 50 percent to 85 percent methanol, with the largest number of insecticides eluting in the 75-percent fraction.

The elution characteristics of each insecticide are controlled by their chemical properties, such as hydrophobicity and octanol-water partitioning

(Thurman and Mills, 1998). The octanol-water coefficient (K_{ow} or, more typically, log K_{ow}) for an insecticide represents the concentration in the organic phase divided by the concentration in water at equilibrium. Therefore, the larger the log K_{ow}, the more likely the insecticide will partition into methanol rather than water and, therefore, the larger percentage of methanol that is required to elute that compound from the cartridge. A plot of weighted mean of methanol (percent) versus log K_{ow} (table 2) shows the expected relation (fig. 3). In addition, the low log K_{ow} of dimethoate (log K_{ow} of 0.71; Tomlin, 1994) (reflecting its low hydrophobicity) explains why it does not sorb to the C-8 resin and, therefore, is not removed by the extraction process. In contrast, permethrin has a high log K_{ow} (log K_{ow} of 6.1; Tomlin, 1994) and high hydrophobicity. Not only is the permethrin readily sorbed by the C-8 SPE cartridge, but it is bound so strongly that it is not removed by elution with methanol.
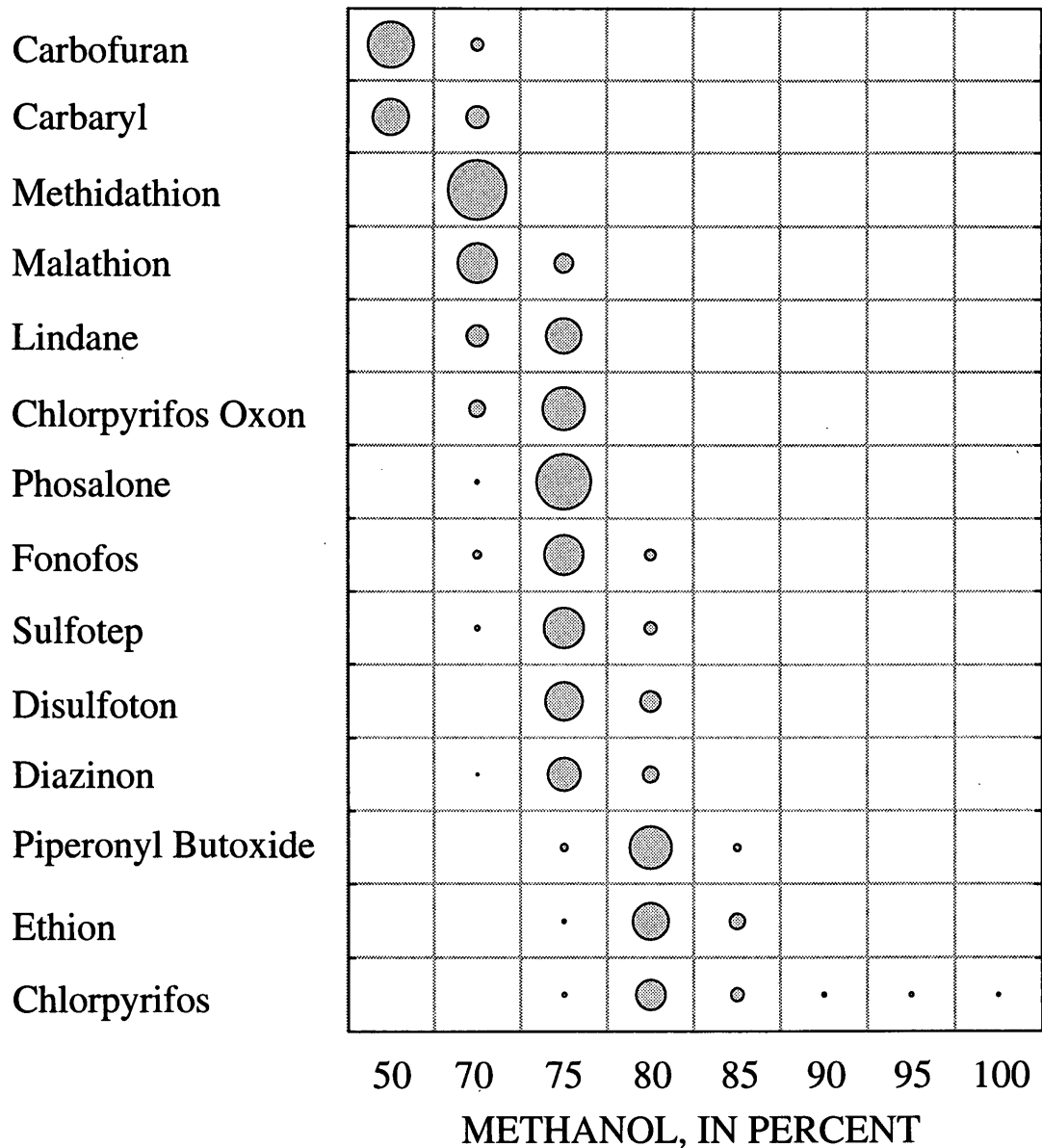
Figure 2. Recoveries of insecticides in consecutive methanol:water fractions. Diameter of circle is proportional to recovery in that fraction normalized to the total recovery for that compound. The circle diameter for methidathion (70 percent methanol) represents 100 percent recovery.
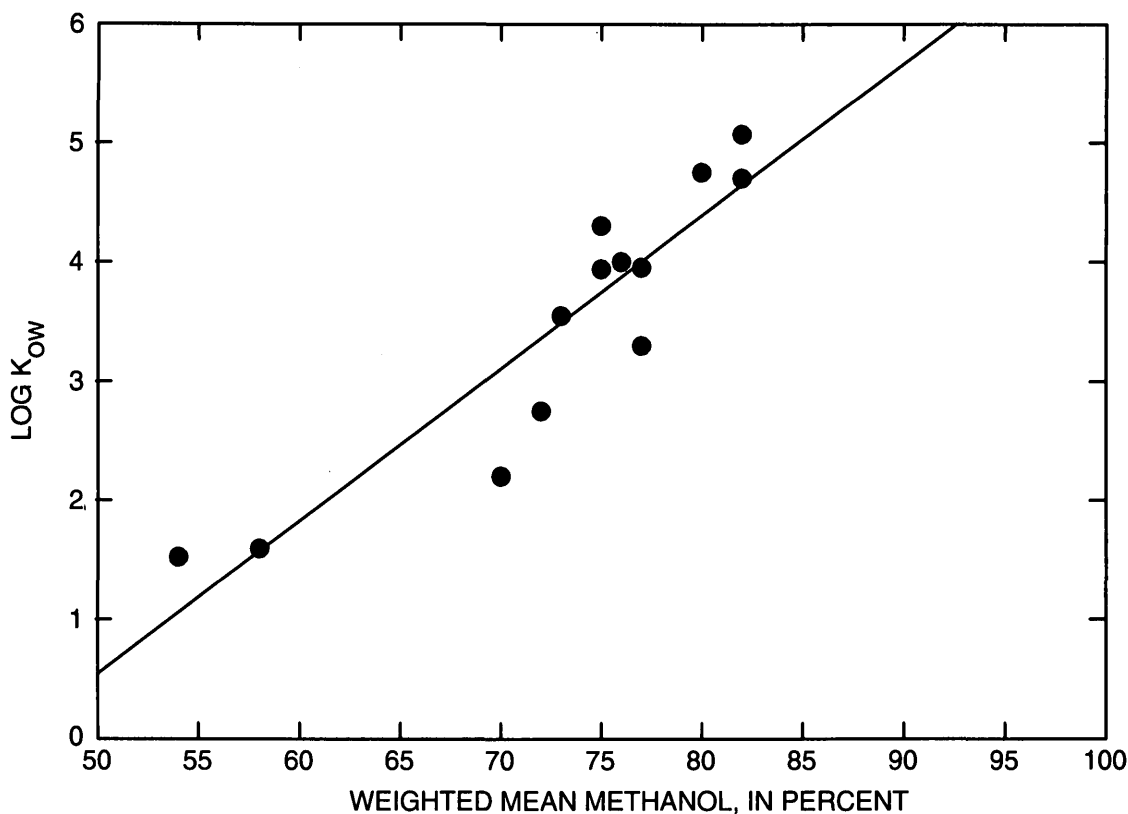
Figure 3. Log K_{ow} versus weighted mean of methanol (percent) for the select insecticides during Phase II of the toxicity identification evaluation procedures

SUMMARY AND CONCLUSIONS

A schematic summarizes the expected response of the select insecticides in an ambient water sample to TIE procedures (fig. 1). In the laboratory study, dimethoate was not removed by extraction on a C-8 SPE cartridge, suggesting that toxicity in an ambient water sample caused by dimethoate also would not be removed by pumping through a C-8 SPE cartridge. In contrast, permethrin was removed by extraction but not recovered with methanol elution in the laboratory study. This suggests that toxicity during a Phase I TIE, which was removed but not recovered, could be caused by permethrin. Chlorpyrifos also was removed by extraction but only partially recovered with methanol elution (62 percent). A TIE resulting in quantitative removal but only partial recovery could be caused by chlorpyrifos. The other 13 insecticides were removed quantitatively by extraction and recovered by elution in the laboratory experiment and would be expected to respond the same way in an ambient water sample.

The purpose of Phase II was to identify the possible toxic compound(s) by separating the ambient water sample into different fractions. In the laboratory study, the 14 insecticides were recovered primarily in four fractions (50, 70, 75 and 80 percent methanol:water) with seven of the insecticides recovered primarily in the 75-percent methanol:water fraction. This suggests that a Phase II TIE on an ambient water sample that results in toxicity in the 75-percent methanol:water fraction would not be useful in narrowing the identification of the toxic compound(s). In contrast, a Phase II TIE could be used to distinguish between toxicity caused by carbofuran or carbaryl as opposed to piperonyl butoxide, ethion, or chlorpyrifos. Increasing the number of fractions in the methanol:water gradient or using a different type of SPE cartridge or column (Bailey and others, 1996) might help in separating more of the insecticides from each other.

The matrix of an ambient water sample could affect the elution characteristics of the C-8 SPE cartridge during the methanol:water gradient, so the

recoveries listed in table 2 may vary slightly with different ambient water samples. For example, fonofos, which was detected primarily in the 75-percent methanol:water fraction, could instead elute primarily in the 70-percent or 80-percent methanol:water fractions. The Phase II results should be evaluated in combination with other TIE information, such as the results of addition of chelating agents or metabolic inhibitors or adjusting the pH, and with chemical analysis of the ambient water (Bailey and others, 1996).

The relation between log K_{ow} and the percentage of methanol:water fraction (fig. 3) could be used to predict the response of untested insecticides to TIE procedures. For example, from the linear regression, an insecticide with a log K_{ow} of 3.15 would be predicted to elute primarily in the 70-percent methanol:water fraction. In addition, the response of dimethoate and permethrin suggests other predictive capabilities of the TIE procedure. Dimethoate, with a log K_{ow} of 0.71 (Tomlin, 1994), was not retained by the SPE cartridge, whereas, carbofuran with a log K_{ow} of 1.52 was quantitatively retained. These results suggest that insecticides with log K_{ow} of approximately 1.0 or less will not be retained by the SPE cartridge, similar to dimethoate. On the other hand, chlorpyrifos, with a log K_{ow} of 4.7, was only partially recovered from the SPE cartridge and permethrin, with a log K_{ow} of 6.1 (Tomlin, 1994), was not recovered at all. These results suggest that insecticides with log K_{ow} of approximately 5.0 or greater will not be quantitatively recovered from the SPE cartridge with methanol.

REFERENCES

Bailey, H.C., DiGiorgio, C., Kroll, K., Miller, J.L., Hinton, D.E., and Starrett, G., 1996, Development of procedures for identifying pesticide toxicity in ambient waters—Carbofuran, diazinon, chlorpyrifos: Environmental Toxicology and Chemistry, v. 15, no. 6, p. 837-845.

California Regional Water Quality Control Board, 1991, San Joaquin watershed bioassay results, 1988-1990: Staff Report, 79 p.

———, 1993, Pesticides in surface water from applications on orchards and alfalfa during the winter and spring of 1991-1992: Staff Report, 79 p.

———, 1995, Insecticide concentrations and invertebrate bioassay mortality in agricultural return water from the San Joaquin Basin: Staff Report, 190 p.

Crepeau, K.L., Domagalski, J.L., and Kuivila, K.M., 1994, Methods of analysis and quality assurance practices of the U.S. Geological Survey Organic Laboratory, Sacramento, California—Determination of pesticides in water by solid-phase extraction and capillary-column gas chromatography/mass spectrometry: U.S. Geological Survey Open-File Report 94-362, 17 p.

Eaton, A.D., Clesceri, L.S., and Greenberg, A.E., eds., 1995, Standard methods for the examination of water and wastewater (19th ed.): Maryland, United Book Press, Inc., 1,040 p.

Kuivila, K.M. and Foe, C.G., 1995, Concentrations, transport, and biological effects of dormant spray pesticides in the San Francisco Estuary, California: Environmental Toxicology and Chemistry, v. 14, no. 7, p. 1,141-1,150.

Montgomery, J.H., 1993, Agrochemicals desk reference—Environmental data (1st ed.): Chelsea, Mich., Lewis Publishers, Inc., 625 p.

Thurman, E.M., and Mills, M.S., 1998, Solid-phase extraction—Principles and practice: New York, John Wiley and Sons, 344 p.

Tomlin, C., ed., 1994, The pesticide manual—A world compendium (10th ed.): Farnham, Surrey, Great Britain, The British Crop Protection Council, The Bath Press, Bath, 1,341 p.

U.S. Environmental Protection Agency, 1991, Methods for aquatic toxicity identification evaluations—Phase I toxicity characterization procedures (2nd ed.): Environmental Protection Agency Report 600/6-91/003, 75 p.

———, 1993, Methods for aquatic toxicity identification evaluations—Phase II toxicity identification procedures for samples exhibiting acute and chronic toxicity: Environmental Protection Agency Report 600/R-92/080, 48 p.