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A Class-Specific Enzyme-Linked Immunosorbent Assay Based on Magnetic Particles for Multiresidue Organophosphorus Pesticides

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A magnetic particle-based enzyme-linked immunosorbent assay (mp-ELISA) to detect multiresidue organophosphorus pesticides (OPs) was developed and evaluated in parallel with a conventional competitive indirect ELISA utilizing the same pair of antibodies. The antibody was immobilized by chemical coupling on the ferroferric oxide nanoparticles coated with 3-aminopropyl triethoxysilane (APTES). Comparative studies demonstrated that mp-ELISA exhibited both significantly improved sensitivity and better class specificity than conventional ELISA, although the reproducibility and repeatability of the two assays were equivalent. As compared with data from the conventional ELISA, the averages of the midpoint inhibition (IC_{50}) decreasing rate of mp-ELISA were 81.1 and 62.1% for the magnetic first antibody (MFA) and magnetic second antibody (MSA), respectively. The class specificity of MFA was broader than that of MSA. The results demonstrated the high potential of mp-ELISA based on the MFA as a tool to improve sensitivity and broad specificity in multiresidue immunoassay.

KEYWORDS: Organophosphorus pesticides; magnetic particle; multiresidue detection; ELISA

INTRODUCTION

Organophosphorus pesticides (OPs) are the most widely used insecticides in the world (*1*). Because of the potential health hazards of these compounds and their possible entry into food chains, it is important to develop sensitive and rapid analytical techniques for environmental monitoring and assessment of human exposure to these compounds. There are many methods to detect OPs, such as high-performance liquid chromatography (HPLC), gas chromatography with electron capture detector (GC-ECD), and gas chromatography with mass spectrometry (GC-MS) (*2*). The procedures for sample preparation in such methods are very complicated and highly sensitive; therefore, highly skilled operations are required. In addition, there is a real need to develop fast, easy-to-use, robust, sensitive, and cost-effective field analytical techniques.

In recent years, immunoassay techniques have begun to gain acceptance as a fast, sensitive, and cost-effective tool to detect environmental contaminants in food (*3*). Immunoassay is an analytical technique based on the specificity of the antigen−antibody reaction. As an alternative to laborious and expensive instrumental methods used to quantify OPs, immunochemical analyses such as enzyme-linked immunosorbent assays (ELISAs) have been successfully developed as a semiquantitative or quantitative screening tool to detect OPs (*4*, *5*). Most assays can be considered specific for a single analyte (*6*, *7*). However, increasing public concern over pesticide contamination of food and the environment has increased demands for broader and stricter pesticide monitoring. Multiresidue immunoassay for OPs has gained increasing attention due to its high sample throughput, short assay time, low sample consumption, and reduced overall cost per assay (*8*−*10*). The most commonly used method to produce a broad-specificity immunoassay is to produce an antibody with broad specificity by using a "generic hapten", which should exhibit common features of all target analytes. Some investigators tried to develop broad-specificity assays for OPs, but the produced broad-specificity polyclonal antibodies had unsatisfactory sensitivity (*4*). Sudi and Heeschen raised antibodies to diethyl phosphate ester conjugates with carrier proteins (*11*). Although the antibodies could detect a number of OPs, Sudi and Heeschen concluded that the use of immunogens based on diethyl thionophosphate esters could lead to a more sensitive assay for most commercial OP pesticides (*12*). Later attempts made by other immunochemists had great improvements in sensitivity (*13*), but the broad-specificity characteristics or uniform responses to all of the analytes of interest were still not desirable (*4*).

The performance of broad-specificity immunoassay is closely related with the type of antibody used in the analysis. If an antibody with higher affinity to the analyte is used, the method sensitivity will be higher (*14*). Magnetic particle-based ELISA (mp-ELISA) is a relatively new immunoassay configuration in which the nanoparticles are the carriers of the antibodies. The principal feature of mp-ELISA is the use of magnetic particles as a solid support for immunoreagent immobilization. The magnetic particle usually has a chemically active group on its surface, such

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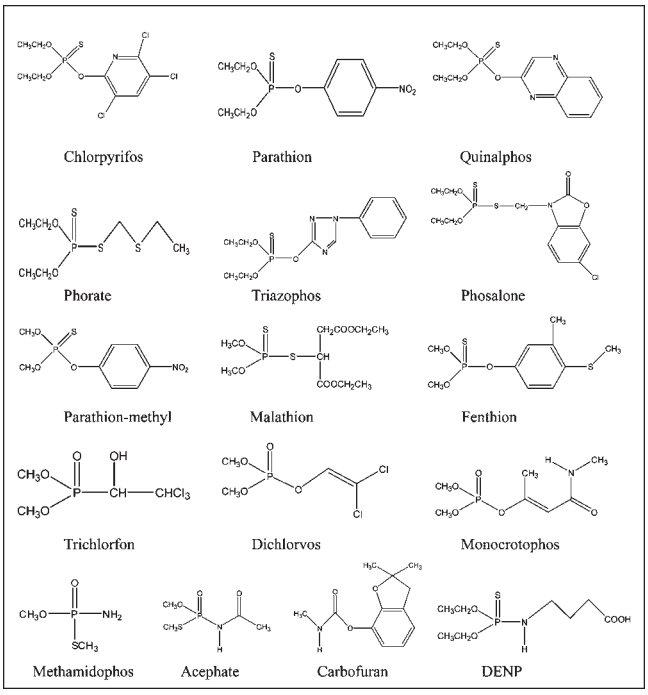

Figure 1. Molecular structures of 15 pesticides and hapten.

as $-CONH_2$ and $-COOH$; therefore, it can be used as a solid antibody. The common magnetic particles are micro/nanospheres of iron oxide (Fe_3O_4 or Fe_2O_3) covered with a polymeric material that allows physical/covalent/affinity attachment, usually of the antibody, onto the particle surface (*15*). The advantages of being coated with a large quantity, having good homogeneity, and a strong binding capacity make the magnetic particle an ideal solid material in immunoassay (*16*).

The mp-ELISAs have been previously described and applied to detect pesticide residues (*17*, *18*). However, there are few studies on the application of mp-ELISA to multiple pesticide residues to improve the sensitivity, broad specificity, and uniform responses to analytes of interest in multiresidue OP immunoassay. In this study, we developed two types of magnetic microparticle antibodies: magnetic first antibody (MFA) and magnetic second antibody (MSA). In addition, two modified indirect competitive ELISA formats based on Fe_3O_4 nanoparticles to multiresidue OP determination were established and evaluated in parallel with a conventional indirect ELISA utilizing the same pair of antibodies.

MATERIALS AND METHODS

Chemicals and Reagents. The OPs chlorpyrifos, parathion, quinalphos, phorate, triazopho, phosalone, methyl parathion, malathion, fenthion, trichlorfon, dichlorvos, monocrotophos, methamidophos, acephate, and carbamate pesticide carbofuran were purchased from the Institute of Agro-environmental Protection, Ministry of Agriculture (Tianjin, China). **Figure 1** shows the chemical structural formula of these OPs. The generic antibody Ab_I against OPs, which was generated by immunizing three New Zealand white rabbits with the hapten−protein conjugate [hapten−bovine serum albumin (BSA)], was kindly provided by Professor Yitong Lu (School of Agriculture and Biology, Shanghai Jiao Tong University). The hapten used was *O*,*O*-diethyl-*N*-(3-carboxypropyl) phosphoramidothioate (DENP) (*19*). The antirabbit goat IgG linked by horseradish peroxidase (Ab_{II}-HRP), dimethylsulfoxide (DMSO), and 3,3′,5,5′-tetramethylbenzidine (TMB) was purchased from Sigma (St. Louis, MO); 96-well ELISA plates were from Corning Costar (Acton, MA). The 3-aminopropyl triethoxysilane (APTES) and all other chemicals were obtained from Sinopharm Chemical Reagent Co. Ltd. (SCRC, Shanghai, China).

Buffers and Solutions. The coating buffer used in the ELISA was 50 mM carbonate buffer, pH 9.6. In addition, 20 mM phosphate buffer saline (PBS; 39 mL of 0.2 M NaH_2PO_4 and 61 mL 0.2 M Na_2HPO_4 dissolved in 1 L of 0.9% NaCl, pH 7.0) was used as a diluent in the preparation of 0.05% Tween 20 in PBS used as a wash solution and 5% skim milk in PBS for blocking. The substrate diluent solution contained 24.3 mL of 100 mM citric acid and 25.7 mL of 200 mM Na_2HPO_4 per 100 mL of Milli-Q-water, pH 5.0. The substrate solution was prepared by adding 400 μL of 1% TMB solution in DMSO and 10 μL of 33% H_2O_2 per 25 mL. As a stop solution, 2 M H_2SO_4 was used.

Preparation of APTES-Coated Fe_3O_4 Magnetic Nanoparticles (APTES-MNPs). The preparation of the APTES-MNPs was based on the method described in the literature with moderate modification (*20*−*22*). First, MNPs were precipitated in an alkali solution of Fe(II) and Fe(III) (molar ratio 2:1) at 85 °C via the standard coprecipitation method. Typically, $FeCl_3 \cdot 6H_2O$ (5.2 g), $FeSO_4 \cdot 7H_2O$ (2.7969 g), and 0.85 mLof concentrated HCl (12.1 mol/L) were dissolved in 200 mL of deoxygenated water. Then, the solution was slowly added dropwise into the previously prepared 250 mL of NaOH solution (0.75 mol/L). Throughout the reaction process, the solution was at 85 °C under N_2 protection and vigorous mechanical stirring. The addition of the Fe^{2+}/Fe^{3+} salt solution resulted in the immediate formation of the black precipitate of MNPs. The reaction continued for another 25 min, and the mixture was cooled to room temperature. The black precipitate was washed with doubly distilled water twice and 95% ethanol twice, and then, the precipitate was diluted into 5 mg/mL with 95% ethanol for storage.

Then, 25 mL of the MNP solution (5 mg/mL) was dispersed in ethanol (150 mL) and water (1 mL) by sonication before APTES (99%, 400 μL) was added to the mixture at room temperature. The mixture was mechanically agitated for 7 h, and the precipitate was separated by centrifugation (10000 rpm or 14336*g* for 30 min). The precipitate was further dispersed in ethanol by sonication and filtered five times by magnetic decantation. The precipitate (APTES-MNP) was stored at room temperature and diluted with 10.5 mg/mL PBS (pH 7.0).

Functionalization of MNPs with Antibodies. The MSA and MFA were prepared according to Gao et al. and Odabas et al. (*22*, *23*). The MNPs were functionalized with Ab_I and Ab_{II}-HRP by cross-linking amine groups on the surface of the MNPs with the amine groups in the antibodies via glutaraldehyde. Then, 500 μL of APTES-MNPs was added into 2 mL of 5% glutaraldehyde solution in PBS (pH 7.0). The mixture was stirred gently for 2 h at room temperature. The MNPs were washed three times with PBS (pH 7.0). Then, either 100 μL of Ab_I or 50 μL of Ab_{II}-HRP was added into the MNPs, and PBS (pH 7.0) was added to reach a final volume of 1.0 mL.

Indirect Competitive ELISA. Microtiter plates were coated overnight at 4 °C with 100 μL/well of 1 μg/mL coating antigen OVA-DENP in 50 mM carbonate buffer (pH 9.6). After the plates were washed with PBST (PBS with Tween 20: 8 g/L NaCl, 1.15 g/L Na_2HPO_4, 0.2 g/L KH_2PO_4, 0.2 g/L KCl, and 0.05% Tween, v/v), the surface of the wells was blocked with 300 μL/well of 5% (w/v) skim milk solution prepared in 20 mM PBS (pH 7.0) for 2 h at 37 °C. After another washing step, 50 μL per well of the appropriate concentration of the first antibody (Ab_I or MFA) and analyte solution was added and incubated for 1 h. Afterward, 100 μL per well of second antibody Ab_{II}-HRP (1:3000 in PBST) or MSF was added and incubated for 1 h at 37 °C. Washing (3 × 300 μL) was performed after each step using a solution of 0.05% (v/v) of Tween 20 prepared in PBS (20 mM, pH 7.0). Finally, the plates were washed again, and 50 μL per well of TMB liquid substrate was added as the chromogen. After that reacted for 20 min at 37 °C, 50 μL per well of 2 M H_2SO_4 was added to stop the reaction. Then, the absorbance was read using 450 nm filters. All experiments were conducted using three- or four-well replicates. The dilutions of antibodies were 1:800 Ab_I (v/v) and 1:2000 Ab_{II}-HRP (v/v) for classic ELISA, 0.5 μg/mL MFA and 1/2000 Ab_{II}-HRP (v/v) for ELISA-MFA, and 1:800 Ab_I (v/v) and 0.75 μg/mL MSA for ELISA-MSA.

Assay Reproducibility. Intra-assay variation was determined by assaying 10 samples of varying concentration, with each sample assayed on four wells within a plate. Four results were obtained for each sample. The coefficient of variation (CV; in percent) between each of these results for each antibody was calculated and averaged. To determine the variation from assay to assay, the samples (n = 15) were run in three separate assays, and the CV was calculated.

Calibration Curves for ELISA. Spectrophotometric ELISA standard curves were obtained using chlorpyrifos and quinalphos standard solutions prepared in 5% methanol−PBS solution. Each experiment was performed in triplicate, and the mean of each value was used for curve fitting. The calibration curves (absorbance at 450 nm versus antigen concentration) were obtained by fitting data to a four-parameter logistic equation as follows $y = \{(A - D)/[1 + (x/C)^B]\} + D$, where A is the maximum absorbance with no analyte present, B is the curve slope at the inflection point, C is the concentration of analyte giving IC_{50}, and D is the minimum absorbance at infinite concentration (*24*).

To allow the indirect comparison of different calibration curves, absorbance values were converted into their corresponding test inhibition values (B/B_0, %) as follows: $\%B/B_0 = (B_0 - B)/(B_0 - B_{ck}) \times 100$, where B is the absorbance value of competitions. B_{ck} and B_0 are the absorbance values corresponding to the blank control and the noncompetition antigen, respectively.

The limit of detection (LOD) was assayed using pollution-free lettuce. The LOD was calculated as the concentration corresponding to 10% of B/B_0. The IC_{50} was evaluated as the concentration of pesticides at 50% B/B_0. The work range was evaluated as the toxin concentration that gives the test inhibition values of 20 and 80% of B/B_0. The slopes obtained from the regression analysis were used to evaluate the matrix effect and the recovery of the assay.

Cross-Reactivity (CR). Data were obtained from standard curves of 12 OPs from the alkoxy group (methoxy/ethoxy), methamidophos/acephate representative of other OP, and carbofuran representative of other pesticides. Each compound was prepared in 5% methanol in PBS and tested in the concentration range from 0.001 to 250 mg/L. The analyte yielding the lowest IC_{50} is referred to as the main analyte and is the basis for the calculation of cross-reactivities of the other analytes according to the following equation (*25*).

$$\text{cross-reactivity } (\%) = \frac{IC_{50} \text{ main analyte}}{IC_{50} \text{ cross-reacting analyte}} \times 100$$

Sample Preparation. For recovery studies, chlorpyrifos (or quinalphos) was spiked into the pesticide-free lettuce, and mp-ELISA and conventional indirect ELISA were done to determine recoveries. Solutions of pesticide in methanol to be used to fortify the samples were prepared at 0.1, 1, and 10 mg/L; 1 mL of the fortifying solution was added to 1 g of finely chopped lettuce leaves. After the leaves were set aside for 24 h, they were then incubated in 5 mL of methanol for 10 min with four vigorous shakes, and the solution was filtered through a filter paper. The container with residue was rinsed with 5 mL of methanol, and the liquid was filtered. The filtrate was then combined with the previous filtrate. Methanol was evaporated to dryness under reduced pressure, and the residue was extracted with 10 mL of 5% methanol−PBS. The mp-ELISA and conventional indirect ELISA were done to analyze the extract. Recovery was determined using the standard curve obtained from the standards in methanol−PBS.

RESULTS AND DISCUSSION

Immunological Characteristics of mp-ELISA. It has been proven that the immobilization procedures of the antibodies on the surfaces of magnetic particles play an important role in constructing the mp-ELISA. A brief study of the specific literature demonstrated that there is still no consensus regarding the most efficient antibody immobilization procedure, which is probably because each antibody is a unique entity, and its behavior after immobilization cannot be rigorously predicted (*14*). Therefore, we utilized magnetic microparticles as first and second antibodies to establish the different models of indirect competitive ELISA to be determined by chlorpyrifos and quinalphos.

Table 1 shows the comparisons between the features of the conventional antibody (Ab) and magnetic antibody (MFA and MSA) for chlorpyrifos and quinalphos. The results show that magnetic antibody not only improves the detection sensitivity but also enlarges the linear working range. As compared with Ab, the LOD and IC_{50} values of the magnetic antibodies, especially MFA, significantly decreased. The IC_{50} values of chlorpyrifos for MFA and MSA decreased by 59.2 and 11.7%, respectively. LOD decreased by 76.7 and 6.9%. In the case of quinalphos, the IC_{50} values for MFA and MSA decreased by 66.1 and 42.5%, respectively, while the LOD decreased by 78.7 and 73.8%. The LODs of MFA for quinalphos and chlorpyrifos were 0.13 and 0.27 mg/L, respectively. The MRLs of quinalphos from Japan's Positive List System for citrus, orange, and grapefruit were 0.5, 0.8, and 0.8 mg/kg, respectively. According to the extraction procedure and the dilution of extract provided by National Standard of China, the values were 0.17, 0.27, and 0.27 mg/L, which were greater than the LOD of MFA. The MRLs of chlorpyrifos for asparagus, apples, banana, broccoli, and Chinese cabbage were 5, 1, 3, 1, and 1 mg/kg, respectively. Likewise, the values were 1.67, 0.33, 1, 0.33, and 0.33 mg/L after dilution, which were also greater than the LOD of MFA. Anyway, this theoretical result should be corrected by extraction procedure and the dilution of extract. Further testing of the application of ELISA to actual sample analysis is needed. For the linear working range of chlorpyrifos and quinalphos, Ab is only about 22- and 31-fold (1.16−26.14 and 0.61−18.70 mg/L), while MFA is 64−80-fold (0.27−17.36 and 0.13−10.34 mg/L) and MSA is 20−158-fold (1.08−21.77 and 0.16−25.37 mg/L). The calibration curves of the three ELISA methods were established based on $B/B_0 - \lg C$. The curves correspond to the four-parameter logistic curves (**Figure 2**).

Table 1. Features of the Conventional ELISA and mp-ELISA Immunoassay[a]

		mg/L		
pesticide	antibody	LWR[b]	IC_{50}[c]	LOD
chlorpyrifos	Ab	1.16−26.14	6.76 (1)	1.16 (1)
	MFA	0.27−17.36	2.76 (−59.2%)	0.27 (−76.7%)
	MSA	1.08−21.77	5.97 (−11.7%)	1.08 (−6.9%)
quinalphos	Ab	0.61−18.70	4.07 (1)	0.61 (1)
	MFA	0.13−10.34	1.38 (−66.1%)	0.13 (−78.7%)
	MSA	0.16−25.37	2.34 (−42.5%)	0.16 (−73.8%)

[a] Data in brackets denote the percentage of decrease as compared with Ab. [b] LWR, linear working range. [c] IC_{50}, midpoint value.

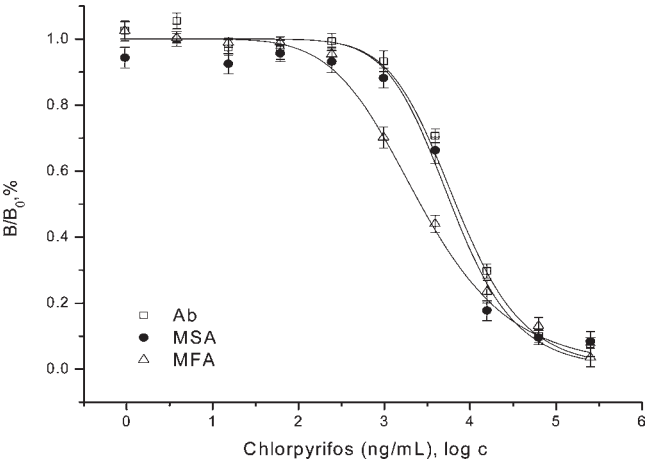

Figure 2. Standard curves of chlorpyrifos performing three types of antibody for the optimum experimental conditions. ELISA assay conditions are as follows: conventional ELISA [Ab (1/800, v/v), OVA-DENP (1 μg/mL), and Ab_{II}-HRP (1/2000, v/v)], mp-ELISA (MFA) [MFA (0.5 μg/mL), OVA-DENP (1 μg/mL), and Ab_{II}-HRP (1/2000, v/v)], and mp-ELISA (MSA) [Ab (1/800, v/v), OVA-DENP (1 μg/mL), and MSA (0.75 μg/mL)]. Data represent means ± SDs of three experiments in the different concentrations ($n = 3$).

Table 2. ELISA Cross-Reactivity for OPs against the Magnetic Antibody

		Ab		MFA			MSA		
		IC_{50}		IC_{50}			IC_{50}		
no.	pesticides	(mg/L)	CR[a] (%)	mg/L	−CK[b] (%)	CR[a] (%)	mg/L	−CK[b] (%)	CR[a] (%)
1	quinalphos	4.07	100	1.38	66.1	100	2.34	42.5	100
2	chlorpyrifos	6.76	60.2	2.76	59.2	50	5.97	11.7	39.2
3	parathion	47.6	8.5	5.96	87.5	23.2	11.1	76.7	21.1
4	phorate	56.3	7.2	3.87	93.1	35.7	8.08	85.6	28.9
5	triazophos	38.3	10.6	3.38	91.2	40.8	17.8	53.5	13.1
6	phosalone	19.0	21.4	3.63	80.9	38.2	6.19	67.4	37.8
7	methyl parathion	65.5	6.2	22.7	65.3	6.1	37.6	42.7	6.3
8	malathion	54.5	7.5	9.86	81.9	14	16.8	69.2	13.9
9	fenthion	115	3.5	11.5	90.0	12	8.22	92.8	28.5
10	trichlorfon	43.9	9.3	1.50	96.6	92	8.87	79.8	26.4
11	dichlorvos	23.7	17.2	3.06	87.1	45.1	5.57	76.5	42
12	monocrotophos	30.4	13.4	7.83	74.2	17.6	16.2	46.4	14.4
13	methamidophos	>1000		>1000			>800		
14	acephate	>1000		>200			>100		
15	carbofuran	>1000		>1000			>1000		

[a] CR denotes cross-reactivity. [b] −CK denotes the percentage of IC_{50} decrease as compared with Ab.

ELISA CR. To further characterize the nature of the antibody recognition, CR, which is a crucial analytical parameter regarding specificity and reliability of immunoassay, was determined. Antibodies with high CR have helped develop ELISAs to screen the group of OPs (*26*). Fourteen commonly used OPs that possess the phosphate and thiophosphate groups and carbamates were used to evaluate the class specificity of the obtained magnetic microparticle antibodies. The obtained IC_{50} values were used to calculate the CR. Quinalphos showed the best recognition pattern; therefore, this analyte was selected as the reference to establish the CR of other compounds of the OP family.

The results show that the RC values were higher by mp-ELISA than those by conventional ELISA. As compared with Ab, the averages of the IC_{50} decreasing rate were 81.1 and 62.1% for MFA and MSA, respectively. One of the main features of the recognition of mp-ELISA was its broad specificity toward a large number of OPs. MFA showed broader specificity than MSA. These results suggest that the recognition is specific for the phosphate group, as the antibody did not bind to methamidophos, acephate, and carbofuran (**Table 2**). MFA recognized OPs that possess the *O*,*O*-diethyl phosphorothioate group (such as chlorpyrifos) and *O*,*O*-dimethyl phosphate group (such as trichlorfon), better than those with *O*,*O*-dimethyl phosphorothioate group (such as methyl parathion).

It has been proven that method performance is closely related with the type of antibody used in the analysis. Method sensitivity will be higher if an antibody with higher affinity to the analyte is used. In the case of mp-ELISA, the antibody was attached covalently via the −COOH groups grafted on the particle surface and the $-NH_2$ groups of the antibody structure. Therefore, an efficient coverage of the magnetic particle surface with the antibody was obtained. Blocking of the active sites of the antibody was also avoided (*14*). In this study, mp-ELISA based on magnetic MFA was the most sensitive immuno-method for multiresidue OP determination as compared with the conventional ELISA and mp-ELISA based on MSA. This is a direct consequence of using magnetic particles. Immobilization of the antibody on the magnetic particle surface allowed the convenient distribution of the antibody into the volume of the microtiter well, improving the efficiency of the immuno-affinity interaction.

Recovery. Spike recovery is a useful tool to monitor the accuracy of an analytical method or immunoassay. Recovery greater than 100% indicates that the measured values for a matrix are higher than the nominal value of the spike, and a recovery less than 100% indicates that the measured values for a matrix are lower than the nominal value of the spike (*27*). If there were no interferences or matrix effects, and the variability was low, one could expect the recovery to be close to 100%. The average recovery of the fortification experiments at each level of fortification must be in the range of 70−120%, with a variation coefficient of ±20% to fulfill the standards of the Environmental Protection Agency (EPA) (*28*). To estimate reliability, recovery tests were performed using pesticide-free lettuce bought from the supermarket. **Table 3** shows the recovery of chlorpyrifos and quinalphos from lettuce. The recoveries in MFA (78−107% of chlorpyrifos and 77−110% of quinalphos) were satisfactory in the three adding concentration levels. The variation coefficients were below 20% at each level of fortification. However, the other two methods did not have good results in detecting pesticides with lower concentration, which were either too high or too low, and this indicated bias in methods. The results show that the mp-ELISA based on MFA could be a useful residue analysis method for multiresidue OP determination.

Table 3. Recovery of Chlorpyrifos and Quinalphos Spiked into Lettuce Samples

		Ab		MFA		MSA	
pesticide	spiked (mg/kg)	recovery (%)	CV[a] (%)	recovery (%)	CV[a] (%)	recovery (%)	CV[a] (%)
chlorpyrifos	0.1	234	43	85	17	190	39
	1.0	124	25	107	13	87	15
	10.0	114	17	78	7	83	16
quinalphos	0.1	232	38	77	20	20	36
	1.0	145	13	90	19	8	21
	10.0	110	21	110	11	88	14

[a] CV = standard deviation (SD)/mean × 100; $n = 3$.

Assay Reproducibility. The reproducibility of the mp-ELISA was assessed by obtaining both intra- and interassay variability data. The intra-assay variability was given by the average of six replicated wells in one microplate. The interassay variability was given by the average of five replicated microplates at different times. **Table 4** shows that the intra-assay average variation coefficients were 9.5, 7.1, and 9.5% for Ab, MFA, and MSA,

Table 4. Comparison of Variability of Intra-assay and Interassay

	Ab				MFA				MSA			
	intra-assay		interassay		intra-assay		interassay		intra-assay		interassay	
concn (mg/L)	B/B_0 (%)	CV (%)	B/B_0 (%)	CV (%)	B/B_0 (%)	CV (%)	B/B_0 (%)	CV (%)	B/B_0 (%)	CV (%)	B/B_0 (%)	CV (%)
1.0	91.9	13.3	95.6	15.1	70.0	9.5	76.8	18.9	90.9	12.1	89.5	19.4
10.0	39.7	9.7	42.7	16.7	26.7	6.7	31.4	13.2	36.0	9.3	37.2	16.1
100.0	6.9	5.4	7.9	12.5	7.9	5.1	6.6	10.8	5.7	7.2	7.5	12.9
mean		9.5		14.8		7.1		14.3		9.5		16.1

respectively, which indicates that measured values from identical microplates are highly repeatable. In the five duplicated microplate assays, the average variation coefficients of the calibration curve in different concentrations were 14.8, 14.3, and 16.1% for Ab, MFA, and MSA, respectively. These results show that there is no significant difference of assay reproducibility between conventional and magnetic ELISA.

ABBREVIATIONS USED

ELISA, enzyme-linked immunosorbent assay; mp-ELISA, magnetic particle-based ELISA; OPs, organophosphorus pesticides; APTES, 3-aminopropyl triethoxysilane; MFA, magnetic first antibody; MSA, magnetic second antibody; HPLC, high-performance liquid chromatography; GC-ECD, gas chromatography with electron capture detector; GC-MS, gas chromatography with mass spectrometry; DMSO, dimethylsulfoxide; HRP, horseradish peroxidase; MNPs, magnetic nanoparticles; LOD, limit of detection; DENP, *O*,*O*-diethyl-*N*-(3-carboxypropyl) phosphoramidothioate.

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