Analysis of Pesticide Exposure Pads Using Selective Absorption and Elution of Reversed-Phase Solid Support

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A sensitive, inexpensive procedure has been developed for the rapid analysis of applicator exposure pads. The pads were extracted with methanol or ethanol, the pesticides were selectively adsorbed and eluted on a reversed-phase solid support (SEP-PAKs) (a concentration step of 50:1), and the elutate was analyzed by high-pressure liquid chromatography. The technique has been field tested with two insecticides, carbaryl and diflubenzuron, providing a sensitivity of 50 and 240 ng of residue/pad (103.2 cm^2), respectively. Multiple samples can be worked up at the same time, and the procedure eliminates the need for more costly organic solvents, liquid/liquid extractions, or solvent evaporation steps.

INTRODUCTION

Analytical methods for pesticides usually require costly high-purity halogenated solvents and time-consuming liquid/liquid extractions and evaporation steps. Also, halogenated solvents used in the procedures need to be disposed of in an environmentally acceptable manner. Any methods that can result in shorter analytical procedures and less use of high-purity organic solvents would be less expensive and more environmentally desirable. Applicator exposure studies generate large numbers of samples (exposure pads) that usually contain low background levels. The purpose of this study was to develop rapid, reliable, inexpensive procedures for the analysis of exposure pads.

Wolkoff and Creed (1981), Lynch and Weiner (1979), and Schauwecker et al. (1977) demonstrated the usefulness of using a reversed phase solid support (SEP-PAK C_{18} cartridge) for trace enrichment of a number of compounds including several pesticides dissolved in water. These studies showed that SEP-PAKs absorbed essentially completely the compounds being tested, but the reversed phase absorption columns have limited capacities and eventually a breakthrough can be expected even with pure materials. Saner and Gilbert (1980) compared a methylene chloride liquid/liquid extraction procedure with a SEP-PAK C_{18} cartridge absorption technique using chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate] in environmental water samples; they showed that SEP-PAKs adsorbed chlorpyrifos efficiently and were superior to a liquid/liquid extraction procedure. Jones et al. (1982) demonstrated the usefulness of using SEP-PAKs for storing field-collected aqueous samples containing carbaryl (1-naphthyl *N*-methylcarbamate) and 1-naphthol. Trace enrichment of azinphos-methyl [*O,O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl] phosphorodithioate] and its oxygen analogue were achieved with SEP-PAKs from vegetable and fruit extracts, and partial cleanup was demonstrated by selective solvent elution of the reversed-phase solid support (Wilson and Bushway, 1981). West et al. (1983) used two SEP-PAKs connected in series to assay volumes up to 500 mL of water. A number of investigators have developed high-pressure liquid chromatography (HPLC) procedures for carbaryl and diflubenzuron [1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea] in crops, soil, and water (Corley et al., 1974; DiPrima et al., 1978; Lawrence, 1977, 1981; Pieper, 1979). Recently Zweig et al. (1984) directly analyzed acetonitrile extracts of exposure pads containing carbaryl by HPLC. We now report the use of SEP-PAKs to develop a rapid, inexpensive HPLC method that does not use costly organic solvents for analysis of applicator exposure pads. Multiple samples can be worked up at the same time. The technique has been applied to exposure pads from a study of incidental and indirect exposure of persons using state parks or other public use areas that have been sprayed with the insecticides, carbaryl, and diflubenzuron (Cameron et al., 1985).

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EXPERIMENTAL SECTION

Sample Extraction Apparatus. Two SEP-PAK C_{18} cartridges (Waters Associates, Inc., Milford, MA) were connected together in series. This was accomplished by forcing a piece of 4-mm (0.d.) glass tubing into the inlet end of one cartridge and the outlet end of another cartridge. The cartridges were conditioned by attaching the free inlet end to a 10-mL glass syringe reservoir and washing with solvent. Ten milliliters of methanol (Fisher, certified grade) was added to the reservoir and the methanol allowed to percolate through the cartridges until about 2 mL had been recovered. The rest of the solvent was then pushed through the cartridges by applying pressure to the syringe plunger. When the reservoir had emptied, but before the cartridge went dry, 10 mL of distilled, deionized water (Milli-Q Water Purification System, Millipore Corp., Bedford, MA) was added to the Reservoir and allowed to percolate through the cartridges as described above. The activated cartridges were then connected to the extraction apparatus (Figure 1) so that the solvent flow went from inlet to outlet ends of the cartridges. At no time from activation until the end of the extraction were the cartridges allowed to go dry.

Samples. The samples consisted of 103.2-cm^2 Johnson and Johnson Topper dressing sponges and ethanol hand rinses. The exposure pads were basted to clothing that was then subjected by the wearer to exposure to the chemical. Similar exposure pads were tacked to table tops the day of spraying, and pads were used to wipe the tables at subsequent sampling dates. The hand rinses were collected after a period of exposure to the sprayed areas at each sampling date. All samples were stored at −20 °C until analysis. [For a more detailed description, see Cameron et al. (1985).]

Sample Extractions and Concentration. For analysis the exposure pads were cut into pieces of less than 1 $in.^2$ and then placed into 250-mL Erylenmyer flasks. The pad pieces were extracted by shaking for 10 min on a Burrell wrist shaker with the appropriate solvent. The ethanol hand washes were reduced in volume by rotoevaporation

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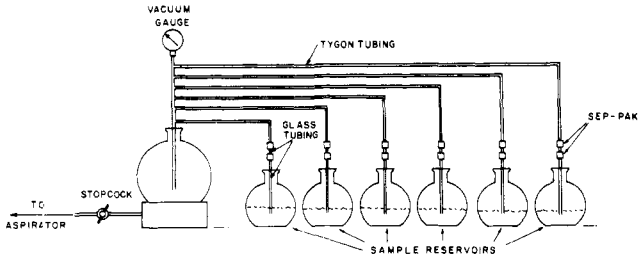

Figure 1. Extraction apparatus used to extract diflubenzuron or carbaryl from an aqueous–methanol extract of applicator exposure pads.

at 40 °C to a volume of 100 mL or less.

The carbaryl pads were extracted in 80 mL of methanol. A 40-mL portion was then decanted into a 250-mL glass-stoppered graduated cylinder, and 160 mL of Milli-Q water was added and thoroughly mixed by vigorous shaking. The methanol–water solution (80% water was then pulled through the dual SEP-PAK C_{18} cartridges with a vacuum of 20 in. of mercury (see Figure 1). The rate of flow through the system was ca. 8–12 mL/min. After all the solution was drawn through the cartridges, an additional 20–30 mL of Milli-Q water was drawn through as a cleanup step. The cartridges were removed from the extraction apparatus and dried by positioning the cartridges vertically, with the outlet end down, on a vacuum source (20 mmHg) and drying the cartridges by passage of air through the cartridge over a 10-min period. The cartridges were then removed from the vacuum and connected by the inlet end to a glass syringe reservoir. A partial cleanup of the sample was achieved by selectively removing the nonpolar interferences with hexane. Hexane (10 mL) was added to the reservoir and percolated through the cartridges. After approximately 2 mL of hexane had dripped through, the remaining hexane was pushed through the cartridges using syringe plunger pressure. Four milliliters of 70:30 (v/v) hexane–ethyl ether was then added to the reservoir and allowed to percolate through the cartridges into a collection vial. Again, after some hexane–ether had dripped into the collection vial, syringe plunger pressure was used to push all the hexane–ether through. The solvent was evaporated under nitrogen, and the residues in the vials were redissolved in methanol for subsequent HPLC analysis.

The diflubenzuron pads were extracted with 80 mL of 95% ethanol. Diflubenzuron is more soluble in ethanol and is a better solvent than methanol in this case. The extracting solvent and pad material were poured into a 125-mm glass funnel containing a folded 185-mm Whatman #40 filter paper. The filtered extracting solvent was collected into a 1000-mL flask, the extracting flask was rinsed twice with 25-mL portions of ethanol, and these rinses were drained through the funnel. All ethanol fractions were combined, and the volume was measured. Milli-Q water was added to the ethanol in the flask (ratio of 4 volumes of water to 1 vol of ethanol) and mixed thoroughly by stirring. The ethanol–water (80% water) was then pulled through SEP-PAK C_{18} cartridges in the same manner as the carbaryl samples. However, after washing the cartridges with an additional 20–30 mL of Milli-Q water and drying by vacuum, the diflubenzuron residue was removed from the cartridges in 4 mL of methanol. No cleanup was necessary since there were no interfering substances at the wavelength used for detection. The methanol was then either concentrated under a stream of nitrogen or diluted as needed.

HPLC Analysis. The samples were analyzed with a Model ALC/GPC 244 high-pressure liquid chromatograph equipped with Model 6000A pump, WISP-710 B automatic injector, a Lambda Max 480 variable-wavelength spectrophotometer, and a Data Module integrator–recorder (Waters Associates, Inc.). The column used for analysis was a 25 cm × 4 mm (i.d.) metal column packed with 5 μm C_{18}-bonded phase (S5 ODS, Supelco, Inc., Bellefonte, PA) with a 2-cm guard column packed with 37–75 μm Porasil B (Waters Associates, Inc.). The mobile phase was acetonitrile/Milli-Q water, 60:40 for carbaryl and 50:50 for diflubenzuron, at a flow rate for both of 1.2 mL/min. The detector was set at 220 nm for carbaryl and 254 nm for diflubenzuron.

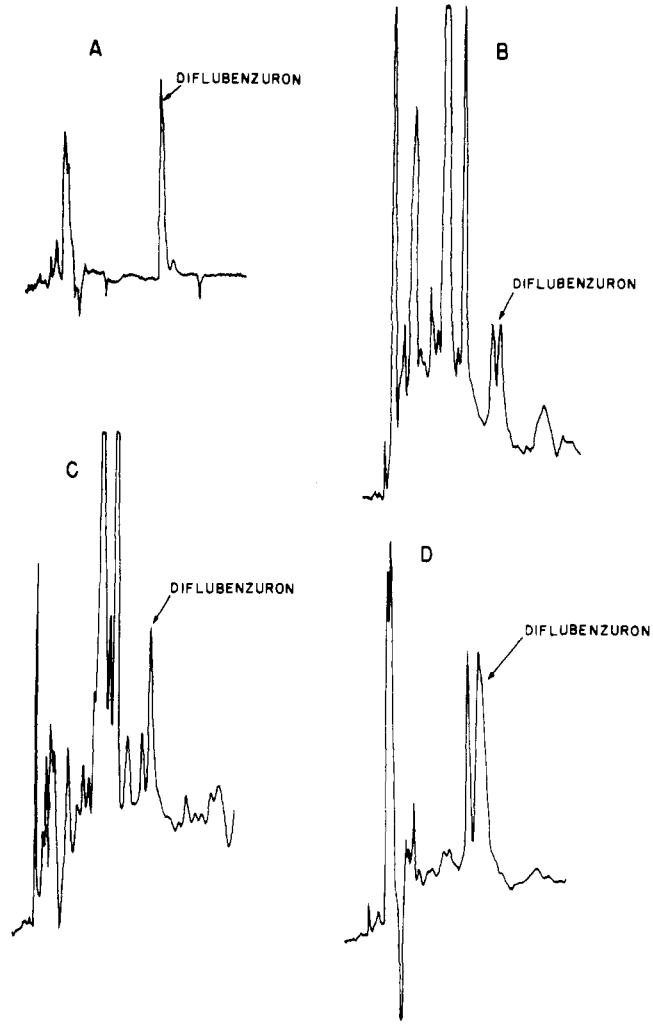

Figure 2. High-pressure liquid chromatograms of various solvent extracts of exposure pad material and diflubenzuron spike: standard diflubenzuron (A); methylene chloride extract (B); hexane extract (C); methanol extract (D). Acetonitrile-water (50:50) at a flow rate of 1.2 mL/min was used with a 5-μm C_{18}-bonded phase (S5 ODS) column. Detection was at 254 nm.

RESULTS AND DISCUSSION

Figure 2 shows the background materials extracted from the exposure pads by various solvents after rotoevaporation as analyzed by high-pressure liquid chromatography. Methanol or ethanol extracted the desired compound and gave the least background. The methanol or ethanol extracts, after dilution with water, were adsorbed on the SEP-PAK C_{18} cartridges, eluted with methanol (2 mL), and analyzed by HPLC. No further cleanup was necessary with diflubenzuron samples since no major interferences were observed at 254 nm. The carbaryl-containing samples needed to be cleaned up, because of interference, by washing the SEP-PAK containing the adsorbed sample and carbaryl with hexane (Figure 3). The ratio of meth-

Table I. Effects of the Ratio of Methanol to Water on the Retention of Diflubenzuron and Carbaryl on SEP-PAK C_{18} Cartridges

% methanol	% water	% chem retained on SEP-PAK[a]	
		diflubenzuron	carbaryl
100	0	0	0
80	20	0	0
70	30	0	0
60	40	0	22
50	50	0	42
33	67	86	75
25	75	90	88
20	80	100	100

[a] 10 μg of chemical and 100 mL of solution. Data represent means of two experiments.

Table II. Retention of Diflubenzuron and Carbaryl on the First or Second SEP-PAK C_{18} Cartridges Connected in Series

sample	% recovery			
	1st SEP-PAK cartridge		1st and 2nd SEP-PAK cartridges in series	
	diflubenzuron	carbaryl	diflubenzuron	carbaryl
1.0 μg without pad matl	99.2 ± 1.1	78.8 ± 3.2	93.2 ± 0.5	98.2 ± 1.2
1.0 μg with pad matl[a]	59.9 ± 11.2	46.2 ± 6.1	99.6 ± 4.2	91.5 ± 2.2
10.0 μg without pad matl	98.3 ± 0.7	56.2 ± 3.8	99.5 ± 1.9	97.8 ± 2.2
10.0 μg with pad matl[a]	38.6 ± 5.4	28.6 ± 4.4	97.7 ± 1.3	90.5 ± 6.8

[a] Pads were extracted with enthanol for diflubenzuron and with methanol for carbaryl samples. SEP-PAKs were eluted with methanol for diflubenzuron and with diethyl ether–hexane (70:30, v/v) following a hexane wash (cleanup) for carbaryl. Data represent means of three experiments.

anol to water for best retention of diflubenzuron and carbaryl on SEP-PAK C_{18} cartridges is at least 1:4 (Table I). The best extracting solution is 100% water, but the chemicals are too insoluble in water (~0.2 ppm at 20 °C for diflubenzuron and ~40 ppm at 30 °C for carbaryl). Thus, a 20% methanol (or ethanol)/80% water solution was used to ensure solubility of the chemicals.

The data presented in Table II demonstrate that the retention of diflubenzuron on SEP-PAK C_{18} cartridges in the absence of any interfering sample material is essentially the same whether trapped on the first SEP-PAK or with two SEP-PAKs connected in series. Under these operating conditions carbaryl required two SEP-PAKs for quantitative recovery even in the absence of pad material. A single SEP-PAK can be easily overloaded when extracting and concentrating actual samples. The two SEP-PAKs provide a reasonable margin of safety and ensure almost total absorption of the pesticides in this case. Table III shows the practical application of this technique to samples of exposure pads used in an incidental exposure experiment utilizing diflubenzuron and carbaryl in a forest ecosystem.

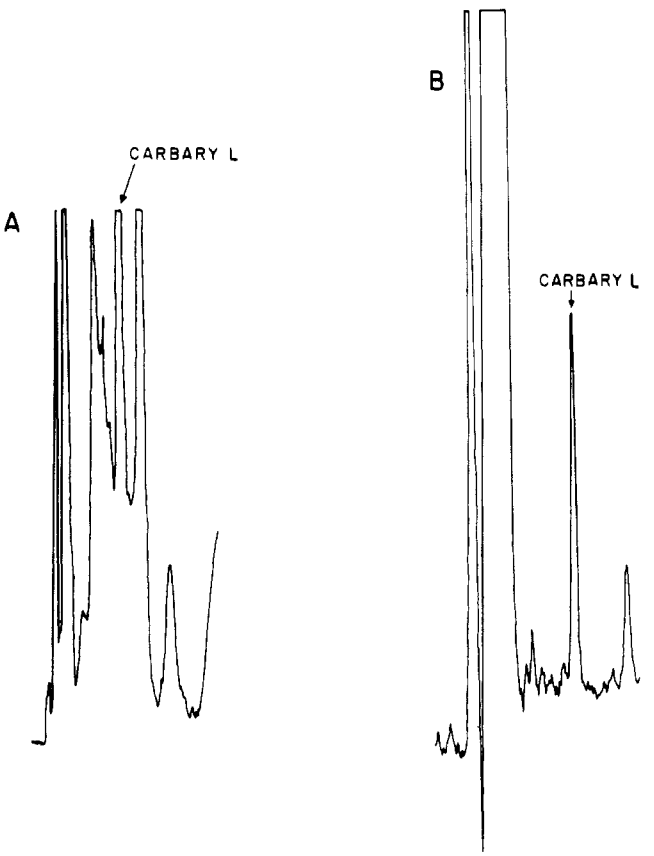

Figure 3. High-pressure liquid chromatograms of methanol extract of exposure pads spiked with carbaryl and adsorbed on SEP-PAKs. Direct hexane-ethyl ether (70:30) wash of SEP-PAK (A) and hexane–ethyl ether (70:30) wash of SEP-PAK after a hexane wash (B). Mobile phase was acetonitrile–water (60:40) at a flow rate of 1.2 mL/min, and detection was at 220 nm.

The single most important step in the use of SEP-PAK cartridges is the conditioning of the cartridges before passing the extracting solution through the cartridge. It is critical that the cartridge never be allowed to go dry. The procedure used in our laboratory was to condition the C_{18} cartridges by first percolating 10 mL of methanol through the cartridge and then 10 mL of Milli-Q water. If at any time during conditioning air was passed through the cartridges, the amounts of chemical retained could vary as much as 75%.

Table III. Residues of Carbaryl or Diflubenzuron Found in Exposure Samples

exposure location	μg of carbaryl per pad[a]				μg of diflubenzuron per pad[a]			
	0 day	7 days	14 days	22 days	0 day	7 days	14 days	21 days
left arm	382.8	1.1	0.8	nd[b]	4.5	nd	nd	nd
right arm	54.5	1.3	1.7	0.1	0.6	nd	nd	nd
left leg	9.3	4.9	0.2	nd	24.5	nd	nd	nd
right leg	54.7	3.0	1.5	2.1	1.8	nd	nd	nd
left buttock	331.7	3.0	2.1	nd	1.0	nd	nd	nd
right buttock	518.0	3.6	nd	0.3	0.4	nd	nd	nd
back	2.4	0.5	0.9	0.3	1.4	nd	nd	nd
head	0.7	nd	nd	nd	14.9	nd	nd	nd
hand washes[c]	28.2	3.0	0.4	2.5	5.0	nd	nd	nd

[a] Data from single individual. [b] nd = not detected, less than 50 ng of carbaryl or 240 ng of diflubenzuron per sample. [c] Ethanol wash of both hands (approximately 300 mL).

With the apparatus used in Figure 1 as many as six samples were simultaneously adsorbed on the SEP-PAKs. Although the chemicals were usually immediately eluted from the SEP-PAKs, they could be stored in this manner for long periods in the refrigerator without noticeable decomposition.

This analytical technique was developed for applicator exposure samples for which we expected to have minimal background material. However, this procedure may have more widespread application, especially for aquatic samples. This procedure provides a rapid, sensitive and inexpensive technique for the analysis of applicator exposure pads, plus multiple samples can be worked up at the same time. The use of methanol and ethanol (certified grade) for extraction eliminates the need for the more expensive pesticide grade organic solvents (halogenated hydrocarbons, acetonitrile, dioxane), which are also environmentally sensitive and require proper waste disposal along with the accompanying costs. The lower limit of detection for carbaryl is 0.5 ng and for diflubenzuron 3.0 ng. The procedure concentrates the extract (50:1), ensuring a sensitive technique, 50 ng of carbaryl or 240 ng of diflubenzuron per 103.2-cm^2 sample pad, which is necessary for applicator exposure experiments since large multiplication factors are typically used to convert pad residues to appropriate skin surface area. The procedure eliminates the need for liquid/liquid extraction or solvent evaporation steps typically found in most residue analysis procedures.

Registry No. Carbaryl, 63-25-2; diflubenzuron, 35367-38-5.

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Chlorinated Dibenzo-*p*-dioxins, Chlorinated Dibenzofurans, and Pentachlorophenol in Canadian Chicken and Pork Samples

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Analysis of 144 chicken and pork tissue samples for pentachlorophenol (PCP) showed more than 60% of fat samples contained greater than 10 parts per billion (ppb; ng/g) PCP while chicken liver had a lower (27%) incidence of positives, and all pork livers contained values over 50 ppb. With the use of new methodology capable of determining all tetra- to octachlorinated dibenzo-*p*-dioxins (CDDs) and chlorinated dibenzofurans (CDFs), the incidence of positives in selected samples of chicken fats for hexa-, hepta-, and octachlorinated CDDs was 50, 62, and 46% with averages of 27, 52, and 90 parts per trillion (ppt; pg/g), respectively. Similar levels of hexa- and heptachlorinated CDFs were also found in some of these samples but tetra- and pentachlorinated CDDs and tetra-, penta-, and octachlorinated CDFs were not detected. A comparison between the chicken tissues and PCP-treated wood with regard to specific isomers and congeners of CDDs and CDFs and their relative proportions showed a marked similarity, indicating that PCP was the source of contamination of the food samples.

INTRODUCTION

Chlorinated dibenzo-*p*-dioxins (CDDs) and chlorinated dibenzofurans (CDFs) are two classes of toxic environmental contaminants that arise from a variety of sources including chlorophenols. The most common chlorophenol, pentachlorophenol (PCP) used as a wood preservative, is known to contain a variety of CDDs and CDFs (Firestone, 1977; Associate Committee on Scientific Criteria for Environmental Quality, 1981) in the high (over 100, sometimes over 1000) parts per million (ppm; μg/g) concentration, with the higher (hexa-, hepta-, octa-) chlorinated congeners predominating. Ryan and Pilon (1982a) detected the higher CDDs in chicken tissues from an incident in which birds were raised in contact with PCP-contaminated wood shavings. This relationship was further documented by Newsome et al. (1984), who, in a controlled laboratory experiment, measured CDDs, pre-CDDs, and

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