Gas Chromatographic Determination of Organophosphorus Insecticides and Their Dialkyl Phosphate Metabolites in Liver and Kidney Samples

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A method is described for the quantitative determination of organophosphorus insecticides and six dialkyl phosphate metabolites in animal tissues. The procedure relies on solvent extraction, automated gel permeation chromatography cleanup, and gas chromatographic analysis with flame photometric detection. Tetrabutylammonium hydroxide is utilized as the derivatization reagent for dialkyl phosphate metabolite analysis. Method detection limits ranged from 0.02 to 0.05 ppm for the compounds studied. The method was applied to the analysis of liver and kidney samples of pigeons exposed to diazinon and parathion. Residue levels were confirmed by mass spectrometry, using selected ion monitoring.

INTRODUCTION

Organophosphorus compounds (OPs), widely used as insecticides, frequently pose a hazard to livestock and wildlife. The exposure to OPs can be assessed by a number of methods, including determination of blood cholinesterase levels, residues of the intact compounds in blood and tissues, and urinary alkyl phosphate metabolites. Depression of blood cholinesterase levels has been routinely used as a measure of exposure but has been found to be of low sensitivity and specificity (Bradway et al., 1977; Shafik et al., 1973). Moderate depression of cholinesterase is difficult to ascribe to a specific cause, because numerous factors may affect its activity (Kachmar et al., 1976). Determination of dialkyl phosphate levels provides a more accurate and sensitive assessment of exposure to OPs (Mount, 1984; Drevenkar et al., 1991; Brokopp et al., 1981). Quantities of these metabolites excreted in the urine have been found to correspond to the OP insecticide dose (Franklin et al., 1981; Reid and Watts, 1981). Analysis for intact OP insecticide residues in animal tissues has been a common practice in veterinary diagnostic investigations (Osweiler et al., 1985). Measurement of dialkyl phosphates (DAPs) together with the residues of intact insecticides in biological tissues could provide a powerful tool in assessing pesticide exposure, but it has not been used—primarily because the metabolites are difficult to analyze for. They are highly water soluble and thus hard to extract from tissues with common organic solvents. They cannot be gas chromatographed without derivatization, owing to their low vapor pressures and polar nature (Fest and Schmidt, 1973). There have been several approaches to the development of methods for the multiresidue analysis of dialkyl phosphate metabolites in urine. The method of Shafik et al. (1973) can detect all six of the common OP metabolites in urine samples at levels above 5 ppb, with recoveries from urine spiked at 100 ppb above 95%. However, isomer mixtures of thionate and thiolate esters are formed in irregular proportions when *O,O*-dimethyl phosphorothioate (DMTP) and *O,O*-diethyl phosphorothioate (DETP) are derivatized (Takade et al., 1979; Bradway et al., 1981; Fenske and Leffingwell, 1989). The analysis for DETP using diazoalkanes gave a 41% relative standard deviation, and the GC peaks were obscured by alkylated inorganic phosphate (Reid and Watts, 1981). In addition, the diazoalkane reagents employed by this method are carcinogenic and explosive.

Methods for isolating the dialkyl phosphate metabolites from urine using ion-exchange resin or ion pairing with quaternary ammonium cation have also been developed (Brokopp et al., 1981; Lores and Bradway, 1977; Draper, 1991; Bradway et al., 1981). The method by Bradway et al. (1981) utilized pentafluorobenzyl bromide as the derivatization agent. The disadvantage of this method was the decomposition of the thio compounds with heating. Recoveries ranged from 40 to 100% at the 0.5 ppm spike level. Arylalkyltriazenes have been recommended as alkylating reagents by Takade (1979), but resulting N-alkylated toluidine was an unacceptable interference in the method. Moody et al. (1985) have shown that DMTP can be derivatized on-column with trimethylanilinium hydroxide (TMAH). The use of TMAH increased sensitivity 10–100-fold in the determination of DMTP levels in urine of workers exposed to fenitrothion.

In most existing methods the variable factor in the analysis of alkyl phosphates is the derivatization step. Derivatization creates many interferences, coming from matrix chemicals that are derivatized along with alkyl phosphates. Removal of matrix from sample extracts is essential for good chromatography and reproducible derivatization. In the majority of the existing methods the cleanup step is often eliminated or limited to allow for rapid analysis (Franklin et al., 1981; Moody et al., 1985). Gel permeation chromatography (GPC), used in the present paper, has been successfully applied to cleanup of various oily extracts for organophosphorus insecticide analyses (Holstege et al., 1991; Ault et al., 1979). It is well suited as a multiresidue cleanup method. Another problem commonly encountered by many researchers (Bradway et al., 1981; Brokopp et al., 1981; Weisskopf and Seiber, 1989)

Table I. Chemical Names and Abbreviations of Dialkyl Phosphate Metabolites and Organophosphorus Insecticides Studied

abbrev or common name	chemical name	CAS Registry No.[a]
DMP	*O,O*-dimethyl phosphate	813-78-5
DEP	*O,O*-diethyl phosphate	598-02-7
DMTP	*O,O*-dimethyl phosphorothioate	28523-79-7
DMDTP	*O,O*-dimethyl phosphorodithioate	16001-68-6
DETP	*O,O*-diethyl phosphorothioate	5871-17-0
DEDTP	*O,O*-diethyl phosphorodithioate	3454-66-8
diazinon	*O,O*-diethyl *O*-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] phosphorothioate	333-41-5
parathion	*O,O*-diethyl *O*-(4-nitrophenyl) phosphorothioate	56-38-2

[a] Supplied by the authors.

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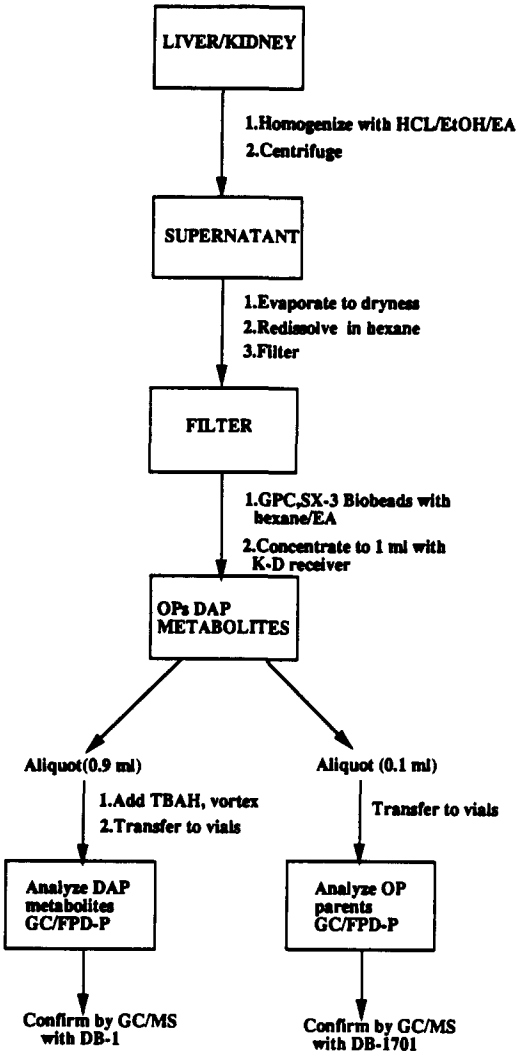

Figure 1. Flow diagram of the overall analytical procedure.

is incomplete derivatization of *O,O*-dimethyl phosphate (DMP) and *O,O*-diethyl phosphate (DEP). A derivatization method that overcame some of the complications previously encountered was developed by Weisskopf (1990). Butylation of dialkyl phosphates using tetrabutylammonium hydroxide reagent was achieved in the GC injection block at 310 °C. This derivatization method was used in the present study, with some modifications.

An objective of this study was to develop a method that could be used to quantitatively determine residues of both parent organophosphorus insecticides and their dialkyl phosphate metabolites in animal tissues rather than urine, which has been the target for most previous work on OP diagnostic metabolites. Any analytical method suitable for post-mortem diagnostic analysis for OP metabolites in large animals would be equally applicable to forensic investigations where OP poisoning is suspected and urine is not available. The method described below is designed for relatively quick turnaround of large sample sets.

EXPERIMENTAL PROCEDURES

Apparatus and Equipment. A Hewlett-Packard (HP) Model 5890 gas chromatograph equipped with flame photometric detector (FPD) and phosphorus filter (525 nm) was used. A glass, cup-type splitter injector port liner (HP part 18740-60840) was used for dialkyl phosphate determination and a glass, splitless, 2 mm i.d. liner (HP part 18740-80220) with 0.5 cm of loosely packed silanized glass wool for parent OP analysis. A Hewlett-Packard Model 7673 autosampler, 2-μL injection volume was used in all GC analyses. The analytical columns were a 30 m × 0.53 mm × 1.5 μm DB-1 (J&W Scientific) and a 30 m × 0.53 mm × 1.0 μm DB-17 (J&W Scientific) used for dialkyl phosphate metabolites and parent organophosphorus insecticides analysis, respectively. Helium carrier gas flow rates were 11 and 12 mL/min, respectively. Operating temperatures were as follows: detector, 275 °C; column programs, 115 °C for 0.5 min, 7 °C/min to 190 °C; run time, 11.21 min, split/splitless injection valve off at 0.8 min. Injector temperature was 310 °C for dialkyl phosphate analysis. The column temperature program for parent OP analysis was as follows: 60 °C for 0.5 min, 30 °C/min to 160 °C, 5 °C/min to 280 °C, 280 °C for 4.2 min; run time, 32 min; split/splitless injection valve off at 0.5 min. Injector was 240 °C for OP analysis.

Quantification was done by comparison with external standard peak areas generated by a Turbochrom 3 data acquisition system with 900 series interface (Perkin-Elmer-Nelson) and HP Model 3393A and Perkin-Elmer Model LCI-100 electronic integrators.

Mass spectral analyses were done using a HP Model 5988A mass spectrometer interfaced with HP Model 5890 GC and controlled by HP ChemStation. Spectra were obtained at 70 eV, with mass range scanned from 40 to 400 amu. A 5 m × 0.53 mm × 1 μm DB-1701 capillary column (J&W Scientific) was used for diazinon confirmation analysis. The carrier gas was helium, flow rate 10 mL/min. Operating temperatures were as follows: injector 240 °C; ion source, 220 °C; transfer line (interface) 280 °C; GC oven program, 60 °C for 0.5 min, 30 °C/min to 160 °C, 10 °C/min to 275 °C. Run time was 15.3 min. All GC conditions for confirmation analyses of dialkyl phosphates were the same as described for FPD with the exception of GC oven program: 120 °C to 200 °C at 7 °C/min, 200 °C for 5 min; run time, 16.5 min.

A gel permeation chromatograph, Model 1002A Autoprep GPC (ABC Laboratories), with 5-mL sample loops was used. The GPC column was 160 mm × 25 mm i.d., packed with 38 g of SX-3 Bio-Beads, 200–400 mesh with exclusion limit of 2000 MW (Bio-Rad Laboratories). The mobile phase was hexane–ethyl acetate 7:3, flow rate 5 mL/min, 15-min dump cycle, 17-min collect cycle, 5-min wash cycle.

A Polytron Model PT 10/35 (Brinkman Instruments, Inc.) tissue homogenizer and French, square homogenization vessels, 250 mL with Teflon-lined caps (Fisher Scientific), were used for tissue homogenizations.

An N-Evap analytical evaporator (Organomation Associates Inc., Berlin, MA) was used for nitrogen evaporation.

Table II. Extraction Efficiency of Dialkyl Phosphates from Spiked Avian Liver Samples with Different Solvents at pH 2

	% recovery			
DAP	5% ethanol in ethyl acetate	acetonitrile	methanol	acetone
DMP	98	80	227[a]	48
DEP	103	100	158[a]	41
DMTP	103	98	102	109
DMDTP	100	99	778[a]	100
DETP	104	95	91	82
DEDTP	100	98	79	99

[a] Large interferences.

Table III. Average Recoveries (n = 3) and Coefficients of Variation (% CV) of Diazinon, Parathion, and Dialkyl Phosphate Metabolites from Fortified Avian Liver and Kidney Samples by the Overall Procedure

	% recovery (% CV)		
	liver		kidney
	0.25 μg/g	0.5 μg/g	0.5 μg/g
DMP	104 (12)	88 (1)	89 (6)
DEP	102 (3)	111 (3)	101 (3)
DMTP	91 (26)	74 (7)	94 (17)
DMDTP	52 (5)	93 (17)	99 (15)
DETP	87 (20)	89 (12)	102 (2)
DEDTP	40 (12)	30 (5)	39 (17)
diazinon		100 (3)	
parathion		101 (2)	

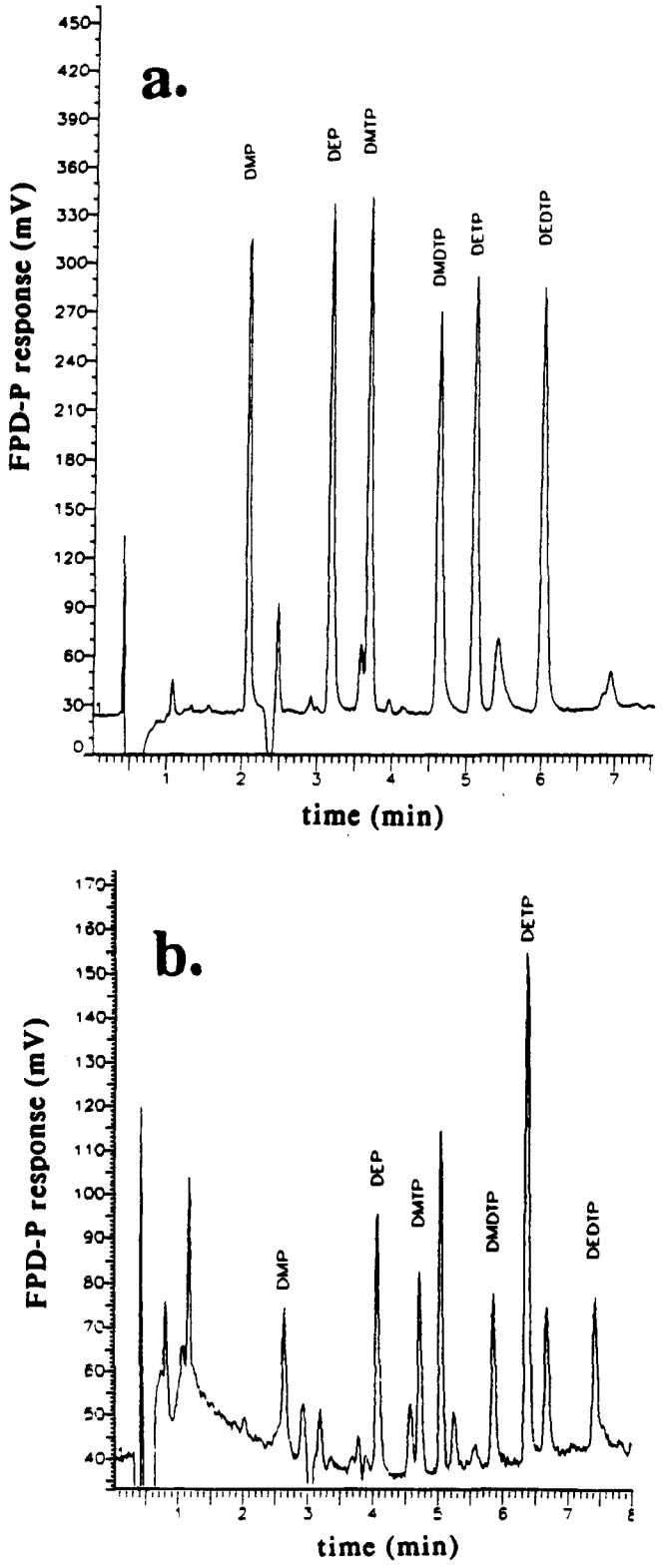

Figure 2. GC-FPD chromatograms of the TBAH derivatives of dialkyl phosphate standards: (a) 0.2 ng each in ethyl acetate; (b) 0.01 ng each in 1 mg of kidney matrix. Retention times for corresponding peaks in parts a and b are different due to change in chromatographic conditions.

An IEC Centra-7 refrigerated centrifuge (International Equipment Co.) and a Bransonic 221 (Branson Corp., Shelton, CT) sonication bath were also used.

Filters were Acrodisk CR PTFE, 0.45 μm, from Gelman-Sciences.

Reagents. Tetrabutylammonium hydroxide (TBAH), 1.0 M in methanol, was from Aldrich Chemical Co. All solvents were of pesticide grade (Fisher Scientific).

Table IV. DEP Metabolite Levels in Liver and Kidney Samples of Pigeons Exposed to Diazinon and Parathion

exposure level	sample ID	DEP,[a] ppm
control	liver 34	ND[b]
	kidney 34	ND
2 mg/kg diazinon	liver 39[c]	0.08
	liver 36	0.05
	kidney 36	0.09
3 mg/kg diazinon	liver 27	0.29
	kidney 27	0.06
	liver 35	0.15
	kidney 35	0.06
0.6 mg/kg parathion	liver 29	0.26
	kidney 29	ND
	liver 30	0.06
	kidney 30	ND
	liver 33	0.17
	kidney 33	0.07

[a] None of the samples showed levels of DMP, DMTP, DMDTP, DETP, or DEDTP above the MDL (0.05 ppm) or diazinon and parathion above the MDL (0.02 ppm). [b] ND, not detected, MDL = 0.05 ppm. [c] Pigeon identification numbers (kidney extract from pigeon 39 was lost during sample preparation).

Table V. Selected Ion Monitoring Program for Simultaneous Confirmation of Butyl Derivatives of Six Dialkyl Phosphates[a]

DAP	acquisition group	start time, min	ions, *m/z*
DMP	1	3.0	153, 127, 109
DEP	2	6.5	181, 155, 127, 99
DMTP	3	7.5	198, 169, 142
DMDTP	4	9.0	214, 159, 158, 125
DETP	5	9.6	226, 197, 170, 143
DEDTP	6	10.0	242, 186, 153

[a] Run time, 16.5 min. TBAH interfering peak: retention time, 6.61 min; *m/z* 185, 143, 142, 100.

Preparation of Standard Solutions. All analytical standards were obtained from the Environmental Protection Agency, Pesticide and Industrial Chemicals Repository. Dialkyl phosphate standards were supplied as the acid form or as sodium or barium salts. Individual standard solutions of 1000 μg/mL of organophosphorus insecticides and acid equivalents of dialkyl phosphates were made in methanol. Two stock standard solution mixes were made at 50 μg/mL in ethyl acetate. Mix 1 contained the parent organophosphorus insecticides: diazinon, parathion, and other compounds listed in Table VII. Mix 2 contained the six dialkyl phosphate metabolites listed in Table I. These standard solution mixes were stable for 6 months, when stored at 5 °C in dark, 50-mL Quorpack bottles. Subsequent dilutions of the above mixes were made in ethyl acetate. Mix 1 was diluted to 0.25 μg/mL with ethyl acetate for analysis of parent organophosphorus insecticides. It was prepared daily from the stock solution. Mix 2 was diluted to 5 μg/mL in ethyl acetate. It was used to prepare working standard mixes ranging from 0.01 to 0.5 μg/mL in a control liver sample extract for analysis of dialkyl phosphates.

Derivatization. This is the modification of the butylation of dialkyl phosphates in the injection block developed by Weisskopf (1990). Forty microliters of TBAH is added for each 1-mL portion of extracts dissolved in ethyl acetate. Manual injections using the "hot needle" technique are replaced with automatic injections by the autosampler.

Procedure. An outline of the overall method is presented in the flow diagram (Figure 1). The details are as follows.

(*a*) *Extraction.* Weigh 5 g of frozen, finely chopped tissue samples into homogenizing vessels. Add 0.5 mL of 50% HCl (v/v) and 100 mL of 5% ethanol–ethyl acetate (v/v) into each vessel. Homogenize for 2 min at high speed. Centrifuge the extracts at 1200 rpm (235 g) for 5 min. Pipet 20-mL aliquots of the extracts into test tubes, add 2 drops of 5% decanol in acetone, and evaporate just to dryness using a nitrogen evaporator at 40

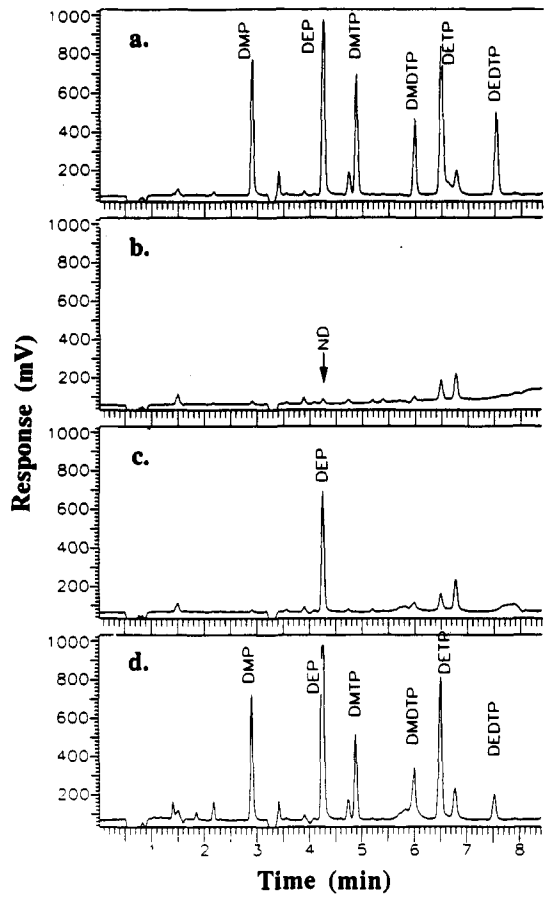

Figure 3. Typical GC-FPD chromatograms of TBAH derivatives of dialkyl phosphates injected onto a 30-m DB-1 megabore column by an autosampler: (a) standard mix in liver matrix, 0.5 ng each; (b) control liver with no detectable metabolites; (c) liver 27 from pigeon exposed to 3 mg/kg diazinon; (d) liver spiked at 0.5 μg/g of each metabolite. One milligram of matrix was injected each time.

°C. Immediately add 10 mL of hexane–ethyl acetate 7:3 (GPC solvent) and sonicate for 1 min to redissolve the residues. Filter samples through 0.45-μm Acrodisk filters.

(*b*) *Gel Permeation Chromatographic Cleanup.* Load sample extracts into the GPC. Each sample loop holds 5 mL of sample extract. However, it is necessary to inject at least 8 mL of each sample. The first 3 mL rinses the system from the previous injection, thus eliminating the possibility of cross-contamination. Run the GPC program as stated above, collecting the eluant into 125-mL Erlenmeyer flasks. Add 3 drops of 5% decanol–acetone (v/v) and evaporate to 5 mL as above. Quantitatively transfer the residue to a 15-mL Kuderna-Danish receiving flask, rinsing with three 2-mL portions of ethyl acetate. Concentrate the samples to 1 mL (final sample concentration 0.5 g/mL). Vortex for 30 s.

(*c*) *Gas Chromatographic Determination of the Parent OPs (Diazinon/Parathion).* Transfer 0.1-mL aliquots of the samples into 0.25-mL Hewlett-Packard autosampler vials. Inject 2 μL of analytical standard (0.5 μg/mL) and the samples (0.5 g/mL) into the GC-FPD with DB-17 megabore column for parent OP determination. Confirm the residues using GC/MS with a DB-1701 megabore column and the selected ion monitoring (SIM) acquisition. Quantitate using external calibration based on injections of 0.1–1.0 ng of OP insecticide standards.

(*d*) *Gas Chromatographic Determination of Dialkyl Phosphates.* Transfer 0.5-mL aliquots of the samples into 2-mL Hewlett-Packard autosampler vials (store the remaining 0.4-mL aliquots at 5 °C for possible confirmation analysis). Add 20 μL of TBAH to all samples, reagent blank, spikes, controls, and dialkyl phosphate standard mixes. Vortex in capped vials for 30 s. Inject 2 μL of dialkyl phosphate analytical standard mixes (concentrations ranging from 0.01 to 0.5 μg/mL of each compound) and samples (0.5 g/mL) into GC-FPD with a 30-m DB-1 megabore column. Confirm residues by GC/MS using identical GC conditions as above and SIM program. Quantitate using external calibration based on injections of 0.05–0.25 μg/mL of dialkyl phosphate standard mix. If confirmation analysis cannot be achieved during 24 h after derivatization, use the additional aliquots of samples stored at 5 °C.

Table VI. Results of Mass Spectral Confirmation of DEP in Livers of Pigeons Exposed to Diazinon—Summary of DEP Ion Ratios

sample ID	% of total ion current		
	m/z 127	*m/z* 155	*m/z* 181
DEP standard, 1 ng in liver matrix	47	50	3
liver 27, 1 mg	49	48	3
liver 39,[a] 1 mg	53	44	3
liver control, 1 mg	ND[b]	ND	ND
liver spike, 0.5 μg/g DEP, 1 mg	48	49	3

[a] Level very close to MDL of 0.05 ppm. [b] ND, not detected.

Table VII. Preliminary (n = 1) Recoveries of Other Organophosphorus Insecticides from Avian Liver Fortified at 0.5 μg/g Level and Analyzed by the Overall Procedure

compd	CAS Registry No.[a]	% recovery
coumaphos	56-72-4	109
butifos	78-48-8	106
ethion	563-12-2	108
fonofos	944-22-9	99
methyl parathion	298-00-0	101
mevinphos	7786-34-7	98
phorate	298-02-2	96
phosalone	2310-17-0	111
terbufos	13071-79-9	104

[a] Supplied by the author.

Treatment. The analytical method was evaluated by analyzing 13 tissue samples, obtained from pigeons (*Columba livia*) exposed to diazinon and parathion, for the residues of the parent OPs and dialkyl phosphate metabolites. The tissue samples utilized here were a part of another experiment on the avian exposure to OPs [see Fry et al. (1992)]. The pesticides used, exposure levels, numbers of birds in each treatment group, and pigeon identification numbers are listed in Table IV. A single, oral dose of compounds (purity >95%) in poly(propylene glycol) was administered to each treatment group by gastric lavage. The animals were sacrificed by cervical dislocation 24 h after dosing, and liver and kidney samples were collected from each animal. Pooled chicken livers from a grocery store were used for method development and standard preparations. All samples were kept frozen at −20 °C for 4 months until analyzed.

RESULTS AND DISCUSSION

The method described here proved to be suitable for quantitative analysis of parent organophosphorus insecticides and dialkyl phosphate metabolites in liver and kidney samples. Several extraction techniques were investigated during the course of method development. Solvent extractions with acetonitrile and 5% ethanol–ethyl acetate resulted in the best spike recoveries of dialkyl phosphates (Table II), with minimum coextractives. Overall a mixture of 5% ethanol–ethyl acetate was chosen in the multiresidue method, because it provided better recoveries for parent OPs and had sufficient polarity to extract the highly polar dialkyl phosphates. In addition, it was important to adjust the pH with hydrochloric acid. The use of sulfuric acid resulted in large interferences throughout the procedure.

A challenging aspect of this work was the development of a cleanup method that would provide satisfactory resolution of analytes from the phospholipid coextractives of complexed sample matrixes. Preliminary studies with a variety of solid-phase extraction (SPE) columns proved satisfactory only for parent OP analysis. The derivati-

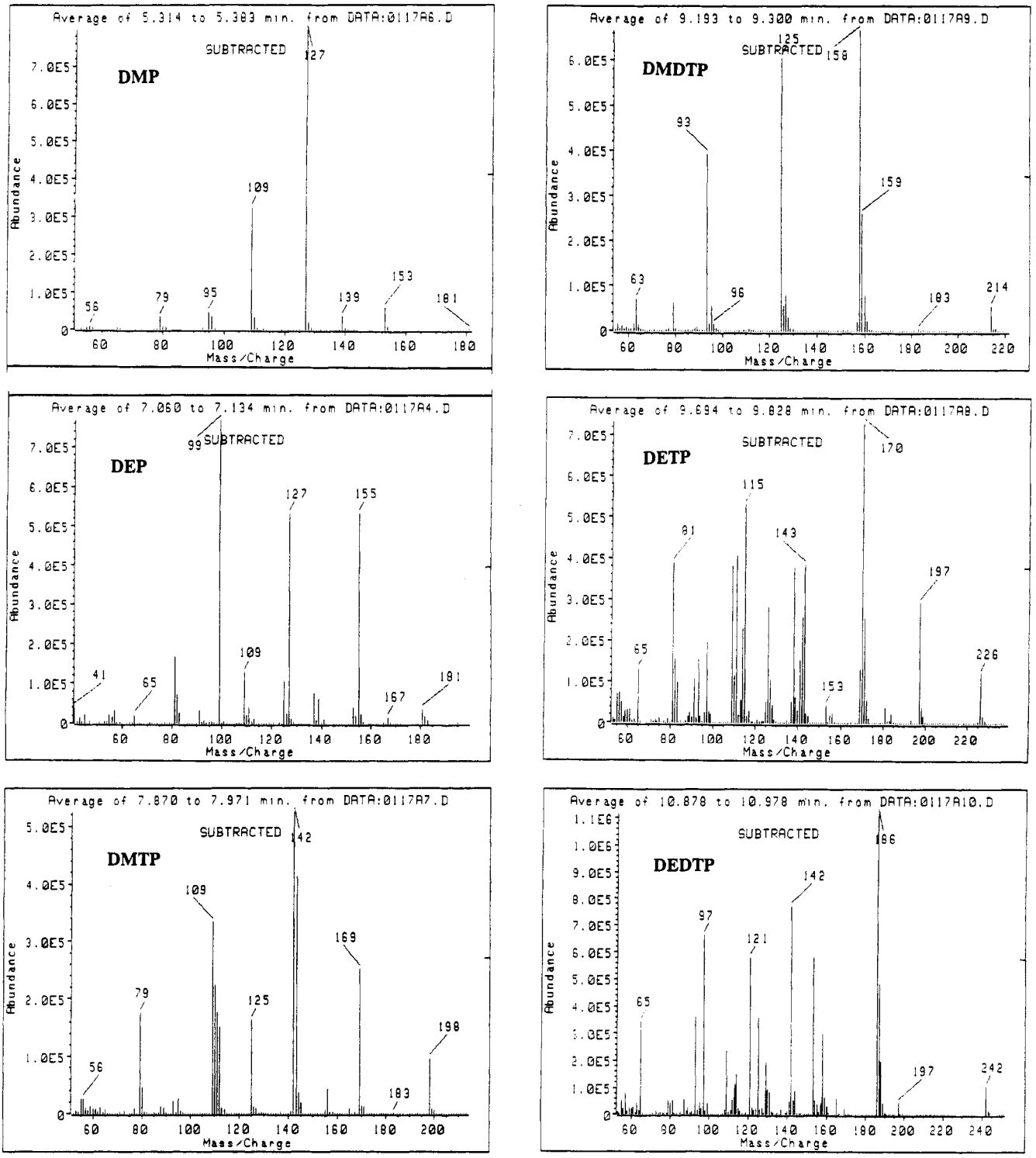

Figure 4. Electron impact ionization mass spectra of butylated (a, top left) DMP, (b, middle left) DEP, (c, bottom left) DMTP, (d, top right) DMDTP, (e, middle right) DETP, and (f, bottom right) DEDTP.

zation step, necessary for dialkyl phosphate metabolite determinations, resulted in large interferences and variable derivatization efficiencies. GPC gave excellent removal of lipids and other large molecules from sample extracts. It produced an extract with the least amount of interferences causing chromatographic difficulties during GC-FPD and GC/MS confirmations of derivatized matrix. A typical chromatogram of DAP standard mix in ethyl acetate and detection limit standard in kidney matrix is presented in Figure 2 to illustrate the cleanup efficiency. One limitation of the GPC cleanup is consistently low recovery of *O,O*-diethyl phosphorodithioate (DEDTP). Table III summarizes the overall spike recoveries from control chicken liver and kidney samples fortified at 0.25 and 0.5 ppm with six dialkyl phosphates and at 0.5 ppm with diazinon and parathion.

Results of Pigeon Exposure Study. None of the liver or kidney samples of exposed pigeons contained the parent organophosphorus insecticides diazinon or parathion at the detection limit of 0.02 ppm. Although throughout the analysis of diazinon-exposed pigeon livers a peak was observed having a retention time very close to that of diazinon, mass spectral confirmation for diazinon ions at m/z 304, 248, and 179 was negative at a detection limit of 0.05 ppm. A more concentrated sample is needed to establish the identity of the peak, possibly a diazinon

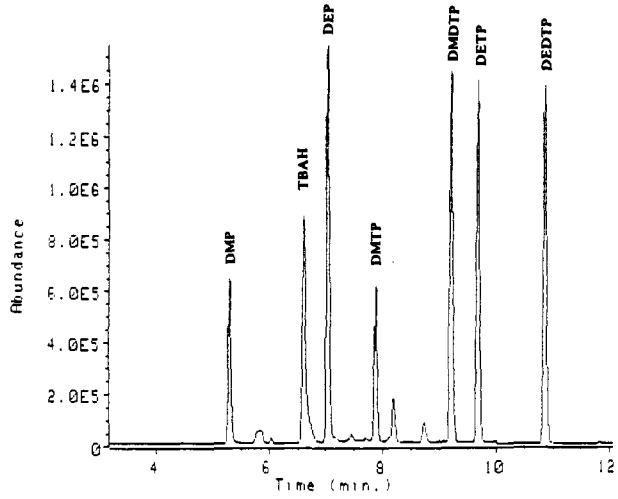

Figure 5. Total ion current chromatogram of dialkyl phosphate standard mix, using SIM program listed in Table V.

metabolite. The analysis of the samples for dialkyl phosphate metabolites resulted in the detection of DEP in liver and kidney samples in the majority of the samples from all of the exposure groups (Table IV). The results presented in Table IV were obtained to establish the applicability of the method to complex tissue matrixes and are not intended to represent a thorough study of these compounds in pigeon tissues. Due to the pilot nature of this experiment, a separate detailed study should be conducted to determine the cause of the variability of dialkyl phosphate levels in tissues of treated pigeons.

Figure 3 shows the comparison of the chromatograms of standard mix of all six dialkyl phosphates in 1 mg of liver matrix, control liver, pigeon liver 27, and liver spiked at the 0.25 μg/mL level of dialkyl phosphate mix. The peak corresponding to DEP has a retention time of 4.26 min. The confirmation of the DEP residues was done by mass spectrometry, by comparing spectra with those of six individual standards of butyl derivatives of dialkyl phosphates (Figure 4). Choosing three ions from each spectra, a SIM program was designed to achieve the sensitivity needed for the confirmation of the dialkyl phosphate residue levels. The program consisted of six acquisition groups (Table V). Using this program, confirmation of each of the six dialkyl phosphate metabolites can be simultaneously achieved at the level of 0.05 ppm. A typical chromatogram of the dialkyl phosphates by the SIM program is presented in Figure 5. A comparison of the ion chromatograms of the DEP standard and pigeon liver 27 (Figure 6) clearly shows the presence of this metabolite in the sample. Further, the DEP ion ratios of the samples matched the ones in standard and spike (Table VI). The confirmation results were within 10% of the value of the original FPD analysis. The smallest amount of DEP positively confirmed was 0.08 ppm (liver 39). The sensitivity of the confirmation analysis compares favorably to that of mass spectral or NMR confirmation of dialkyl phosphates by Takade (1979) and thermospray analysis by Draper et al. (1989), where only large amounts of dialkyl phosphates could be confirmed.

Matrix and different solvents created variability in derivatization yields. Derivatization of alkyl phosphates in ethyl acetate or ethyl acetate blank liver extracts with the injector block of the GC above 300 °C gave the best reproducibility among other solvents and conditions examined in this study. The use of an autosampler gave results comparable with those of the manual hot needle injection technique. The automatic injection technique

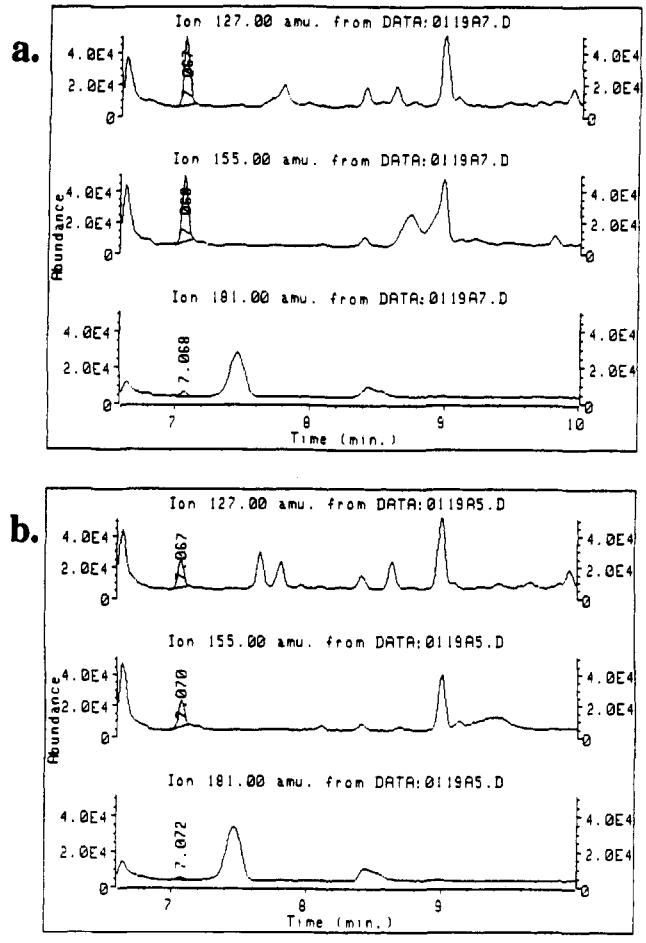

Figure 6. Extracted single ion chromatograms of DEP from (a) liver spike at 0.5 μg/g DEP and (b) pigeon liver 27.

minimized derivatization variables due to the operator and greatly sped up the analysis. The FPD response for injections ranging from 0.010 to 1 ng was linear for all five dialkyl phosphates tested. Retention times over the course of the study varied by no more than 3% and the peak areas for standard injections by less than 15%.

The sensitivity of the method compares with that of existing methods for dialkyl phosphates in urine (Bradway et al., 1981; Moody et al., 1985; Reid and Watts, 1981). The limits of detection of the metabolites in liver and kidney samples were sufficiently low to indicate the exposure of the pigeons to diazinon and parathion even when the presence of parent OPs was not detected. The drawback of the method is that it has low spike recoveries for DEDTP, although the recoveries are relatively consistent. Preliminary trials from liver samples fortified with other parent OPs listed in Table VII showed good recoveries (94–109%), at the 0.5 ppm level, suggesting the potential applicability of our method for multiresidue screens. The chromatographic resolution of the mix of six metabolites by the capillary GC column is superior to those reported previously for packed columns (Reid and Watts, 1981; Takade et al., 1979; Shafik et al., 1973). After 70 injections of liver extracts by the autosampler, there was no sign of a general decrease in column, instrument, or detector performance. The sources of errors were minimized by reducing operator manipulation of the sample and by maximizing automatization.

CONCLUSIONS

A method was developed for determining dialkyl phosphate metabolites in tissues (liver and kidney) of animals

exposed to organophosphorus insecticides. The method was validated by spiking experiments and also by application to analysis of tissues from pigeons exposed to OPs. Both the sensitivity (LD 0.02–0.05 ppm) and specificity (GC and GC/MS) of the method will enable it to be applied to diagnosing potential OP exposure in poisoned animals, for which urine may not be readily available and postmortem tissues may provide the only source for diagnostic OP residue analysis. While we did not explore the application, the method could conceivably handle a variety of biological tissues. The GPC cleanup used in conjunction with SIM-MS could accommodate both parent OPs and metabolites at relatively low concentration levels.

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