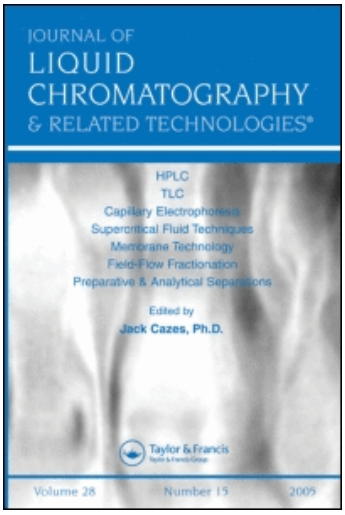

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A Comparison of Sample Preparation Techniques for the Determination of an Organo-phosphorous Pesticide from Water Using Reverse-Phase HPLC

William A. Saner[a]; James Gilbert[a]
[a] Development Center Avery Point, U.S. Coast Guard Research, Groton, Connecticut

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A COMPARISON OF SAMPLE PREPARATION TECHNIQUES FOR THE DETERMINATION OF AN ORGANO-PHOSPHOROUS PESTICIDE FROM WATER USING REVERSE-PHASE HPLC

William A. Saner and James Gilbert
U.S. Coast Guard Research and Development Center
Avery Point, Groton, Connecticut 06340

ABSTRACT

A methylene chloride liquid/liquid extraction and Sep-Pak C_{18} cartridge adsorption techniques were used to quantify the pesticide, Dursban, in contaminated environmental water samples. Results showed a large disparity between Dursban levels using these two techniques, due primarily to the presence of a large adsorbed fraction of pesticide. A Sample Clarification Kit was used to isolate the particulate fraction, which can subsequently be stripped of its adsorbed pesticide compliment by means of a methanol rinse. Lastly, the filtrate from the Sample Clarification Kit may be trace enriched on a Sep-Pak C_{18} cartridge to isolate the dissolved fraction of pesticide.

INTRODUCTION

An accidental release of the organo-phosphorous pesticide, Dursban (O-O-diethyl-O-[3,5,6-trichloro-2-pyridyl]-phosphothioate, figure 1) to the environment resulted from a fire in a chemical packaging plant in Florida. The ensuing ground contamination and leaching necessitated determining the extent of contamination in a nearby waterway to allow a spill mapping of concentration gradients at the packaging plant site for clean-up work.

However, due primarily to its extreme hydrophobicity, elevated concentrations of the pesticide can exist in water only if it is either adsorbed onto particulates suspended in the water

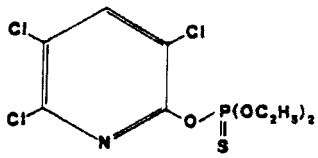

FIGURE 1

Structure of Dursban

column or dissolved in the hydrophobic portion of an oil/water emulsion. Henry et al. (1) found that when a similar organophosphorous pesticide, Abate, was added to pond water, this resulted in its adsorption onto suspended organic matter. This adsorption caused an initial rapid decrease in the concentration of dissolved Abate. Otsuki et al. (2) also recognized the adsorption phenomenon of Abate and relied upon the use of an emulsifier to form micelles to keep a homogeneous pesticide/water solution and avoid adsorption onto suspended particulates such as bacteria and algae.

The possibility of Dursban behaving similarly to Abate, i.e., becoming adsorbed onto particulates, particularly for high-concentration environmental samples, in addition to also existing in a dissolved (non-adsorbed) condition, necessitated extraction techniques which could address both adsorbed and dissolved physical states of the pesticide. Furthermore, in some cases, on-scene clean-up could be made both more effective and less complicated, simply by mechanical filtration of the contaminated water body to remove a substantial fraction of the adsorbed pesticide. This can be determined only if the sample preparation techniques reflect both potential physical states of the contamination at hand. These techniques included a liquid/liquid extraction using methylene chloride, trace enrichment using Sep-Pak C_{18} cartridges, and mechanical filtration followed by extraction of retained particulates using a Sample Clarification

Kit. Reverse-phase liquid chromatography was used to isolate and quantitate the Dursban contents.

EXPERIMENTAL

Apparatus

A Perkin-Elmer Series 3 liquid chromatograph was used in combination with a Waters 440 Absorbance Detector, Micromeritics 725 Auto-injector, and a Perkin-Elmer Model 56 recorder. A Dupont ZORBAX-ODS analytical column (0.46 cm x 50 cm) together with a Whatman guard column (0.46 cm x 7 cm), packed with CO:PELL ODS, comprised the liquid chromatographic supports.

Operating Conditions

A four-segment gradient from water to acetonitrile, at a flow-rate of .1 mL/min, was used:

T_1 -- 5 minutes -- curve 0.5 -- 0.1% to 30% acetonitrile
T_2 -- 25 minutes -- curve 1.0 -- 30% to 50% acetonitrile
T_3 -- 20 minutes -- curve 3.0 -- 50% to 80% acetonitrile
T_4 -- 15 minutes -- curve 1.0 -- 80% to 99.9% acetonitrile

A solvent purge of 99.9% acetonitrile for 15 minutes duration followed the above gradient prior to returning to 99.9% water for 15 minutes equilibration prior to the next injection.

The strip chart operated at 0.5 cm/min. The UV detector monitored effluent at 280 nm. All separations were carried out at room temperature.

Reagents

Baker HPLC water was used as received; spectroquality acetonitrile (MCB AX 142) was first filtered through Millipore 0.5 mu filters (FHUP04700) prior to use in the chromatograph.

Spectroquality methanol (MCB MX488) and methylene chloride (MCB DX831) were used for sample preparation in conjunction with a Millipore Sample Clarification Kit (equipped with FHLP 01300 filters) and Waters Sep-Pak C_{18} cartridges. An authentic Dursban standard was obtained from the Environmental Protection Agency, Triangle Park, NC. The clay mineral standard Attapulgite #43, from #3 Pit, Attapulgus, GA, was obtained from Ward's Natural Science Establishment, Rochester, NY.

Sample Preparation

Liquid/Liquid Extraction Using Methylene Chloride

Twenty-five mL aliquots of five environmental water samples were withdrawn from near the top portions of the sample containers after allowing the previously inverted samples to stand for about 20 minutes. (This permitted large pieces of insoluble residue to settle out and not be included with the sub-samples taken for pesticide extraction.) The aliquots were extracted in a 125 mL seperatory funnel by shaking twice with two 20 mL portions of methylene chloride. The two combined extracts for each water sample were filtered through Drierite (indicating mesh size 8) contained in a glass-fiber filter (Reeve Angel grade 934AH). (The Drierite and filters were pre-extracted using methylene chloride rinses.) The dried extracts were concentrated by rotary evaporation at 35°C and 15 in. mercury vacuum. The concentrated extracts were adjusted to 2.5 mL volumes with methylene chloride before chromatographing 100 uL amounts.

Trace Enrichment Using Sep-Pak Cartridges

The cartridges were rinsed prior to use with 10 mL portions of acetonitrile, then methanol. (This serves to wet the C_{18} packing and remove from it any organic contamination.) Ten mL of

Baker HPLC water served as a final rinse to remove residual organic solvent left from the pre-rinses so that no dilution of the actual water sample by the pre-rinse solvents could occur during the first part of the enrichment step. This allowed the adsorption of Dursban onto the C_{18} packing to take place for the entire 20 mL sample. (The use of a water pre-rinse is unnecessary for larger sampling volumes since the dilution becomes insignificant.) The water samples were inverted just prior to removal of the 20 mL aliquots from each so that a proportional sampling of all entrained particulates, including "large" pieces, was included. The water samples were applied to the cartridges at about 20 mL/minute flow by means of a hand-held syringe. Ten mL of room air was applied to the cartridges to remove residual water, followed by a 2 mL methanol rinse to recover adsorbed compounds from the cartridge. 100 µL injections were made from these final methanol rinses.

Preparation of an Attapulgite Clay - Dursban - Contaminated Suspension and Extraction of the Clay Fraction for Adsorbed Dursban and the Water Fraction for Dissolved Dursban

Attapulgite clay mineral standard was pre-cleaned of organic contamination by means of successive rinses with methylene chloride, methanol, and acetonitrile. The air-dried clay was then suspended in 100 uL of deionized water on a magnetic stirrer to result in a 0.5% suspension. Dursban concentration of 1 ppm in the suspension was made by the addition of 100 µL of a 1000 ppm Dursban-in-methanol standard solution. The pesticide suspension was stirred for forty minutes prior to removal of aliquots for determination of adsorbed pesticide. Fluoropore filters (0.5 mu), as supplied for use with a Sample Clarification Kit, were used for filtering the clay fraction from the water after first extracting the filters (and pre-filters) with methylene chloride then acetonitrile. 10 mL aliquots of the Dursban suspension were removed and filtered. The particulates isolated from the water

aliquots on the filter discs were extracted for adsorbed Dursban first with one mL of methanol, then with one mL of methylene chloride. 100 µL of the combined 2 mL rinse solutions were injected into the liquid chromatograph.

The particulate-free filtrates (20 mL) from two of the above described filtrations were combined and extracted for dissolved Dursban using trace enrichment through a Sep-Pak C_{18} cartridge described earlier.

RESULTS AND DISCUSSION

Determination of Extraction Efficiency for Dursban from Water Using Methylene Chloride Extraction and Sep-Pack C_{18} Cartridge Adsorption

In order to determine the extraction efficiencies of the above-mentioned techniques for removal and recovery of Dursban from water samples, the extraction efficiency for "dissolved" (non-adsorbed) Dursban was determined using particulate-free deionized water. A Dursban standard in methanol (1000 ppm) was used to adjust concentrations of Dursban in water, i.e., 10 ppm, 1 ppm, and 100 ppb. Table 1 lists the extraction efficiencies

TABLE 1

Extraction Efficiencies (%'s) for Methylene Chloride Liquid/Liquid Extraction and Sep-Pak C_{18} Adsorption Techniques for Dursban in Deionized Water

PPM Dursban in Water	$MeCl_2$ (25 mL sampling volume)	Sep-Pak C_{18} (20 mL sampling volume)
10.0	97.4	89.7
1.0	89.1	104.5
0.1	91.3	90.8
	$\bar{x}$ = 92.6	$\bar{x}$ = 95.0
	% rel st dev = 4.3	= 8.7%

for the two techniques. Both are very comparable, and nearly equally effective at removing Dursban from particulate-free water. Assuming a negligible effect on the efficiency of either extraction technique due to the possible presence of an adsorbed pesticide fraction, then closely similar calculated concentrations of Dursban in the environmental water samples should result.

Comparison of Methylene Chloride Liquid/Liquid Extraction and Sep-Pak C_{18} Cartridge Adsorption for Dursban in Environmental Samples

Figure 2 shows the liquid chromatograms resulting from 100 µL injections of the Sep-Pak adsorption of Dursban from environmental samples 2 and 3 and the methylene chloride extractions of the same samples. Since injection volume, detector sensitivity, and concentrating factor (the volume of extraction solvent relative to the volume of water extracted, i.e., X10 for both techniques) are the same in all chromatograms, then the heights of the eluting Dursban peak should be the same for any particular water sample. However, the chromatogram of sample 3, Sep-Pak-adsorbed, contains a tremendously increased amount of Dursban relative to the methylene chloride extract. On the other hand, the heights for the Dursban peaks for sample 2 are much more comparable.

Dursban concentrations calculated from the methylene chloride extractions and Sep-Pak adsorptions for the five environmental samples are listed in table 2. Results show large variability between samples, yet consistently greater concentrations of Dursban for the water samples using the Sep-Pak adsorption technique. The greatest discrepancy between the two techniques is for sample 3, where the Sep-Pak concentration is a factor of 50X greater than that for the methylene chloride. The results for water sample 4, however, show only a factor of 1.25X increase of Dursban from Sep-Pak over that in the methylene chloride extract.

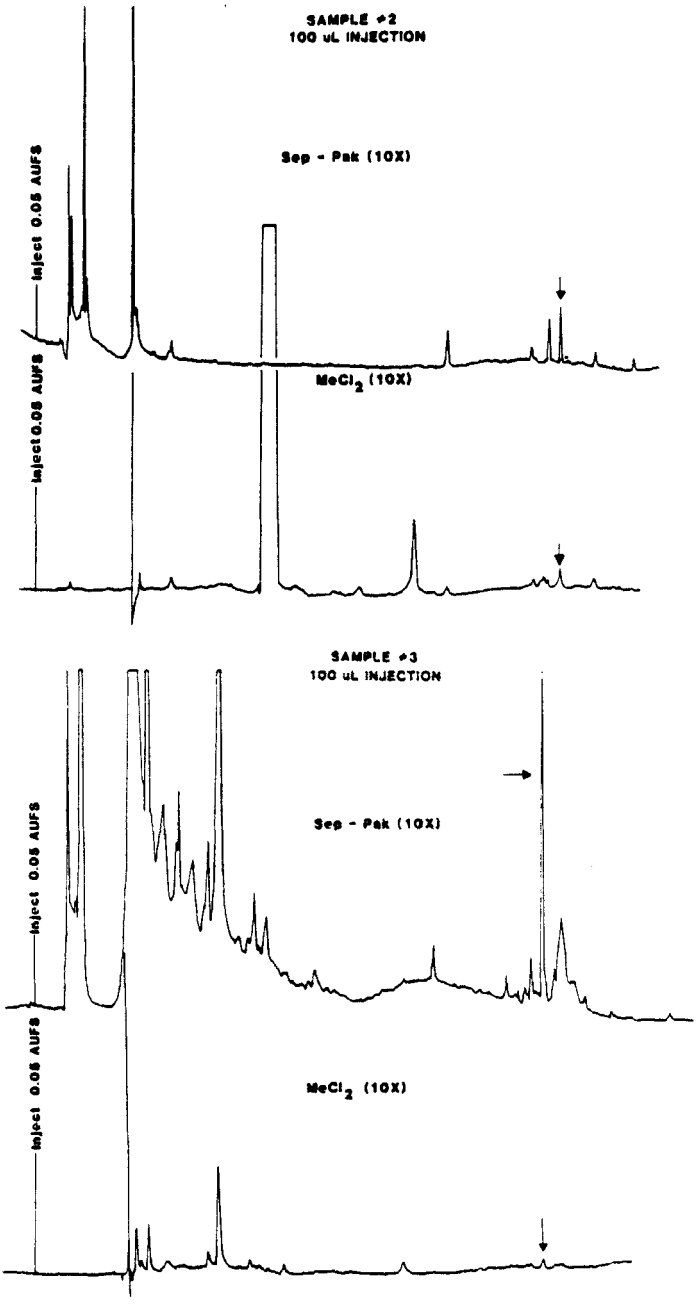

FIGURE 2. Sep-Pak Adsorption and Methylene Chloride Extraction of Dursban (see arrows) from Water Samples 2 and 3. (The 10X notation denotes the concentrating factor for Dursban above the original Dursban concentration in water.

TABLE 2

Dursban Concentrations (ppb) Determined in Environmental Water Samples Using a Methylene Chloride Liquid/Liquid Extraction and Sep-Pak C_{18} Cartridge Adsorption Techniques

	SAMPLE				
	1	2	3	4	5
$MeCl_2$	4,240	80	40	40	70
Sep-Pak	14,100	250	1,900	50	200

These differences in Dursban levels, dependent on the sample preparation, must ultimately be traceable to the presence of an adsorbed Dursban fraction since the extraction efficiency for non-adsorbed Dursban from particulate-free water, over a comparable Dursban concentration range, showed minimal differences for these two extraction techniques (table 1). The reduced methylene chloride extraction capability for Dursban could be due to either (or both) a loss of some of the larger particulates to sedimentation before removal of the aliquots for extraction (with methylene chloride) and/or the reduced capability of this solvent to strip suspended particles of their adsorbed Dursban compliments.

Conversely, the Sep-Pak cartridges acted not only to adsorb Dursban from the water fraction of the sample (dissolved and emulsified), but also acted as mechanical filters by retaining the particulate fraction. This resulted in an accumulation of sediment at the cartridge inlet. The particulates were subsequently co-extracted for adsorbed organic compounds simultaneously with the C_{18} packing in the body of the cartridge by means of the 2 mL methanol rinse originally intended to desorb organic compounds only from C_{18} packing itself.

Determination of Adsorbed and Dissolved Dursban from a Water/Clay Suspension

Neither the organic nor inorganic particulate fractions of the environmental samples were exactly comparable from one sample

to the next. Furthermore, it was realized that the organic particulates, especially, are prone to variability and consequently approximating this portion of the particulate fraction in the laboratory in order to study its Dursban-adsorption capability would be of doubtful applicability. On the other hand, the inorganic content of the particulate fraction from the environmental water samples can be approximated, and should have included a considerable fraction of clay. The particulate clay mineral, Attapulgite, is found in a very localized geographical area of the continental United States, primarily encompassing southern Georgia and northern Florida. Since the environmental samples were collected in Jacksonville, which location is within this geological clay deposit, the likelihood of Attapulgite clay's presence in the samples is very high. Consequently, a study to determine any changes in extraction efficiency for Dursban using methylene chloride extraction and Sep-Pak adsorption was undertaken using a particulate-contaminated water which contained, with high probability, a major particulate present in the original environmental water samples.

In addition, the probable presence of carbonates in the environmental samples was also realized. However, due to a vast reduction in surface area for adsorption (relative to the clay) and a surface charge distribution which is very non-conducive to adsorption of neutral molecules, carbonates were not included. The preparation and extraction of Dursban from 0.5% Attapulgite clay suspension in water was performed as previously described.

The determination of both dissolved and adsorbed Dursban from the clay suspension was originally planned to be determined by both the methylene chloride extraction and Sep-Pak adsorption techniques. However, the Sep-Pak cartridges allowed passage of some of the clay fines during the enrichment step, apparently due to some fines being too small to be filtered out by the cartridge. Therefore, in order to isolate any adsorbed pesticide fraction, a mechanical filtering of 10 mL aliquots of the Dursban-spiked suspension was performed using a Sample Clarification Kit,

described earlier. (Due to the buildup of particles on the clarification kit filters, and ensuing increase in resistance, the sampling volumes were limited to 10 mL of the clay suspension.)

The Sample Clarification Kit filters (two filters, 20 mL of suspension) provided a recovery of 7.5 ug pesticide per filter (15.0 ug of pesticide from 20 mL of suspension). Since a total of 20.0 ug of pesticide was present in 20 mL of suspension (at the 1 µg/mL, 1 ppm Dursban concentration level specified for the suspension) then a recovery of 15.0 ug from the clay fraction represents at least 75% of the total Dursban present in an adsorbed physical state.

Recently, Rogers et al. (3) have demonstrated the adsorption of benzene on montmorillonite clay. In addition to adsorption of ions, Attapulgite clay has also been shown capable of adsorption of neutral molecules. The adsorption of non-polar compounds, such as hydrocarbons, onto Attapulgite, was demonstrated by Nederbragt (4). Consequently, the adsorption of Dursban by Attapulgite clay is not totally unexpected. However, careful consideration should be given to the choice of extracting solvent since some solvents are incapable of thoroughly wetting the external, and particularly, the internal channels of the clay. As a consequence, the stripping of adsorbed organic compounds from the clay may be substantially incomplete. The choice of methanol as a stripping solvent is particularly appropriate due not only to its miscibility with water, but also to its particularly effective clay-wetting ability owing to the alcoholic hydroxyl group-clay interaction.

The Sep-Pak adsorption of the 10 mL filtrates passed by the Sample Clarification Kit filters resulted in a recovery of 5.3 ug of Dursban. Thus a total of 20.3 ug pesticide (15.0 ug adsorbed and 5.3 ug non-adsorbed) represents complete recovery of all Dursban using these two complimentary techniques.

The efficiency of extraction of Dursban from the clay/water suspension was also determined for the methylene chloride liquid/

liquid extraction technique, using 25 mL (containing 25 ug pesticide) of the same 1 ppm Dursban-in-clay suspension. An overall extraction of 82% was obtained, with a recovery of 20.5 ug. However, 75% of the pesticide present was adsorbed onto the clay. The remaining non-adsorbed fraction (25%) represents 6.3 ug pesticide. Using a 93% (table 1) extraction efficiency for the methylene chloride extraction of Dursban from particulate-free water, then 5.8 ug (0.93 x 6.3 ug) of the total recovered Dursban (20.5 ug) existed in a non-adsorbed condition. The remaining 14.7 ug of Dursban (20.5 ug - 5.8 ug) represents a 78% recovery of the total 18.8 ug (0.75 x 25 ug) of adsorbed pesticide present in 25 mL of the water/clay mixture.

CONCLUSION

The disparity in calculated Dursban concentrations between methylene chloride liquid/liquid extraction and Sep-Pak adsorption techniques is due primarily to the presence of a large adsorbed fraction of the pesticide in the environmental water samples. The lower concentrations resulting from the methylene chloride extraction could be due to a partial loss of particulates (and their compliment of adsorbed pesticide) to sedimentation of the water samples prior to removal of the aliquot for extraction. Additionally, methylene chloride demonstrated a reduced capability to strip adsorbed pesticide from a clay mineral sediment relative to its ability to extract the non-adsorbed pesticide from water (78% versus 93%).

Since a large proportion of pesticide may exist in an adsorbed state, no settling of the particulates should be allowed to occur before removal of any sub-samples for analysis.

The use of a Sep-Pak for adsorption of non-adsorbed Dursban resulted in nearly complete recovery. However, a filtration of the insoluble fraction from the water samples and buildup at the inlet side of the cartridge during the sampling (enrichment) step itself was the primary mode of removal of Dursban from the water

samples, and not one of enrichment on the cartridge's C_{18} packing.

The utilization of both a Sep-Pak cartridge for adsorption after a preliminary filtering step using a Sample Clarification Kit, allows the determination of both adsorbed and non-adsorbed fractions of the pesticide with effectively complete recovery.

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