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Method for the Analysis of 4-Nitrophenol and Parathion in Soil Using Supercritical Fluid Extraction and Immunoassay

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Analysis of trace residues of pesticides and their breakdown products in the environment is a continuing need. Recent advances in analytical systems have allowed detection of even lower levels of pesticides. Two recent techniques, supercritical fluid extraction (SFE) and enzyme-linked immunosorbent assay (ELISA), have been integrated for the analysis of 4-nitrophenol and parathion in soil. Carbon dioxide SFE gave recoveries equivalent to liquid extraction with ethyl acetate for soils spiked at 10, 1, and 0.1 ppm of each compound. Quantitation of the recovered compounds by ELISA agreed well with analysis by gas chromatography. Parathion extracted from soil was analyzed as 4-nitrophenol by ELISA after oxidation using dimethyldioxirane to paraoxon followed by hydrolysis. Dimethyldioxirane is an easily prepared, volatile, oxidizing reagent that should generally be applicable in converting thionophosphates to their corresponding oxons for analysis. Extraction and analysis by SFE ELISA resulted in greater sample throughput, allowing for rapid screening of a large number of environmental samples. The extraction and derivatization techniques presented here demonstrate a general principle in immunoassay of using volatile extraction solvents and derivatizing reagents to minimize interference with the subsequent immunoassay.

INTRODUCTION

Nitrophenol derivatives play a major role in the chemical industry and in consumer products, having wide application in agriculture as herbicides, insecticides, and fungicides (Hartter, 1985). Organophosphate derivatives of nitrophenol, such as methyl and ethyl parathion, are used for control of pests in agriculture and public health. Although parathion is one of the less persistent of the organophosphate pesticides, its usage does raise environmental concern because of the high toxicity of the parent chemical and its oxon conversion product and its tendency to form other toxic products including 4-nitrophenol, aminoparathion, and diethyl thiophosphate (Sethunathan et al., 1977). Nitrophenols are also key intermediates in the breakdown of aromatic compounds by microorganisms. The presence of extremely low concentrations of phenolic compounds, including nitrophenols, in water is of aesthetic concern since they may impart undesirable flavors or odors in water sources and must be degraded further in water treatment. Nitrophenol derivatives are also introduced into the soil environment by other means, both intentional and inadvertent, and monitoring their presence and persistence in soil is important in determining possible contamination, fate, and transport to untreated areas.

Detection levels of pesticides in environmental samples have been decreased due to advances in analytical systems. Parathion was originally analyzed after alkaline hydrolysis by colorimetric determination of 4-nitrophenol, giving detection limits of approximately 30 ppm (Ramakrishna and Ramachandran, 1978). By contrast, parathion can now be detected by gas chromatography (GC) using specific detectors at parts per billion and lower levels (Barles et al., 1979; Nigg et al., 1979). Phenols are usually derivatized prior to GC analysis for increased sensitivity by formation of an ester or ether derivative (Cohen, 1970; Seiber et al., 1972).

New detection methods have been developed that are less time-consuming and more sensitive than GC. Enzyme-linked immunosorbent assays (ELISAs) have been used to detect a number of pesticides, such as organochlorines, organophosphates, sulfonylureas, phenoxy acids, triazines, and others in the environment (Jung et al., 1989). The advantages to using ELISAs include speed of analysis, low cost, and ability to run analyses without extensive sample workup, particularly for water and soil. ELISAs for parathion (Ercegovich et al., 1981) and paraoxon (Hunter and Lenz, 1982) have been developed and used to screen environmental samples.

An ELISA that detects for 4-nitrophenols and a wide number of monosubstituted phenols has recently been developed which can be used as a good screening system for the metabolites of many organophosphate pesticides of commercial interest (Li et al., 1990). By use of this system, phenols such as 4-nitrophenol, 2-chloro-4-nitrophenol, and 3-methyl-4-nitrophenol can be screened as breakdown products of methyl and ethyl parathion, dicapthon, and fenitrothion, respectively, in the environment.

Extraction of contaminants from soil with solvent may be quite complex, requiring a number of partitioning steps, pH adjustments, and solvent evaporation (Barles et al., 1979; Kliger and Yaron, 1975). Supercritical fluid extraction (SFE) is a new and powerful alternative to conventional organic solvent extraction. Supercritical fluids provide increased rates of extractions compared to subcritical liquid solvents, and since most supercritical fluids (CO_2, N_2O) are gases at ambient conditions, solvent removal is much simpler. The low critical temperature (31 °C for CO_2) means low extraction temperatures can be used to recover thermally unstable solutes. SFE has recently been applied to pesticides in soil and plant material (Capriel et al., 1986; Wheeler and McNally, 1989; Janda et al., 1989), organochlorine and organophosphate pesticides from sand (Lopez-Avila et al., 1990), natural products from vegetative material (Engelhardt and Gross, 1988; Saito et al., 1989; Xie et al., 1989), polynuclear aromatic hydrocarbons, polychlorinated biphenyls, dioxins, and DDT in soil and dust particulates (Hawthorne et al., 1988; Levy et al., 1989; Schantz and Chesler, 1986; King et al., 1989).

In this study, SFE was compared to conventional solvent extraction for recovery of parathion and its breakdown

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Table I. Reiff Fine Sandy Loam Soil Characteristics

% moisture	3.1	% sand	65.6
pH	6.6	% silt	24.3
% organic C	0.4	% clay	8.2

product, 4-nitrophenol, from soil. Analyses of soil extracts by ELISA and GC were also compared. SFE was coupled to an ELISA developed specifically for nitrophenols. Since ELISAs require an aqueous environment, sample preparation can be simplified by supercritical fluid extraction with CO_2. By use of SFE, solvent-exchange steps could be eliminated and sample concentration facilitated. The SFE ELISA technique for analyzing nitrophenols in soil could be used to analyze for 4-nitrophenol or the parent compound, parathion, after its hydrolysis.

MATERIALS AND METHODS

Soil Spikes. EPA analytical grade standard parathion (99.9%) and 4-nitrophenol (analytical standard) from Aldrich were used to spike soil. Fine sandy loam soil (3 g) (Table I) was spiked with an ethyl acetate solution of 4-nitrophenol or parathion at 10, 1, and 0.1 ppm levels. Soil was mixed to homogeneously incorporate the chemicals, and residual solvent was evaporated under nitrogen.

Soil Extractions. For liquid extraction, parathion and 4-nitrophenol were extracted from 3 g of soil with 5 mL of "resi-analyzed" (J. T. Baker) ethyl acetate three times, with agitation for 20 min for each extraction on a rotary shaker. After each extraction, the soil was allowed to settle for 10 min and the solvent was decanted through anhydrous sodium sulfate (Aldrich). The ethyl acetate extracts were combined for subsequent analysis.

For supercritical fluid extraction, soil spiked with 4-nitrophenol or parathion was placed in a 3-mL stainless steel extraction cell (Keystone, Bellefonte, PA) and extracted for 25 min by using CO_2 modified with 5% methanol (v/v) at 50 °C and 2000 psi and a flow rate of approximately 1 mL/min. The pressure was maintained in the extraction cell by using a 22 cm, 50 μm i.d., deactivated fused silica capillary flow restrictor. To prevent freezing within the restrictor, the restrictor was butt-connected to a 10-cm length of deactivated megabore (530 μm i.d.) fused silica capillary, which was used to direct the effluent containing 4-nitrophenol or parathion into 2 mL of methanol at 0 °C.

Gas Chromatographic Analysis. 4-Nitrophenol extracts were analyzed by using a Hewlett-Packard 5890 GC equipped with a 15 m × 0.53 mm i.d. DB-1 column and an electron capture detector (ECD). GC conditions were as follows: column flow, 15 mL/min helium; makeup gas, 55 mL/min 10% methane/argon; detector temperature, 300 °C; injector temperature, 250 °C; column temperature, programmed at 8 °C/min from 125 to 150 °C and then at 18 °C/min to 210 °C.

Parathion was analyzed on a Hewlett-Packard 5710 GC equipped with a 30 m × 0.53 mm i.d. DB-5 column with an NP thermionic specific detector. The GC conditions were as follows: carrier flow, 10 mL/min helium; makeup flow, 10 mL/min helium; air flow, 63 mL/min; hydrogen flow, 2.9 mL/min; injector, detector, and column temperatures were 250, 250, and 220 °C, respectively.

SFE extracts of parathion and 4-nitrophenol from 10 ppm, 1 ppm, and 100 ppb spikes in methanol trapping solvent were diluted as necessary and analyzed. Liquid extracts in ethyl acetate at 10 ppm were directly analyzed, while 1 ppm and 100 ppb samples required concentration under nitrogen before analysis.

Enzyme Immunoassay and Competitive Enzyme Immunoassay Procedure. The procedure was similar to that previously described (Li et al., 1990). Briefly, microtiter plates (96 well; Molecular Devices, Menlo Park, CA) were coated with coating antigen, nitrophenylacetyl ovalbumin conjugate (2 μg/mL, 100 μL/well), in 0.1 M carbonate–bicarbonate buffer (pH 9.6) and incubated overnight at 4 °C. The samples or standards were mixed with antiserum AB1812 against 2-hydroxy-4-nitrobenzyl bovine serum albumin, with the appropriate dilution in phosphate-buffered saline solution containing 0.05% Tween 20 and 0.02% sodium azide (PBSTA), pH 7.4, and were incubated overnight at room temperature. The following day, the coated plates were washed and the samples or standards mixed with AB1812 were added to the wells (50 μL/well). After incubation for 3 h at room temperature, the plates were washed and goat anti-rabbit IgG conjugated to alkaline phosphatase, diluted 1:2500 with PBSTA, was added. Plates were incubated for an additional 2 h at room temperature and washed, and 100 μL of a 1 mg/mL solution of 4-nitrophenyl phosphate in 10% diethanolamine buffer was added to the plates. The plates were incubated for 20–60 min, and the optical density was read with a V_{max} microplate reader (Molecular Devices) at 405 with 560 nm in the dual-wavelength mode. The incubation curves were analyzed by using a four-parameter logistic curve fitting procedure which calculated I_{50} values (molar concentration giving 50% inhibition).

Ethyl acetate extracts of 4-nitrophenol at 10 ppm were diluted for analysis by ELISA, while 1 and 0.1 ppm samples were concentrated under nitrogen. 4-Nitrophenol SFE extracts in methanol were diluted as necessary or analyzed directly.

Parathion Oxidation. Parathion in the soil extracts was oxidized to paraoxon by using dimethyldioxirane prepared according to the method of Adam et al. (1987). Dioxirane solution (50 mM) in acetone was added to 1 mL of the ethyl acetate or methanol extracts, with 200 M excess dioxirane, and 15 min was allowed for completion of the reaction. The oxidized product was then hydrolyzed by adding 1 mL of 0.2 N NaOH and incubating in a water bath at 45 °C for 30 min. The extracts were neutralized with 0.2 N HCl to pH 7 and analyzed by ELISA. Ethyl acetate extracts were first concentrated under nitrogen and the solvent was exchanged with PBSTA buffer, while SFE extracts were directly oxidized and hydrolyzed.

Table II. Recoveries of Parathion and 4-Nitrophenol from Soil by Supercritical Fluid Extraction

compd	extraction time, min	methanol modifier, %	pressure, psi	% recovery[a]
parathion	15	0	2000	62.8
	15	5	2000	93.9
	20	5	2000	87.8
	15	5	3000	98.5
	20	5	3000	108
4-nitrophenol	20	0	2000	24.6
	10	5	2000	29.4
	15	5	2000	74.8
	20	5	2000	88.4
	15	10	2000	79.7
	20	10	2000	82.7

[a] 10 ppm spike.

RESULTS AND DISCUSSION

Comparison of Extraction Techniques. Recoveries of 4-nitrophenol and parathion from soil with supercritical carbon dioxide were investigated by extracting 3-g aliquots of soil spiked separately with 10 ppm of either compound. Initial extractions were performed to determine the length of time required for quantitative recoveries using CO_2, with and without methanol modifier (Table II). Initial studies showed low recoveries with unmodified CO_2. Adding 5% methanol to the CO_2 resulted in complete recovery of parathion in 15 min at 2000 psi; increasing the pressure to 3000 psi did not increase recovery. 4-Nitrophenol was completely recovered from soil within 20 min with 5% methanol in CO_2 at 2000 psi. On the basis of these results, subsequent soil spikes containing 4-nitrophenol and parathion were extracted at 2000 psi with 5% methanol in CO_2 for 15 and 20 min, respectively. Recoveries of 4-nitrophenol from soil at 0.1, 1, and 10 ppm as measured by GC were in the range 88–98% by liquid extraction and 82–97% by SFE (Table III). Parathion recoveries from soil measured by GC were similar: 90–97% by liquid extraction and 85–92% by SFE (Table III).

Table III. Percent Recoveries of 4-Nitrophenol and Parathion from Soil by Solvent and Supercritical Fluid Extraction and GC and ELISA Determination

spiking conc, ppm	ethyl acetate extract[a]		supercritical fluid extract[a]	
	GC	ELISA	GC	ELISA
4-nitrophenol				
10	87.9 ± 3.3	96.5 ± 4.5	97.1 ± 7.4	100 ± 5.9
1	92.1 ± 8.0	86.4 ± 8.6	82.3 ± 4.8	79.5 ± 6.8
0.1	97.9 ± 2.0	92.4 ± 14.5	86.7 ± 8.8	84.8 ± 9.6
parathion[b]				
10	90.1 ± 9.6	93.9 ± 8.5	85.4 ± 2.2	103.5 ± 7.1
1	97.1 ± 5.5	87.9 ± 9.0	89.7 ± 6.2	92.6 ± 4.4
0.1	91.8 ± 5.0	97.0 ± 13.0	92.1 ± 4.3	99.9 ± 28.9

[a] Mean and standard deviation of triplicate samples. [b] Parathion analyzed by ELISA as 4-nitrophenol.

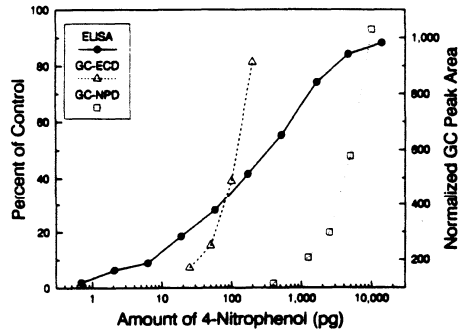

Figure 1. Comparison of 4-nitrophenol standard curves by GC (nitrogen–phosphorus and electron capture detectors) and ELISA.

The major advantage of SFE over the liquid extraction was ease of extraction and sample preparation prior to analysis by GC. Supercritical extraction of 4-nitrophenol or parathion from soil required a total of 20–25 min, and methanol trap extracts could be directly injected on GC without further concentration. Liquid extraction of the two compounds required a 20-min extraction followed by the time needed to allow soil to settle before filtration followed by, in some cases, the time needed to concentrate the extracts to 2–3 mL prior to analysis on GC. There were some problems with soil extraction by SFE. The restrictor became plugged with organic residue extracted from the soil by CO_2, which resulted in drastic drops in flow rate. This problem was easily solved by using new restrictors after four to five extractions; used restrictors could be reclaimed after they were flushed with organic solvents.

The number of samples that could be extracted in a given time was a disadvantage in the setup we employed for SFE. Several ethyl acetate extractions of soil could be done simultaneously, limited only by glassware availability and space. However, the SFE setup used in this study extracted one sample at a time, and only 8–10 soil samples could be extracted and analyzed by GC per day. For routine analysis, it is possible that multiple SFE extractions could be done by using commercially available extractors that can handle as many as six samples at a time.

Comparison of GC to ELISA. The sensitivity of ELISA for 4-nitrophenol was compared to GC analysis. The standard curves for GC (ECD, NPD detection) and ELISA are shown in Figure 1. The steepness of the GC curves shows that better precision of analyses may be obtained by GC than by the current ELISA format. By contrast, the ELISA curve had a wider working range (10 pg–1 ng). Also, ELISA can be more sensitive than GC; the detection limit for GC is 10 ppb by ECD (liquid solution), while the detection limit for ELISA is 0.2–1 ppb in aqueous samples.

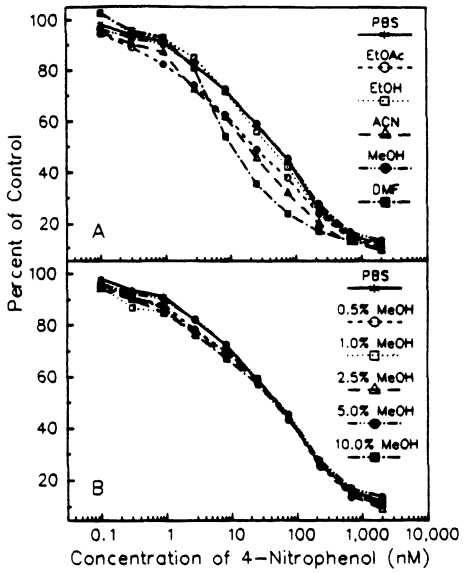

Figure 2. (A) Effect of solvents on ELISA standard curves for 4-nitrophenol. Solvents tested were ethyl acetate (EtOAc), ethanol (EtOH), acetonitrile (ACN), methanol (MeOH), and dimethylformamide (DMF) against the PBS buffer standard. Average percent coefficients of variation (CV) for the curves were 3.4% for ethyl acetate, 6.9% for ethanol, 5.5% for acetonitrile, 5.0% for methanol, 5.5% for PBS, and 3.5% for dimethylformamide. (B) Effect of 0.5–10% methanol (MeOH) on ELISA standard curves for 4-nitrophenol. ELISAs were run with 0.5% MeOH, 1.0% MeOH, 2.5% MeOH, 5.0% MeOH, and 10% MeOH. Percent CV were 3.1% for 0.5% MeOH, 2.9% for 1% MeOH, 3.0% for 2.5% MeOH, and 3.3% for 10% MeOH.

Since water-miscible solvents must be used for ELISA analysis, the effects of solvents on the ELISA standard curve were investigated. Standard curves of 4-nitrophenol were constructed by using 5% solutions of several solvents in PBSTA buffer (Figure 2A). There was little difference between the standard curve with buffer and the standard curve with the water-soluble solvents ethanol, acetonitrile, methanol, and dimethylformamide. Any of these solvents may be compatible with ELISA and thus may be used as a trapping solvent for SFE. Methanol was selected for SFE extracts since it is compatible with both GC and ELISA so that no change in solvent was required in either case. Even 5% ethyl acetate showed little deviation from the buffer solution, so that concentrated organic samples could be simply diluted in PBSTA for ELISA analysis. The effects of the solvents, methanol and ethyl acetate, used for liquid extraction and SFE extraction ELISA were tested more extensively. Methanol (0.5–10%) and 0.5–5% ethyl acetate showed only slight deviations from the PBSTA standard curves (Figure 2B). Up to 25% methanol did not adversely affect ELISA, so that the 0.1 ppm level SFE extracts could be analyzed without sample concentration. SFE produced highly concentrated extracts in a water-soluble solvent, which was a great advantage in integrating the technique with ELISA. All three SFE extracts could be directly analyzed by ELISA with no further sample preparation. By contrast, although the 10 ppm 4-nitrophenol ethyl acetate extracts required dilution with PBS buffer for analysis, the 1 and 0.1 ppm extracts required sample concentration and exchange of the solvent.

4-Nitrophenol in both ethyl acetate and SFE extracts was analyzed by both ELISA and GC (Table III). Both

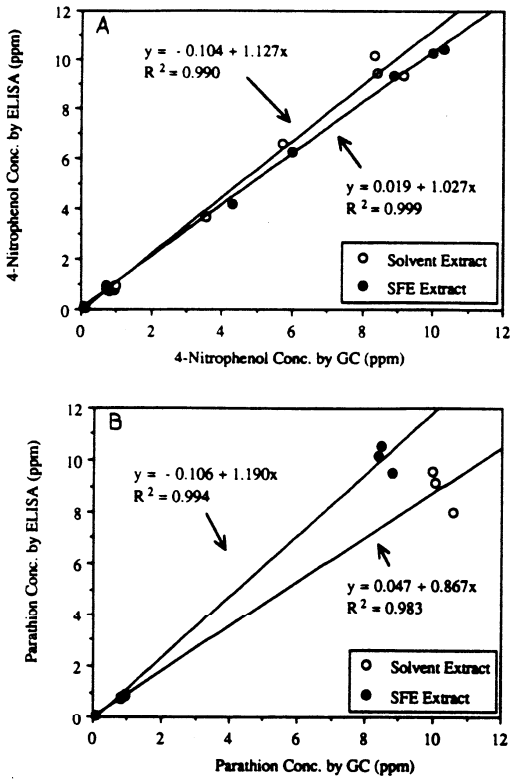

Figure 3. (A) Correlation of GC vs ELISA analysis for 4-nitrophenol extracts by liquid extraction and supercritical fluid extraction. (B) Correlation of GC vs ELISA analysis for parathion extracts by liquid extraction and supercritical fluid extraction.

analytical techniques gave comparable results, with a mean difference of 6.6% for ethyl acetate extracts and 2.7% for SFE extracts. The mean standard deviations for the SFE extracts were also similar by GC (7%) and ELISA (7.5%). The mean standard deviation for ethyl acetate samples by ELISA (9.2%) was higher than that by GC (4.4%). Linear relationships between GC and ELISA had a slope of 1.13 and a correlation coefficient (r^2) of 0.99 for ethyl acetate extracts, while a slope of 1.03 and an r^2 of 0.999 were obtained for the SFE extracts (Figure 3A). Thus, SFE ELISA analyses gave a better precision than ethyl acetate-ELISA and a better correlation with GC; this was likely due to the minimal sample preparation needed for SFE ELISA and possible ethyl acetate interference.

Parathion extracts could not be directly analyzed by ELISA because the antibody for 4-nitrophenol will not recognize parathion. Also, the I_{50} for paraoxon (3000 nM) was much higher than the I_{50} for 4-nitrophenol (60 nM). This 50-fold decrease in sensitivity would allow analysis of only the 10 ppm samples with a paraoxon-based assay. Any contamination of the paraoxon hydrolysis product 4-nitrophenol in a paraoxon sample would skew the analysis since the antibody is so much more sensitive to 4-nitrophenol.

Thus, parathion was quantitatively analyzed after hydrolysis to 4-nitrophenol for more sensitive detection by ELISA. Since the rate of hydrolysis of the paraoxon ($t_{1/2}$ = 26 min; Ramakrishna and Ramachandran, 1979) is faster than that of parathion ($t_{1/2}$ = 103 min; Qian et al., 1985) under basic conditions at room temperature, the parathion samples were first oxidized to the paraoxon by using the oxidizing reagent dimethyldioxirane. Dimethyldioxirane is an efficient oxidizing reagent that reacts with a number of functional groups, including thiophosphonates, under mild and neutral conditions (Sanchez-Baeza et al., 1990). Dimethyldioxirane, being volatile, is easily removed from the sample matrix and would not interfere with the subsequent immunoassay. Oxidation of parathion was complete within 10 min at room temperature with the reaction monitored by the appearance of the paraoxon peak and disappearance of the corresponding parathion peak by GC. The paraoxon was then hydrolyzed at 45 °C for 30 min with completion of the hydrolysis reaction indicated by monitoring the visible absorbance of 4-nitrophenol at 405- and 560-nm wavelengths.

Table III compares the recoveries of parathion as determined by GC and analyzed after conversion to 4-nitrophenol by ELISA. Parathion recoveries were calculated on the basis of molar equivalents to 4-nitrophenol. Recoveries of parathion in the ethyl acetate extracts analyzed by GC and ELISA differed on the average by only 5%, while SFE recoveries by GC and ELISA differed by only 6%. The mean standard deviation of the ethyl acetate samples analyzed by GC was 7%, which was slightly less than that by ELISA (9.8%). The SFE extracts in methanol had standard deviations by GC (4.2%) that were again lower than those by ELISA (12.9%). Linear relationships between GC and ELISA for parathion showed a slope of 0.87 (r^2 = 0.98) for solvent extracts and a slope of 1.19 (r^2 = 0.99) for SFE extracts (Figure 3B). The correlation between GC and ELISA for parathion was not perfect, perhaps due to the additional oxidation and hydrolysis steps required to analyze parathion by ELISA. Parathion analysis by ELISA was also more complicated by ethyl acetate extraction because parathion could not be converted to 4-nitrophenol without a solvent-transfer step. Overall, although standard deviations were higher, ELISA parathion results were comparable to those by GC, even when the extra steps required for analysis were considered.

The major advantage of ELISA over GC analysis was that it was very easy to run a large number of samples rather quickly. As many as 15 plates (more than 90 samples) can be run in 2 8-h days, each plate with a standard curve and six samples at two concentrations, and all samples and standards analyzed in quadruplicate. Analysis by GC took more time because standards and samples required duplicate injections with typical GC runs lasting from 10 to 15 min. Only 10–15 samples with the appropriate number of standards could be analyzed in an 8-h day.

An advantage of GC over ELISA is that all compounds that respond to the detector (ECD or NPD) will be seen as different peaks, giving information on sample composition and amounts, and retention times that can be used to confirm peak identity (Seiber et al., 1990). Also, hypothetically both 4-nitrophenol and parathion could be analyzed in a single GC trace. Unfortunately, this advantage of GC may also be its disadvantage since coeluting background interferences may not allow quantitation of all peaks of interest. While 4-nitrophenol and parathion standards could be resolved easily on a 15-m DB-1 column in real samples, background interferences did not allow quantitation of the parathion peak using the ECD without sample cleanup (Figure 4). Parathion extracts were analyzed by using the more selective NPD. By comparison, although it can be used for *p*-nitrophenol and a number of its isomers, this ELISA is not very sensitive to paraoxon and cannot detect parathion at all. Thus, conversion to 4-nitrophenol was required.

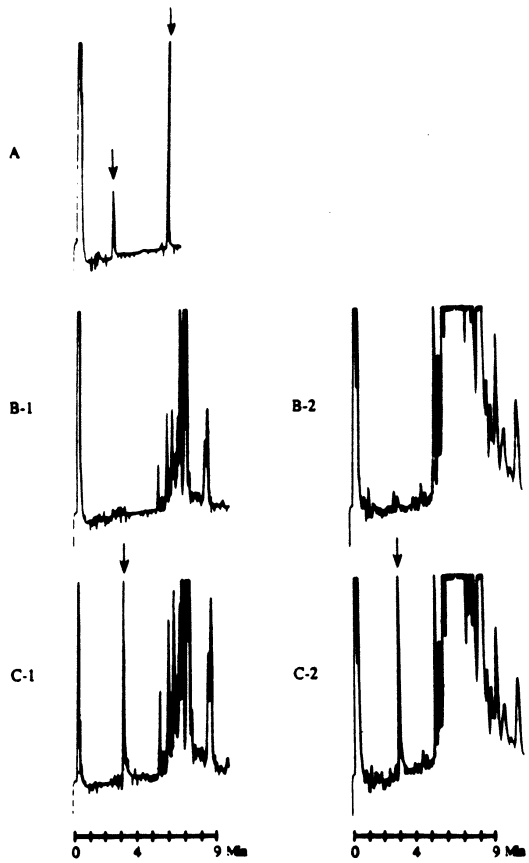

Figure 4. Analysis of parathion and 4-nitrophenol by gas chromatography. Chromatogram A shows 4-nitrophenol and parathion standards, retention times of 3.1 and 6.0 min, respectively, by electron capture detection. Chromatogram B-1 shows a CO_2 extract of a soil blank in methanol trapping solvent, and chromatogram B-2 shows an ethyl acetate extract of blank soil; volumes of extracts were 2 mL. Chromatogram C-1 shows a CO_2 extract of soil (methanol trapping solvent), and chromatogram C-2 shows an ethyl acetate extract of soil, both spiked with 100 ppb of 4-nitrophenol; extract volumes were 2 mL.

CONCLUSIONS

SFE was successfully used to extract 4-nitrophenol and parathion from a soil matrix, giving quantitative recoveries at three spiking levels. SFE extracts were much simpler to prepare than solvent extracts, requiring less time per sample to extract and no subsequent concentration steps. The extracts could then be directly analyzed by either GC or ELISA.

SFE was also an ideal method to extract soil samples for screening by ELISA. Extracts prepared by SFE required no solvent-exchange step and were concentrated enough to be directly analyzed by ELISA. Excellent agreement between GC and ELISA was obtained for 4-nitrophenol and parathion in all of the spiked samples. Both GC and ELISA analyses have advantages and disadvantages, and it may be preferable in some situations to use one technique over the other. Since ELISA has a wide linear range and is relatively rapid, it is ideal as a screening tool for 4-nitrophenol in a large number of samples. With some simple sample manipulations, the ELISA could also be potentially used to analyze for both parathion and 4-nitrophenol in the same sample. For example, half of the soil extract could be analyzed directly for 4-nitrophenol by ELISA. The other half of the extract could be oxidized and hydrolyzed to convert parathion in the sample to 4-nitrophenol which could then be analyzed by ELISA; the difference between the two analyses would account for parathion in the sample. Alternatively, 4-nitrophenol could be separated from parathion by partitioning with mild base solution, with ELISA determinations done separately on the parathion and nitrophenol fractions.

By use of an immunoassay that detects 4-nitrophenol, as well as a number of substituted 4-nitrophenols, this system can be used as a screening tool for breakdown products of environmentally important pesticides such as methyl and ethyl parathion, dicapthon, and fenitrothion. The SFE extract solutions can be divided to analyze both parent compound and metabolites by use of the highly specific and class selective immunoassays, resulting in application of this integrated SFE ELISA system to multiresidue problems.

ABBREVIATIONS USED

CO_2, carbon dioxide; ECD, electron capture detector; ELISA, enzyme-linked immunosorbent assay; GC, gas chromatography; I_{50}, molar concentration giving 50% inhibition; NPD, nitrogen–phosphorus detector; PBSTA, phosphate-buffered saline solution with Tween 20 and sodium azide; SFE, supercritical fluid extraction.

ACKNOWLEDGMENT

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