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Analysis of Organophosphorus Pesticides in Water by Graphitized Carbon Black Extraction and Gas Chromatography-Mass Spectrometry

Ma. Pythias Espino[1]*, Diana S. Aga[2], Mihn H. Nguyen[3], Heinz Singer[4], Michael Berg[4], and Stephan R. Müller[4]

[1]*Institute of Chemistry*
University of the Philippines
Quezon City 1101, Philippines

[2]*Chemistry Department*
University of Nebraska-Kearney
NE, USA 68849

[3]*Centre for Environmental Chemistry*
Hanoi University of Science
Hanoi, Vietnam

[4]*Swiss Federal Institute for Environmental Science & Technology*
EAWAG, Ueberlandstrasse 133
CH-8600 Dübendorf, Switzerland

A solid-phase extraction (SPE) procedure using graphitized carbon black (GCB) was developed to study organophosphorus pesticides (OPs) in natural water samples. Determination was carried out by gas chromatography-mass spectrometry (GC-MS) using single-ion monitoring. The pesticides investigated include heptenophos, diazinon, chlorpyrifos methyl, parathion methyl, paraoxon, fenitrothion, malathion, parathion ethyl, phosalone, and azinphos ethyl. Recoveries in different water samples ranged from 38 to 161% and the standard deviations were from 0.9 to 31%. The detection limits ranged from 0.1 to 8 ng/L. The method described here has practical applications to environmental investigations that require trace analysis of OP compounds in water.

Keywords: organophosphorus pesticides; solid-phase extraction; carbopack; gas chromatography/mass spectrometry; water analysis

INTRODUCTION

Organophosphorus pesticides, such as those listed in Fig. 1, are generally broad-spectrum insecticides that have found increasing use in agricultural practices as a consequence of the ban of organochlorine pesticides in many countries. Some OPs also have important non-agricultural applications that contribute significantly to the total insecticide use. Popular household insect control agents such as malathion, diazinon and chlorpyrifos are used against mosquitoes, ants, roaches and other mammalian parasites [1–4]. Chlorpyrifos is also commonly used in wood treatment [5]. The latest survey shows that about 65% of the total insecticide applied in crops in the United States are OPs [6]. It is not surprising then that there has been an increased frequency of detection of OP residues in surface waters [7]. Although less persistent than the organochlorines, most of these compounds and their degradation products are toxic, teratogenic or cholinesterase inhibitors [8–10]. The U.S. Environmental Protection Agency (USEPA) criteria for acute and chronic exposures for the safety of freshwater aquatic biota are less than 0.1 µg/L for azinophos methyl, chlorpyrifos, malathion, and parathion [11].

The USEPA has recently banned some OP compounds for use in certain crops and in the household such as chlorpyrifos ethyl and parathion methyl. It also restricted the use of azinphos methyl for some fruits and vegetables [12]. The Food and Agriculture Organization (FAO) and the United Nations

*Author to whom correspondence should be addressed.

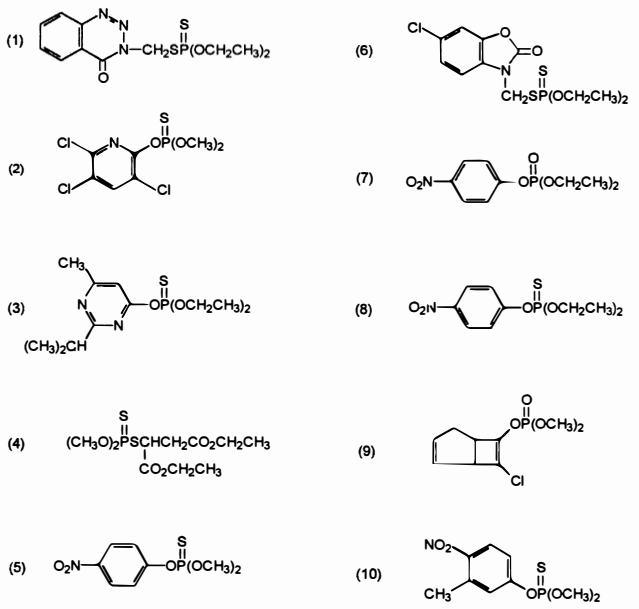

Fig. 1. Structures of OP pesticides investigated. (1) *Azinphos Ethyl;* (2) *Chlorpyrifos Ethyl;* (3) *Diazinon;* (4) *Malathion;* (5) *Parathion Methyl;* (6) *Phosalone;* (7) *Paraoxon;* (8) *Parathion Ethyl;* (9) *Heptenophos;* (10) *Fenitrothion.*

Environmental Programme (UNEP) have included methamidophos, monocrotophos, parathion ethyl, parathion methyl and phosphamidon in the priority list of environmental pollutants [13].

The environmental fate and behavior, and the physicochemical properties of OPs vary considerably. For instance, the log K_{ow} values can range from 0.7 (for dimethoate) to 4.7 (for chlorpyrifos ethyl) and K_{oc} values can be from 27 (for dimethoate) to 8753 (for chlorpyrifos ethyl) [14–15]. While some OPs degrade within one to three days in the aquatic environment, others can persist for several weeks, possibly posing damage to humans and wildlife with prolonged exposure. It is therefore imperative to have analytical methods that will enable the isolation and quantification of low concentrations of these compounds in various environmental matrices.

The analysis of OP compounds in environmental samples is routinely carried out with capillary gas chromatography or high performance liquid chromatography using various types of detector [16]. Solid-phase extraction (SPE) using different adsorbents has been employed for the isolation and concentration of organophosphosphorus compounds from water samples. For example, extraction procedures using C-18 and C-8 bonded porous silica, Empore extraction disks, XAD sorbents, and SDB co-polymer disks have been reported [17–20]. However, for the simultaneous extraction of the most important OPs with their broad range of physicochemical parameters that determine the extraction and elution from a SPE material, a trace analytical method is missing.

This paper describes a method for analyzing OP compounds in natural waters using graphitized carbon black (GCB) for SPE and gas chromatography-mass spectrometric detection for pesticide concentrations below the EU regulations at 0.1 µg/L. This method can be applied to study the fate and transport of OP compounds and their metabolites in the aquatic environment.

EXPERIMENTAL

Reagents and materials. The pesticide standards used were all at least of 96% purity. Azinphos ethyl, diazinon, fenitrothion, heptenophos, malathion, paraoxon, parathion methyl, and phosalone were commercially available from Riedel-de Häen (Seelze, Germany), and parathion ethyl and chlorpyrifos methyl were purchased from Supelco (Bellefonte, PA). The internal standard, deuterated parathion diethyl-D10, for GC-MS analysis was obtained from Cambridge Isotope Laboratories (Anover, MA). Individual stock solutions were prepared at 10 mg/mL by dissolving 100 mg of each compound in 10 mL methanol. The stock solution of parathion diethyl-D10 was prepared at 1 mg/mL by dissolving 10 mg in 10 mL methanol. These stock solutions were stored at -23°C. The working standard solutions and the internal standard solution were prepared from the stock solutions by dilution with methanol for water fortification and with ethyl acetate for direct injection into the gas chromatograph.

Methanol, ethyl acetate and methylene chloride of analytical grade were purchased from Fluka AG (Buchs, Switzerland). Ascorbic acid was purchased from Fluka AG (Buchs, Switzerland) and hydrochloric acid was from Merck (Darmstadt, Germany). Graphitized carbon black cartridges (Supelclean ENVI-Carb SPE tubes, 6 mL, 0.25 g) were purchased from Supelco (Bellefonte, PA). Nitrogen gas was from Carbagas (Rümlang, Switzerland). Nanopure water was prepared with a water purification device (NANOpure 4, Skan, Basel, Switzerland).

Sample preparation. Natural water samples (rainwater, lakewater from lake Greifensee, and riverwater from Kriesbach River, Switzerland) were filtered through a 0.45 µm cellulose nitrate membrane (Advantec MFS, Inc., CA) to eliminate suspended particles. For recovery studies, 1L of each samples (including nanopure water) were spiked with standard solutions to final concentrations of 10, 50, and 100 ng/L. To determine relative recoveries the internal standard was added before extraction, and for absolute recoveries before injection into the gas chromatograph.

Solid-phase extraction. Commercially available cartridges with 250-mg graphitized carbon black were conditioned as follows: 5 mL methanol, 5 mL methylene chloride, 5 mL methanol, 20 mL ascorbic acid, and 10 mL nanopure water [23]. Water samples were passed through the conditioned cartridges at a flow rate of 10–15 mL/min. The solid phase was rinsed with 5 mL nanopure water followed by 0.25 mL

methanol and air-dried for 5 min. Elution of analytes was carried out with 10 mL methylene chloride and these were collected using 10-mL graduated conical vials. The eluates were evaporated and concentrated using a gentle stream of nitrogen at a temperature of 35°C to a final volume of 100 µL. Finally, 150 µL of ethyl acetate was added to each concentrated sample before injection into the gas chromatograph.

Gas chromatography. A general purpose non-polar column (poly 5%-diphenyl-95%-dimethylsiloxane) with the following dimensions: 15 m length, 0.25 mm id, 0.90 µm film thickness, was connected to a Fisons MD800 gas chromatograph. The oven temperature was programmed from 80 to 100°C at 30°C/min, from 100 to 240°C at 5°C/min, and from 240 to 270°C at 20°C/min. The final temperature was held for 3 min. The source and injector temperatures were 200°C and 250°C, respectively. Helium was used as a carrier gas. The mass spectra were obtained using electron impact (EI+) mode at 70 eV with full scan and detection was performed with single ion monitoring (SIM). 1-µL samples were injected in the split/splitless mode.

RESULTS AND DISCUSSION

Packing materials of different polarities like C-8 and C-18 bonded phases, Empore disks and graphitized carbon black have been used for the pre-concentration of many pesticides [16, 24–26]. A number of OP pesticides have been successfully isolated by SPE using C-18 and GCB as solid phases and GC-NPD for analysis of the extracts. The mechanisms involved in GCB extraction are mixed-mode interactions capable of isolating both polar and non-polar analytes in water. OP pesticides cover a broad range of polarities. GCB is a common solid phase for separating acidic and base-neutral pesticides [21–22].

Initial experiments in this study, such as the investigations of various eluting solvents, employed a GC-NPD for analysis of the extracts. The recoveries of OP pesticides using C-18 and GCB for extraction with ethyl acetate as eluting solvent show that GCB is efficient in extracting even polar OP compounds like dimethoate (Table 1). Low recoveries for phosalone and azinphos ethyl using GCB were obtained due to their high adsorption capacity towards the solid phase. Therefore, various eluting solvents were explored to improve

Table 1. Absolute recoveries of OP pesticides from fortified nanopure water using GCB and C-18 with ethyl acetate as eluent and analysis by GC-NPD.

	% Recovery[a] Using GCB	*% Recovery[a] Using C-18*
Dimethoate	99	22
Fonofos	88	81
Diazinon	90	79
Parathion Methyl	100	88
Paraoxon[b]	83	92
Malathion	109	82
Chlorpyrifos Ethyl	71	67
Methidathion	91	84
Phosalone	13	82
Azinphos Ethyl	51	82

[a]Mean values of three determinations; spike level at 1 µg/L;volume of eluent is 10 mL.
[b]Pesticide and metabolite.

Table 2. Absolute recoveries of OP pesticides from fortified nanopure water using GCB and different eluents and analysis by GC-NPD.

	% RECOVERY[a]									
	Solvent System I		*Solvent System II*		*Solvent System III*		*Solvent System IV*		*Solvent System V*	
	EA	EA/H (50:50)	EA/H (80:20)	EA/H (50:50)	EA	$MeCl_2$	EA	EA/ $MeCl_2$ (50:50)	$MeCl_2$	$MeCl_2$/H (50:50)
Dimethoate	66	nd	74	nd	126	nd	54[b]	nd	64[b]	nd
Fonofos	71	2	64	3	74	nd	84	8[b]	98	nd
Diazinon	72	nd	71	2[b]	74	nd	72	5[b]	101	nd
Parathion Methyl	78	nd	78	nd	85	nd	90	11[b]	111	nd
Paraoxon[c]	117	nd	111	nd	120	nd	69	nd	119	nd
Malathion	84	nd	81	nd	84	nd	89	nd	95	nd
Chlorpyrifos Ethyl	55	25	56	14	40	36	59	25	78	11
Bromophos Methyl	23	31	50	18	30	43	35	36	84	12
Methidathion	104	nd	101	nd	108	nd	98	nd	99	nd
Phosalone	nd	nd	nd	12	nd	112	nd	57	97	25[b]
Azinphos Ethyl	8	49	29	41	14[b]	74	nd	64	84	nd

EA, Ethyl Acetate; H, Hexane; $MeCl_2$, Methylene Chloride.
[a]Mean values of 2 determinations; spike level at 1 µg/L; the first value is the recovery from the first 5 mL eluent and the second value is from the succeeding 5 mL eluent.
[b]n=1.
[c]Pesticide and Metabolite.
nd = not detected.

Table 3. Organophosphorus pesticides under investigation, their retention times and ions monitored and recoveries in nanopure water by GCB-SPE and GC-MS.

	Spike Level (ng/L)	*Retention Time* (min)	*Masses*[a] (m/z)	*% Absolute Recovery*[b]	*(SD)*	*% Relative Recovery*[b]	*(SD)*
Heptenophos	10	9.4	124, **250**	91	(9)	98	(1)
	50			73	(13)	79	(5)
	100			87	(16)	81	(5)
Diazinon	10	13.6	276, **304**	67	(4)	72	(4)
	50			66	(8)	69	(6)
	100			75	(10)	71	(3)
Chlorpyrifos Methyl	10	15.3	**286**, 288	66	(4)	72	(8)
	50			64	(10)	72	(4)
	100			77	(14)	76	(4)
Parathion Methyl	10	15.6	109, **263**	95	(7)	99	(6)
	50			103	(10)	108	(4)
	100			102	(5)	112	(10)
Paraoxon[c]	50	16.2	109, **275**	139	(14)	130	(2)
	100			127	(29)	122	(11)
Fenitrothion	10	16.7	125, **277**	106	(6)	111	(4)
	50			108	(13)	109	(5)
	100			120	(30)	113	(9)
Malathion	10	17.3	158, **173**	96	(4)	97	(8)
	50			86	(11)	89	(8)
	100			102	(13)	103	(8)
Parathion Ethyl	10	17.4	109, **291**	95	(5)	96	(7)
	50			100	(12)	98	(6)
	100			112	(24)	107	(8)
Phosalone	50	27.1	182, **367**	146	(31)	146	(10)
	100			153	(26)	158	(6)
Azinphos Ethyl	10	28.7	**132**, 160	139	(9)	161	(21)
	50			132	(28)	143	(8)
	100			127	(21)	141	(6)

[a]bold number: quantification mass; italicized number: molecular ion.
[b]n=3.
[c]pesticide and metabolite.

recoveries. Different solvent systems of varying compositions were evaluated and good recoveries for all compounds were achieved with methylene chloride (Table 2). Most of the OP compounds under investigation, therefore, are relatively non-polar.

In the succeeding experiments, ten OP pesticides of varying polarities were selected to investigate their behavior in the GCB extraction. In addition, rather than using the GC-NPD, all the GCB extracts were analyzed by GC-MS to obtain maximum sensitivity and selectivity. Table 3 shows the compounds studied, their retention times, and the ions used for monitoring. It also gives the absolute and relative recoveries in nanopure water. Measurements of recoveries were performed using the internal standard technique. Good recoveries (64–139%), both absolute and relative, were obtained for all compounds except for phosalone and azinphos ethyl. Recoveries of most pesticides in water are dependent on several factors including pesticide concentration and the sample volume processed. For this investigation, varying the concentrations from 10 to 100 ng/L do not influence the recovery significantly.

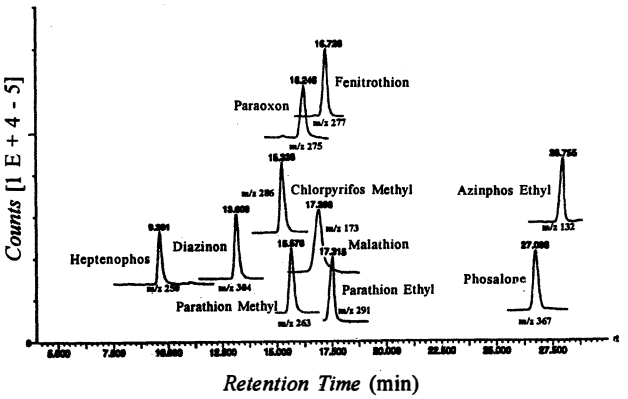

Fig. 2. Mass trace chromatogram of a lakewater sample spiked with 50 ng/L organophosphorus pesticides.

Figure 2 shows the SIM chromatogram (mass traces of the quantification ions) of an extract from a 1L lakewater sample spiked with 50 ng/L of OP pesticide standards. The ion peaks were vertically arranged in one chromatogram for better illustration. The retention times and masses are also given in Table 3. In all water samples, the chosen quantification ions

Table 4. Absolute recoveries and limits of detection in natural waters by GCB-SPE and GC-MS.

	LAKEWATER			RAINWATER			RIVERWATER		
	% Abs. Rec. [a]	*(SD)*	*LOD* (ng/L)	*% Abs. Rec.* [a]	*(SD)*	*LOD* (ng/L)	*% Abs. Rec.* [a]	*(SD)*	*LOD* (ng/L)
Heptenophos	61	(3)	2	44	(2)	1	55	(3)	2
Diazinon	56	(4)	0.5	38	(1)	0.7	59	(1)	0.4
Chlorpyrifos Methyl	60	(3)	0.2	38	(1)	0.1	57	(2)	0.1
Parathion Methyl	118	(1)	0.4	107	(5)	0.4	121	(9)	0.5
Paraoxon[b]	118	(5)	2	109	(3)	0.9	123	(14)	0.9
Fenitrothion	93	(2)	0.4	77	(4)	0.4	92	(5)	0.4
Malathion	68	(4)	0.5	55	(3)	0.7	68	(2)	0.3
Parathion Ethyl	88	(4)	0.5	74	(4)	0.5	88	(4)	0.6
Phosalone	88	(3)	0.9	64	(8)	1	82	(11)	0.4
Azinphos Ethyl	85	(2)	0.9	60	(8)	0.9	79	(12)	0.9

[a]spike level at 50 ng/L. n=3.
[b]pesticide and metabolite.

Table 5. Relative Recoveries and Limits of Detection in Natural Waters by GCB-SPE and GC-MS.

	LAKEWATER			RAINWATER			RIVERWATER		
	% Rel. Rec. [a]	*(SD)*	*LOD* (ng/L)	*% Rel. Rec.* [a]	*(SD)*	*LOD* (ng/L)	*% Rel. Rec.* [a]	*(SD)*	*LOD* (ng/L)
Heptenophos	68	(3)	2	58	(3)	2	59	(4)	2
Diazinon	61	(2)	0.5	49	(2)	0.7	62	(2)	0.3
Chlorpyrifos Methyl	66	(2)	0.2	48	(2)	0.2	58	(3)	0.09
Parathion Methyl	132	(1)	0.5	118	(1)	0.5	124	(3)	0.4
Paraoxon[b]	103	(2)	2	113	(5)	2	122	(7)	0.9
Fenitrothion	101	(0.9)	0.6	86	(2)	0.6	94	(2)	0.8
Malathion	79	(2)	0.6	64	(0.9)	0.6	70	(2)	0.3
Parathion Ethyl	94	(3)	0.6	84	(2)	0.7	92	(1)	0.5
Phosalone	105	(7)	1	71	(10)	1	83	(13)	0.8
Azinphos Ethyl	99	(13)	0.9	69	(10)	0.7	82	(17)	0.8

[a]spike level at 50 ng/L. n = 3.
[b]pesticide and metabolite.

were baseline resolved. The monitored ions gave good peak shapes at low spike levels and were detectable even at low concentrations (down to 0.1 ng/L).

Using the same ions for quantification, the absolute and relative recoveries and the limits of detection (LODs) of these OP compounds in lakewater, riverwater and rainwater were determined and are given in Table 4 and Table 5. The recoveries are lower than the recoveries obtained from nanopure water. This could be attributed to matrix effects. At a spike level of 50 ng/L the LODs in natural water samples are comparable with the LODs in nanopure water that were calculated to range from 0.1 to 8 ng/L. The LODs were calculated using peak intensities from single ion chromatograms of the extracts. The limit of detection is the amount that gives a signal-to-noise ratio of 3:1.

This procedure using solid-phase extraction with GCB and GC-MS for determination can be used to analyze residues of OP compounds in aqueous environmental samples. Preliminary investigations showed trace concentrations of diazinon in lake Greifensee at 4 ng/L, in Kriesbach River at 3 ng/L, and 10 ng/L in rainwater samples.

The method was demonstrated to be useful in the extraction of OP compounds in water at low ng/L levels. This method, therefore, is a promising tool to investigate the fate and behavior of OPs and their degradation products at levels expected in the environment.

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