Development of a Magnetic Particle-Based Enzyme Immunoassay for the Quantitation of Chlorpyrifos in Water

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A competitive enzyme immunoassay for the quantitation of chlorpyrifos in water that utilizes amine-terminated superparamagnetic particles as the solid phase to covalently attach mouse monoclonal anti-chlorpyrifos antibodies was developed. Specificity studies indicate that the monoclonal antibody can distinguish chlorpyrifos from its major degradation products, including 3,5,6-trichloro-2-pyridinol, and structurally similar pesticides. The immunoassay has an estimated limit of detection of 0.10 ng/mL in water and compares favorably with traditional chromatographic methods when water samples are analyzed by both methods ($r = 0.985$). Within- and between-assay percent coefficient of variation values are <13%, and recovery of chlorpyrifos from water averaged 97% across the range of the immunoassay method.

Keywords: *Chlorpyrifos; immunoassay; ELISA; organophosphate; monoclonal antibody; insecticide; water*

INTRODUCTION

Analytical methods for the determination of pesticide residues in food, crops, water, and soil are used for a variety of applications including enforcement of crop tolerances established by federal agencies, water monitoring, and product stewardship. Immunoassay (IA) technology has been applied to the detection of pesticide residues in various matrices both for quantitative analysis and as a field screening technique (Kaufman and Clower, 1995). Traditional analytical testing methods involving gas or liquid chromatography can be time-consuming and expensive and may require specialized instrumentation. Immunoassay methods can provide the analytical chemist with a cost-effective, sensitive, rapid, and reliable alternative for residue detection (Van Emon and Lopez-Avila, 1992).

Chlorpyrifos is a broad spectrum, nonsystemic insecticide used to control a variety of soil insects and anthropods on a wide range of crops including pome, stone, and citrus fruits, vegetables, cereals, maize, and tobacco. The active ingredient of Dursban and Lorsban insecticides (DowElanco, Indianapolis, IN), chlorpyrifos is one of the most widely used insecticides in the United States with 10−15 million pounds applied per year for crop protection and 9−12 million pounds applied per year for nonagricultural uses (U.S. EPA, 1994). Non-crop uses of chlorpyrifos include application to soil surrounding building structures as a barrier to termites and control of household insects such as ants and cockroaches. As a result of crop and non-crop usage, GC-based analytical methods have been developed for chlorpyrifos residues in a variety of environmental and crop matrices.

Magnetic particle-based immunoassays have previously been described and applied to the detection of pesticide residues in water (Lawruk et al., 1992; Gruessner et al., 1995), soil (Lawruk et al., 1993, 1996), crops (Yeung and Newsome, 1995; Lawruk et al., 1995; Selisker et al., 1995), fruit and fruit juice (Itak et al., 1993, 1994), wine (Lawruk et al., 1994), and meats (Nam and King, 1994). Other previously described immunoassays for chlorpyrifos (Manclús et al., 1994; Hill et al., 1994) utilized antibody passively adsorbed to polystyrene plates or tubes which adversely affects assay sensitivity and precision (Howell et al., 1981). In comparison, the magnetic particle-based immunoassay system described here provides covalent coupling of the antibody to the magnetic solid phase for precise antibody addition and non-diffusion-limited reaction kinetics (Newman and Price, 1991), thus eliminating the imprecision associated with antibody-coated tubes and microtiter plates. The chlorpyrifos magnetic particle-based ELISA described combines a monoclonal antibody specific for chlorpyrifos with an enzyme-labeled chlorpyrifos conjugate. The assay takes <1 h to perform and requires no sample preparation for the analysis of water samples.

MATERIALS AND METHODS

Immunochemicals. To prepare the chlorpyrifos immunogen, 4-(3,5-dichloro-pyridinyl-2,6-dioxy)valeric acid (0.02 mmol, DowElanco) and sodium carbonate (0.04 mmol) in *N*,*N*-dimethylformamide were stirred with diethyl chlorothiophosphate (0.046 mmol, Aldrich Chemical Co., Milwaukee, WI) for 2 h at room temperature. The reaction mixture was slowly added to a cooled, premixed solution of porcine thyroglobulin (120 mg) in 4 mL of deionized water and 0.1 mL of 1.0 N sodium hydroxide. The solution was stirred in an ice bath for 1 h and then at room temperature for 1 h. The solution was transferred to dialysis membrane tubing (Spectrapor, MWCO 6000−8000, Los Angeles, CA) and dialyzed against deionized water (2 L) for 24 h (two changes of water) and then lyophilized. The resulting chlorpyrifos immunogen (Figure 1) was used to immunize female Balb/c mice. The antibody-secreting cells produced were fused with myeloma cells to produce hybridoma cells. The hybridoma was injected (ip) into mice to form ascites fluid containing the monoclonal antibodies (Harlow and Lane, 1988). The resulting antibodies were covalently coupled to amine-terminated superparamagnetic

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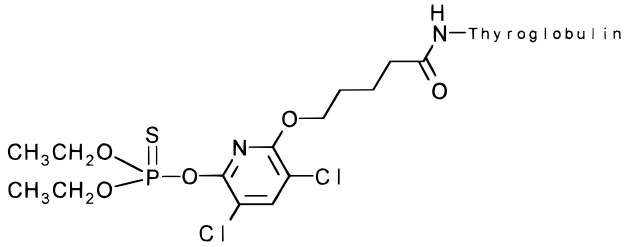


Figure 1. Structure of the chlorpyrifos immunogen used in the production of monoclonal antibodies.

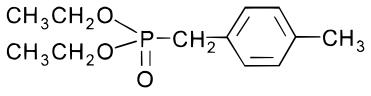


Figure 2. Structure of diethyl (4-methylbenzyl)phosphonate used as a stable chlorpyrifos analog standard material in aqueous buffers.

particles of approximately 1-μm diameter (Perseptive Diagnostics Inc., Cambridge, MA) by glutaraldehyde (Sigma Chemical Co., St. Louis, MO) activation of the solid phase (Rubio et al., 1991). Efficiency of the antibody coupling to the magnetic particles exceeded 90%. Superparamagnetic particles of this size separate quickly (<2 min) in magnetic fields but have no magnetic memory, allowing for repeated separations and resuspensions. The small particle size permits the particles to remain suspended in solution for over 1 h once resuspended. The antibody-coupled magnetic particle stocks were diluted 1:1000 in Tris-buffered saline (pH 7.4) containing 0.1% gelatin and 1 mM EDTA for use in the immunoassay.

Immunoassay Method Standardization. The major degradation pathway for chlorpyrifos is the hydrolysis to 3,5,6-trichloro-2-pyridinol. The rate of chlorpyrifos hydrolysis was found to be consistent over a pH range of 1.0−7.5 with an average half-life of 77 days (Racke, 1993). Hydrolysis rates increase dramatically above this pH level with pH 8.1 and 9.8 having 23 and 11 day half-lives, respectively (Racke, 1993). Hydrolytic degradation of chlorpyrifos prohibited the preparation of stable aqueous analytical standards compatible with the immunoassay; therefore, a stable organophosphate analog, diethyl (4-methylbenzyl)phosphonate (Figure 2; Lancaster Synthesis, Inc., Windham, NH), was used to prepare standards for the immunoassay (patent pending). This compound has a stable carbon−phosphorus chain in place of the oxygen−phosphorus bond which is subject to the hydrolysis to 3,5,6-trichloro-2-pyridinol. Various concentrations of diethyl (4-methylbenzyl)phosphonate were prepared in acetate-buffered saline with 0.1% gelatin at pH 4.0 and compared to chlorpyrifos standards in the same buffer. Concentrations of 10, 50, and 200 μg/mL diethyl 4-methylbenzylphosphonate were found to be equivalent to 0.22, 1.0, and 3.0 ng/mL chlorpyrifos. Diethyl (4-methylbenzyl)phosphonate standards were analytically prepared at these concentrations and used as chlorpyrifos standards of 0.22, 1.0, and 3.0 ppb in all assays described.

Additional Chemicals. Hydrogen peroxide and 3,3′,5,5′-tetramethylbenzidine (TMB) were obtained from Kirkegaard and Perry (Gaithersburg, MD). Horseradish peroxidase (HRP) was purchased from Sigma. The chlorpyrifos−HRP enzyme conjugate was obtained from Ohmicron Environmental Diagnostics (Newtown, PA). Chlorpyrifos and the chlorpyrifos metabolites, 3,5,6-trichloro-2-pyridinol, dechlorinated chlorpyrifos, and chlorpyrifos oxon, were obtained from DowElanco (Indianapolis, IN). Other organophosphate compounds as well as other compounds tested for cross-reactivity were purchased from ChemService (West Chester, PA). All other chemicals were of reagent grade or suitable chemical purity.

Apparatus. The spectrophotometric measurements were determined using the RPA-I Analyzer (Ohmicron). The detailed functions of this instrument have previously been described (Rubio et al., 1991). A two-piece magnetic separation rack (Ohmicron) was utilized, consisting of a test tube holder that fits over a magnetic unit containing permanently positioned rare earth magnets. The two-piece design allows for a 60-tube immunoassay to be set up, incubated, and magnetically separated without removal of the tubes from the holders (Itak et al., 1992). Eppendorf trivolume and repeating pipets (Eppendorf, Hamburg, Germany) were used to dispense liquids.

Immunoassay Procedure. Water samples or standards were assayed by adding 250 μL of sample, 250 μL of chlorpyrifos−HRP conjugate, and 500 μL of magnetic particles, with monoclonal anti-chlorpyrifos antibody covalently attached, to a polystyrene test tube (12 × 75 mm) in the magnetic rack tube holder. The reaction mixture was incubated for 15 min at room temperature and then separated using the magnetic rack. After separation, the magnetic particles were washed twice with 1.0 mL of deionized water to remove unbound chlorpyrifos−HRP and eliminate any potential interfering substances. The colored product was developed for 20 min at room temperature by the addition of 500 μL of a 1:1 mixture of peroxide/TMB solution. Formation of the colored product was stopped and stabilized by the addition of 500 μL of 2 M sulfuric acid. Since the enzyme-labeled chlorpyrifos competes for antibody binding sites with the unlabeled (sample) chlorpyrifos, the color developed is inversely proportional to the chlorpyrifos concentration in the sample. The final concentration of chlorpyrifos in each sample was determined using the RPA-I analyzer by measuring the absorbance of each sample at 450 nm. The RPA-I analyzer was preprogrammed to compare the observed sample absorbances to a linear regression line using the logarithm of the concentration versus logit B/B_0 standard curve (where B/B_0 is the absorbance at 450 nm for a sample or standard divided by the absorbance at 450 nm of the zero standard). The standards were prepared in the zero standard (acetate-buffered saline preserved solution) and contained the equivalent of 0.22, 1.0, and 3.0 ng/mL chlorpyrifos. Samples >3.0 ng/mL were diluted in the zero standard for analysis and sample concentrations calculated by multiplying the results by the appropriate dilution factor.

GC Analysis for Method Comparison. A method comparison study was performed with water samples collected from a field runoff study. The samples were stored refrigerated until an aliquot was taken for extraction and analysis by GC. The remainder of each sample was frozen at −20 °C. For GC analysis, the extraction of chlorpyrifos from the water samples was accomplished by the addition of 2.0 mL of hexane to 40 mL of sample and then shaking for a minimum of 15 min. Following centrifugation to separate the aqueous and organic phases, an aliquot of hexane was transferred to a GC autosampler vial for analysis by capillary GC. Sample analysis was performed on a DB-17 (J&W Scientific, Folsom, CA) capillary column installed in a Hewlett-Packard (Avondale, PA) 5890 Series II GC equipped with a flame photometric detector (phosphorus mode) and a Hewlett-Packard 7673A autosampler. Chromatographic data were collected and peaks integrated using a PE Nelson Access Chrom chromatography data system. For immunoassay analyses, the frozen portions of these samples were thawed, an aliquot was removed, and the analyses were performed as described above.

Specificity. To evaluate specificity, each organophosphate or other compound of interest was weighed analytically and dissolved in an appropriate solvent to a 10 mg/mL concentration. Each compound was then diluted in methanol to obtain 100 μg/mL stock solutions and each further diluted to 10 000 ng/mL in the zero standard. The 10 000 ng/mL stock was diluted serially in the zero standard to obtain 1000, 100, 10, 1.0, 0.1, and 0.01 ng/mL solutions for each compound. All dilutions were prepared volumetrically. For each compound, the 0.01−10 000 ng/mL solutions were assayed and the B/B_0 calculated at each concentration. The B/B_0 (y) versus concentration (x) were graphed on log/logit graph paper and the 90% B/B_0 and 50% B/B_0 concentrations determined for each compound.

RESULTS AND DISCUSSION

Dose−Response Curve and Sensitivity. Figure 3 illustrates the mean dose−response curve for the chlorpyrifos analog standards, linearly transformed

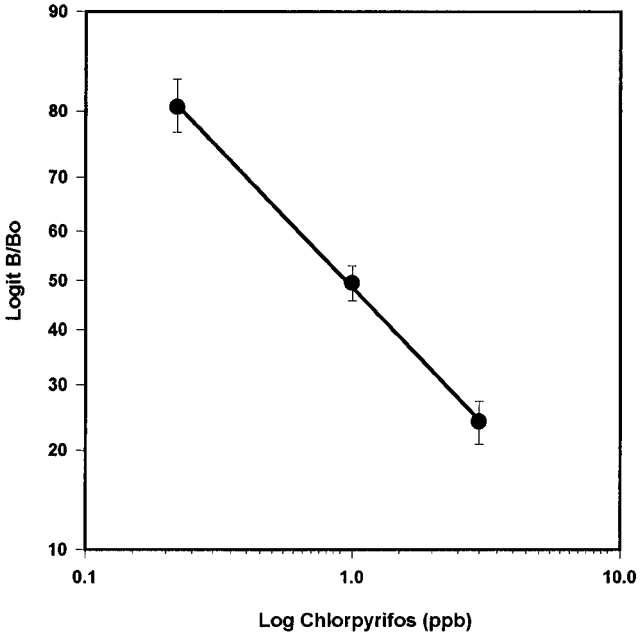


Figure 3. Chlorpyrifos dose−response curve. Each point represents the mean of 67 determinations. Vertical error bars indicate ±2 SD about the mean.

using a log/logit curve fit, collected over 67 assays with error bars representing 2 SD. The error bars at each calibration point represents the assay-to-assay variability expected from small differences in timing, temperature, or reagent age over the 67 assays. A calibration curve was included with every assay to account for this variability.

The immunoassay sensitivity or limit of detection (LOD), the lowest concentration that can be distinguished from zero, was estimated by analysis of 3 sets of 20 replicates of the zero standard. The mean absorbance and standard deviation were calculated for each assay. The mean absorbance value minus 4 SD was equivalent to a 90% B/B_0 concentration (Midgley et al., 1969), which corresponded to an estimated limit of detection of 0.10 ng/mL in water. An alternative approach to determine the limit of detection and limit of quantitation (LOQ) (Keith et al., 1983) was calculated on the basis of the analysis of 40 water samples fortified with 0.25 ng/mL chlorpyrifos, representing the estimated LOQ of the method. The mean concentration determined for the 40 samples was 0.24 ng/mL with a SD of 0.023 ng/mL. The calculated LOD based on 3 times the SD from the analysis of these samples was 0.07 ng/mL. The calculated LOQ based on 10 times the SD from this study was 0.23 ng/mL.

Precision. A precision study was conducted in which two sources of surface and two sources of ground water were fortified with chlorpyrifos at 0.35, 0.70, 1.25, and 2.5 ng/mL and each assayed five times in singlets on 5 different days (Table 1). The within- and between-day variations were determined by analysis of variance (ANOVA) (Bookbinder and Panosian 1986). Coefficients of variation (% CV) within- and between-days were less than 13% and 12%, respectively, for all samples. A component of the between-day variability can be attributed to the degradation of chlorpyrifos in the sample over the 5-day testing period. On day 1 of the precision study, the samples correctly recovered the chlorpyrifos added to the samples (0.31, 0.65, 1.22, and 2.19 ng/mL for sources 1−4, respectively). There was a decrease in sample concentrations over the next 2 days of the study, possibly due to chlorpyrifos hydrolysis or adsorption to glass (Racke, 1993), as reflected in the mean sample concentrations compared to the fortified concentration for each sample. After this decrease in chlorpyrifos concentrations was observed, the water samples were aliquotted and frozen. The individual sample aliquots were thawed and analyzed for 5 consecutive days (new aliquots were thawed each day) and the results used for the ANOVA calculations.

Table 1. Precision of Chlorpyrifos Measurement by Immunoassay[a]

	source			
	1	2	3	4
replicates	5	5	5	5
days	5	5	5	5
N	25	25	25	25
mean (ng/mL)	0.16	0.54	1.10	1.92
% CV (within assay)	13.0	8.4	5.3	5.1
% CV (between assay)	6.2	11.3	10.0	9.5

[a] Water samples were fortified with 0.35, 0.70, 1.25, and 2.5 ng/mL chlorpyrifos and assayed in five singlets each over 5 days. Source 1, municipal water fortified with 0.35 ng/mL; source 2, surface water fortified with 0.70 ng/mL; source 3, ground water fortified with 1.25 ng/mL; source 4, surface water fortified with 2.5 ng/mL.

Table 2. Accuracy of the Chlorpyrifos Immunoassay in Water[a]

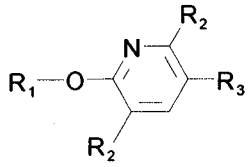
chlorpyrifos added (ng/mL)	mean chlorpyrifos recovered (ng/mL)	*n*	SD (ng/mL)	% recovery
0.35	0.33	12	0.02	93
0.70	0.68	12	0.03	97
1.25	1.31	12	0.09	105
2.50	2.34	12	0.16	94
av				97

[a] Two surface water sources, a municipal water source and a ground water source, were each fortified at the described concentrations and assayed in duplicate in three separate immunoassays in a single day.

Accuracy in Water. The accuracy of the immunoassay was assessed by evaluating four sources of water, each fortified with chlorpyrifos at 0.35, 0.70, 1.25, and 2.50 ng/mL. The water sources included a small pond, a stream, ground water, and municipal drinking water. Each sample was evaluated in three separate assays, in duplicate, on the same day to verify reproducibility and minimize chlorpyrifos degradation. Table 2 summarizes the accuracy of the chlorpyrifos immunoassay in environmental water samples. Added amounts of chlorpyrifos were recovered quantitatively in all cases with an average recovery of 97%. The potential for ground, surface, and municipal water samples to interfere in the assay was evaluated by fortifying 312 water samples collected from around the world with 1.5 ng/mL chlorpyrifos. The average recovery of the fortified water samples was 98% (SD = 7%) with all sample recoveries within the 70−120% recovery range (Mihaliak and Berberich, 1995). The accurate recovery of the fortified water samples suggests that no matrix effects or interferences were present in the water sources analyzed, and the immunoassay was accurate across the range tested.

Method Comparison. Method correlation of 151 runoff water samples obtained by the immunoassay (y) and an established GC method (x) is illustrated in Figure 4. The regression analysis yields a correlation coefficient (r) of 0.985 and a slope of 1.14 between methods. However, when the immunoassay and GC results were compared in the range from 0 to 4 ng/mL,

Table 3. Specificity (Cross-Reactivity) in the Chlorpyrifos Immunoassay



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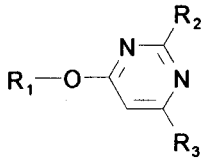

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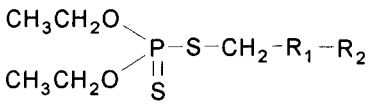

C

compound	structure	R_1	R_2	R_3	LDD[a] (ng/mL)	I_{50}[b] (ng/mL)
chlorpyrifos	A	$P(S)(OCH_2CH_3)_2$	Cl	Cl	0.10	0.94
chlorpyrifos-methyl	A	$P(S)(OCH_3)_2$	Cl	Cl	0.14	3.58
chlorpyrifos, monodechlorinated	A	$P(S)(OCH_2CH_3)_2$	Cl	H	0.14	4.74
chlorpyrifos oxon	A	$P(O)(OCH_2CH_3)_2$	Cl	Cl	13.5	90.4
chlorpyrifos, dechlorinated	A	$P(S)(OCH_2CH_3)_2$	H	H	64.7	1650
3,5,6-trichloro-2-pyridinol	A	H	Cl	Cl	>10,000	>10,000
diazinon	B	$P(S)(OCH_2CH_3)_2$	$CH(CH_3)_2$	CH_3	0.12	7.56
pirimiphos-ethyl	B	$P(S)(OCH_2CH_3)_2$	NCH_2CH_3	CH_3	0.32	57.1
pirimiphos-methyl	B	$P(S)(OCH_3)_2$	NCH_2CH_3	CH_3	19.8	688
disulfoton	C	$(CH_2)_2$	SCH_2CH_3		26.2	688
terbufos	C	S	$C(CH_3)_3$		10.3	2180

[a] Least detectable dose (90% B/B_0). [b] Fifty percent inhibition concentration (50% B/B_0). The following compounds had no reactivity in the chlorpyrifos immunoassay up to 10 000 ng/mL: alachlor, aldicarb, atrazine, benomyl, butachlor, butylate, captan, carbaryl, carbendazim, carbofuran, 2,4-D, dichloropropene, dinoseb, lindane, metolachlor, metribuzin, PCP, picloram, pirimicarb, profenofos, propachlor, terbufos, thiabendazole, and thiophanat-methyl.

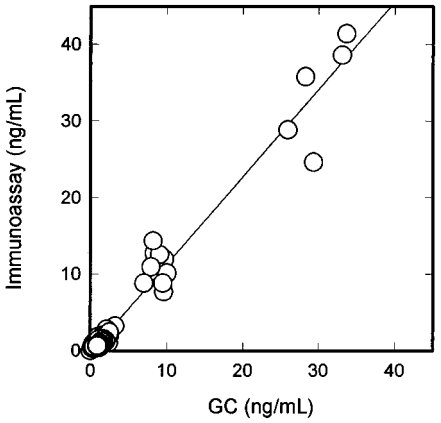

Figure 4. Correlation between chlorpyrifos concentrations in water samples from a field runoff study as determined by the magnetic particle-based immunoassay and GC method. $n = 151$, $r = 0.985$, $y = 1.14x + 0.20$ ng/mL.

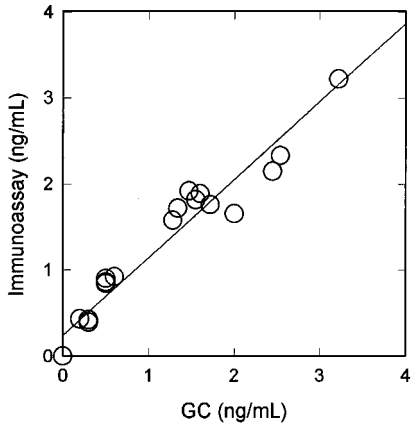

Figure 5. Correlation between chlorpyrifos concentrations in water samples from a field run off study as determined by the magnetic particle-based immunoassay and GC method performed on the same day. $n = 20$, $r = 0.970$, $y = 0.907x + 0.24$ ng/mL.

the correlation coefficient (r) was 0.751 with a regression line of $y = 0.807x + 0.15$ ($n = 137$). It appeared that chlorpyrifos degradation occurred in some water samples during the time from GC sample preparation to immunoassay analysis possibly due to the instability of chlorpyrifos in water (Racke, 1993). Twenty water samples analyzed by GC and the immunoassay on the same day (Figure 5), thus minimizing chlorpyrifos degradation, had an excellent correlation coefficient ($r = 0.970$) and regression line ($y = 0.907x + 0.24$) for samples in the range of 0−4 ng/mL.

Specificity. Table 3 summarizes the specificity of the chlorpyrifos immunoassay with various metabolites and organophosphate pesticides. The least detectable dose (LDD) was determined as the amount of each compound necessary to achieve 90% B/B_0. At the 90% B/B_0 concentration, each compound would provide an apparent chlorpyrifos concentration greater than the LDD of chlorpyrifos. The 50% inhibition concentration (I_{50}) was determined by estimating the amount of each compound necessary for 50% displacement of the chlorpyrifos−HRP conjugate. Reactivity of the major chlorpyrifos degradation product, 3,5,6-trichloro-2-pyridinol was very poor (<0.01%), demonstrating the necessity of the thiophosphate ester moiety for monoclonal antibody recognition. However, reactivity was also strongly influenced by the presence of the chlorinated pyridine ring since monodechlorinated chlorpyrifos (19.8%) was much more reactive than the totally dechlorinated chlorpyrifos metabolite (0.06%). Also, thiophosphate esters that did not retain the chlorinated pyridine ring structure such as disulfoton (0.14%) and terbufos (0.04%) exhibited poor reactivity in the immunoassay. Chlorpyrifos oxon, the biologically active phosphate ester analog of chlorpyrifos, also had poor reactivity in the immunoassay (1.0%), again illustrating the affinity of the monoclonal antibody for the thiophosphate ester substituent.

Variations of the ester moieties illustrated by chlorpyrifos-methyl (26.3%) demonstrate the specificity of the

Table 4. Linearity upon Sample Dilution by Immunoassay

source	dilution level[a] undiluted	1:2	1:4	1:8
municipal water 1				
obtained (ng/mL)	2.37	1.11	0.53	0.29
expected[b] (ng/mL)	2.37	1.14	0.57	0.29
recovery (%)		97	93	100
surface water 1				
obtained (ng/mL)	2.35	1.06	0.53	0.28
expected (ng/mL)	2.35	1.17	0.58	0.29
recovery (%)		91	91	97
surface water 2				
obtained (ng/mL)	2.32	1.12	0.55	0.30
expected (ng/mL)	2.32	1.16	0.58	0.29
recovery (%)		97	95	103
ground water 1				
obtained (ng/mL)	2.18	1.00	0.51	0.24
expected (ng/mL)	2.18	1.09	0.55	0.27
recovery (%)		92	93	89

[a] Samples were diluted in the zero standard. [b] Expected concentrations are derived from the chlorpyrifos concentration obtained in the undiluted sample.

monoclonal antibody used in this immunoassay. Manclús et al. (1994) have developed an indirect competitive chlorpyrifos immunoassay using monoclonal antibodies produced from a similar immunogen design with 3-mercaptopropionic acid linkage at the 6-position of the pyridine ring. However, chlorpyrifos-methyl had substantially greater reactivity in this assay (183%) than chlorpyrifos, presumably because the methyl groups were better accommodated by the antibody binding sites (Manclús et al., 1994). Hill et al. (1994) have previously described a chlorpyrifos immunoassay utilizing a chlorpyrifos-methyl immunogen hapten with protein carrier attachment at the thiophosphate ester to produce polyclonal antisera. Not surprisingly, this immunoassay was also much more reactive for chlorpyrifos-methyl (330%) than for chlorpyrifos and had substantial reactivity to the chlorpyrifos oxon (30%). The magnetic particle monoclonal antibody-based immunoassay described is more reactive with chlorpyrifos than with chlorpyrifos-methyl (26%) with minimal reactivity to the major degradation products of chlorpyrifos.

Sample Dilution. Water samples that contain detectable concentrations of chlorpyrifos by the immunoassay can be diluted in the zero standard and reassayed to determine parallel dilution. Sample concentrations dilute parallel when the assay response is due to presence of chlorpyrifos in the sample. If a sample contains detectable concentrations of chlorpyrifos by the immunoassay due to specific or nonspecific interferences, the concentrations of the diluted sample would not assay as expected (Jung et al., 1989). Values obtained from four water samples diluted in the zero standard showed agreement between measured and expected values (Table 4). The expected concentrations were derived from the chlorpyrifos concentration of the undiluted sample by the immunoassay.

Interferences. The following compounds were added at 250 μg/mL to water samples containing no detectable chlorpyrifos and to water samples fortified with 1.0 ng/mL chlorpyrifos and evaluated for possible interference in the immunoassay: copper (chloride), magnesium (chloride), nickel (sulfate), zinc (chloride), nitrate (sodium), phosphate (sodium), sulfite (sodium), and thiosulfate (sodium). In addition, calcium (chloride) up to 500 μg/mL, sulfate (sodium) up to 10 000 μg/mL, silicates (sodium meta-) up to 500 μg/mL, sodium chloride up to 0.5 M, and iron (chloride), manganese (chloride), and mercury (chloride) up to 100 μg/mL exhibited no interference in the immunoassay (Table 5). Solvents typically used for chlorpyrifos extraction from various matrices, such as acetonitrile, acetone, dichloromethane, dimethylformamide, and methanol, were tolerated by the immunoassay up to 2.0%, 2.0%, 0.2%, 5.0%, and 10.0% respectively.

Table 5. Effect of Possible Interfering Substances

compound	max concn tolerated[a] (μg/mL)	recovery at max concn tolerated (%)[b]
calcium (chloride)	500	96
copper (chloride)	250	86
iron (chloride)	100	114
magnesium (chloride)	250	86
manganese (chloride)	100	98
mercury (chloride)	100	100
nickel (sulfate)	250	99
zinc (chloride)	250	89
nitrate (sodium)	250	87
phosphate (sodium)	250	87
sulfate (sodium)	10000	102
sulfite (sodium)	250	93
silicates (sodium meta-)	500	99
thiosulfate (sodium)	250	90
NaCl	0.5[c]	83
acetone	2.0[d]	80
acetonitrile	2.0[d]	81
dichloromethane	0.2[d]	100
dimethylformamide	5.0[d]	80
methanol	10.0[d]	83

[a] All unfortified samples assayed as less than the least detectable dose of 0.10 ng/mL chlorpyrifos. [b] Recovery with samples fortified with 1.0 ng/mL chlorpyrifos. [c] Molar. [d] Percent.

Conclusions. The performance characteristics of a magnetic particle-based enzyme immunoassay for the quantitative determination of chlorpyrifos in water samples have been summarized. This immunoassay compares favorably to gas chromatographic methods for the analysis of water samples ($r = 0.985$) and exhibits within- and between-assay variability of <13% with results obtained in <1 h. Studies have shown the magnetic particle-based immunoassay to be accurate in the analysis of water samples without prior sample preparation with an average recovery of 97% demonstrated across the range of the method. The specificity of the monoclonal antibody allows for the detection of chlorpyrifos in the presence of degradation products, other organophosphates, and commonly found water components. The estimated method sensitivity of 0.10 ng/mL makes this immunoassay a valuable method for residue analysis. The LOD and LOQ of the method are comparable to the estimated detection limit of 0.3 ng/mL chlorpyrifos for Method 622, a GC method commonly used for municipal and wastewater analysis (U.S. EPA, 1992). The system is ideally suited for adaptation to the analysis of chlorpyrifos residues in air and agricultural products such as bananas, fruit juices, and cereal grains (Herzog et al., 1995).

LITERATURE CITED

Bookbinder M. J.; Panosian K. J. Correct and incorrect estimation of within-day and between-day variation. *Clin. Chem.* **1986**, *32*, 1734−1737.

Gruessner, B.; Shambaugh, N. C.; Watzin, M. C. Comparison of an enzyme immunoassay and gas chromatography/mass spectrometry for the detection of atrazine in surface waters. *Environ. Sci. Technol.* **1995**, *29* (1), 251−254.

Harlow, E.; Lane, D. P. Monoclonal antibodies. *Antibodies: A Laboratory Manual*; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1988; pp 139−243.

Herzog, D. P.; Mihaliak, C. A.; Jourdan, S. W.; Lawruk, T. S. Immunochemical Measurement of chlorpyrifos in multiple matrices. *J. Clin. Ligand Assay* **1995**, *18*, 150−155.

Hill, A. S.; Skerritt, J. H.; Bushway, R. J.; Pask, W.; Larkin, K. A.; Thomas, M.; Korth, W.; Bowmer K. Development and application of laboratory and field immunoassays for chlorpyrifos in water and soil matrices. *J. Agric. Food Chem.* **1994**, *42* (9), 2051−2058.

Howell, E. H.; Nasser, J.; Schray, K. J. Coated tube enzyme immunoassay: factors affecting sensitivity and effects of reversible protein binding to polystyrene. *J. Immunoassay* **1981**, *2*, 205−225.

Itak, J. A.; Selisker, M. Y.; Herzog, D. P. Development and evaluation of a magnetic particle-based enzyme immunoassay for aldicarb, aldicarb sulfone and aldicarb sulfoxide. *Chemosphere* **1992**, *24*, 11−21.

Itak, J. A.; Selisker, M. Y.; Jourdan, S. W.; Fleeker, J. R.; Herzog, D. P. Determination of benomyl (as carbendazim) in water, soil and fruit juice by a magnetic particle-based immunoassay. *J. Agric. Food Chem.* **1993**, *41*, 2329−2332.

Itak, J. A.; Selisker, M. Y.; Herzog, D. P.; Fleeker, J. R.; Bogus, E. R.; Mumma, R. O. Determination of captan in water, peaches and apple juice by a magnetic particle-based immunoassay. *J AOAC Int.* **1994**, *77* (1), 86−91.

Jung, F.; Gee, S. J.; Harrison, R. O.; Goodrow, M. H.; Karu, A. E.; Braun, A. L.; Li, Q. X.; Hammock, B. D. Use of Immunological Techniques for the analysis of pesticides. *Pestic. Sci.* **1989**, *26*, 303−317.

Kaufman, B. M.; Clower, M. C. Immunoassay of pesticides: an update. *J. AOAC Int.* **1995**, *78* (4), 1079−1090.

Keith, L. H.; Crummett, W.; Deegan, J.; Libby, R. A.; Taylor, J. T.; Wentler, G. Principles of environmental analysis. *Anal. Chem.* **1983**, *55*, 2210−2218.

Lawruk, T. S.; Hottenstein, C. S.; Herzog, D. P.; Rubio, F. M. Quantification of alachlor in water by a novel magnetic particle-based ELISA. *Bull. Environ. Contam. Toxicol.* **1992**, *48*, 643−650.

Lawruk, T. S.; Lachman, C. E.; Jourdan, S. W.; Fleeker, J. R.; Herzog, D. P.; Rubio, F. M. Determination of metolachlor in water and soil by a rapid magnetic particle-based ELISA. *J. Agric. Food Chem.* **1993**, *41* (9), 1426−1431.

Lawruk, T. S.; Lachman, C. E.; Jourdan, S. W.; Scutellaro, A. M.; Fleeker, J. R.; Herzog, D. P.; Rubio, F. M. Detection of procymidone in wine and grapes by a magnetic particle-based enzyme immunoassay. *J. Wine Res.* **1994**, *5* (iii), 205−214.

Lawruk, T. S.; Gueco, A. M.; Jourdan, S. W.; Scutellaro, A. M.; Fleeker, J. R.; Herzog, D. P.; Rubio, F. M. Determination of chlorothalonil in water and agricultural products by a magnetic particle-based enzyme immunoassay. *J. Agric. Food Chem.* **1995**, *43* (5), 1413−1419.

Lawruk, T. S.; Lachman, C. E.; Jourdan, S. W.; Fleeker, J. R.; Hayes, M. C. Herzog, D.P.; Rubio, F. M. Quantitative determination of PCBs in soil and water by a magnetic particle-based immunoassay. *Environ. Sci. Technol.* **1996**, *30* (2), 695−700.

Manclús, J. J.; Primo, J.; Montoya, A. Development of a chlorpyrifos immunoassay using antibodies obtained from a simple hapten design. *J. Agric. Food Chem.* **1994**, *42* (6), 1257−1260.

Midgely, A. R.; Niswender, G. D.; Rebar, R. W. Principles for the assessment of reliability of radioimmunoassay methods (precision, accuracy, sensitivity, specificity). *Acta Endocrinol.* **1969**, *63*, 163−179.

Mihaliak, C. A.; Berberich, S. A. Guidelines to the validation and use of immunochemical methods for generating data in support of pesticide registration. *Immunoanalysis of Agrochemicals*; Nelson, J. O.; Karu, A. E.; Wong, R. B., Eds.; ACS Symposium Series 586; American Chemical Society: Washington, DC, 1995; pp 288−300.

Nam, K. S.; King, J. W. Supercritical fluid extraction and enzyme immunoassay for pesticide detection in meat products. *J. Agric. Food Chem.* **1994**, *42*, 1469−1474.

Newman, D. J.; Price, C. P. Separation techniques. *Principles and Practice of Immunoassay*; Stockton Press: New York, 1991; pp 87−88.

Racke, K. D. The environmental fate of chlorpyrifos. *Rev. Environ. Contam. Toxicol.* **1993**, *131*, 1−154.

Rubio, F. M.; Itak, J. A.; Scutellaro, A. M.; Selisker, M. Y.; Herzog, D. P. Performance characteristics of a novel magnetic particle-based enzyme-linked immunosorbent assay for the quantitative analysis of atrazine and related triazines in water samples. *Food Agric. Immunol.* **1991**, *3*, 113−125.

Selisker, M. Y.; Herzog, D. P.; Erber, R. D.; Fleeker, J. R.; Itak, J. A. Determination of paraquat in fruits and vegetables by a magnetic particle based enzyme-linked immunosorbent assay. *J. Agric. Food Chem.* **1995**, *43*, 544−547.

U.S. EPA. *Methods for the Determination of Nonconventional Pesticides in Municipal and Industrial Wastewater*; U.S. GPO: Washington, DC, 1992; EPA-821/R-92-002, pp 227−245.

U.S. EPA. *Pesticide Industry Sales and Usage: 1992 and 1993 Market Estimates*; Office of Pesticide Programs: Washington, DC, 1994.

Van Emon, J. M.; Lopez-Avila, V. Immunological methods for environmental analysis. *Anal. Chem.* **1992**, *64*, 79−89.

Yeung, J. M.; Newsome, W. H. Determination of chlorothalonil in produce by enzyme immunoassay. *Bull. Environ. Contam. Toxicol.* **1995**, *54 (3)*, 444−450.

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