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Development of an Enzyme-Linked Immunosorbent Assay for the Detection of Pentachloronitrobenzene Residues in Environmental Samples

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A competitive enzyme-linked immunosorbent assay (ELISA) for pentachloronitrobenzene (PCNB), a fungicide and chemical intermediate, was developed using a polyclonal antiserum produced against a hapten−protein conjugate of pentachlorophenoxypropionic acid−bovine serum albumin (BSA). An indirect competitive ELISA of PCNB showed an IC_{50} of 37 ng/mL and a limit of detection (LOD) of 7 ng/mL. The ELISA can tolerate up to 10% (v/v) methanol, 5% (v/v) acetonitrile, or 5% (v/v) acetone without significant fluctuation of A_{max} and IC_{50}. The assay sensitivity showed little change in a range of pH from 6 to 8 and concentrations of 0.05−0.2 M NaCl in the assay buffer. Very low cross-reactivities were observed for some structurally related compounds except for hexachlorobenzene (12%). The average recoveries of PCNB from fortified well water, river water, and soil samples were in ranges of 88−94, 80−91, and 70−81%, respectively. The correlations between the gas chromatographic and ELISA results were excellent ($r^2 \geq 0.97$, slopes from 0.86 to 1.10) for those fortified samples. The ELISA is a good alternative tool for monitoring PCNB residues in environmental samples.

KEYWORDS: Pentachloronitrobenzene; ELISA; fungicide; water; soil

INTRODUCTION

Pentachloronitrobenzene (PCNB) was first synthesized in 1868 and introduced into agricultural use during the 1930s in Germany as a substitute for mercurial pesticides. It was widely used as a potent fungicide against soilborne plant pathogens such as *Rhizoctonia solani* and *Sclerotium rolfsii* (*1*−*3*). In China, PCNB was commonly applied in a treatment of soil drench to protect ginseng crops during cool and wet months (*4*). PCNB is also a chemical intermediate in the dye, medicine, and pesticide industries. PCNB is presumably antiestrogenic due to its ability to antagonize the proliferative effect of 17β-estradiol (E2) (*5*). PCNB has slight acute toxicity to mammals and fishes (*6*, *7*). No information is available regarding carcinogenic effects of PCNB in humans; nonetheless, hepatomas were observed in mice exposed by gavages (*8*, *9*). The half-life of PCNB in the soil is relatively long (5−10 months) (*10*). As a result of its high persistence in soil, residues could be found in agricultural products (*11*−*13*).

In recent years, some concern has arisen about the PCNB residues that may remain in agricultural products and the environment. Current methods for detecting PCNB residues include high-performance liquid chromatography (HPLC) (*14*) and gas chromatography−mass spectrometry (GC-MS) (*15*, *16*). These instrumental methods are accurate but expensive and time-consuming, requiring lengthy sample extraction and cleanup procedures. In contrast, immunoassays generally are more sensitive, simpler, faster, and much less expensive. To our knowledge, no immunoassay for PCNB has been reported before. This study was carried out to develop an immunological method for the analysis of PCNB residues in environmental samples. In addition, the assay can be used for product stewardship to improve its application for control of crop pathogens.

MATERIALS AND METHODS

Reagents. PCNB (99% purity), pentachlorophenol (PCP), hexachlorobenzene (HCB), pentachloroaniline (PCA), 2,3,5,6-tetrachloronitrobenzene, 2,4,6-trichloronitrobenzene, 3,4-dichloronitrobenzene, and PCB 209 were purchased from Aldrich Chemical Co. (Milwaukee, WI). The coupling reagents, goat anti-rabbit (GAR) immunoglobin conjugated to horseradish peroxidase (IgG−HRP), bovine serum albumin (BSA), ovalbumin (OVA), *o*-phenylenediamine (OPD), Freund's complete and incomplete adjuvants, and Tween 20, were obtained from Sigma Chemical Co. (St. Louis, MO).

Instruments. ELISA was carried out on 96-well microplates (Nunc-Immuno plate, MaxiSorp surface, Roskilde, Denmark). The result was

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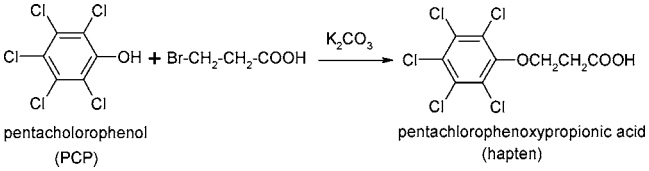


Figure 1. Synthesis scheme for PCNB hapten.

read spectrophotometrically with a Labsystems Dragon Wellscan MK3 microplate reader (Helsinki, Finland). GC analysis was performed on a HP-5890E series II plus GC (Hewlett-Packard, Palo Alto, CA) equipped with dual anode purge (type II) electron capture detectors (ECD), split/splitless injection port with electronic pressure control (EPC) pneumatics, and HPIB communications (INET comm. Optional). A fused-silica capillary column HP-5 MS (30 m × 0.25 mm i.d., 0.25 mm film thickness, Agilent Technologies Inc., Palo Alto, CA) was used with nitrogen as the carrier gas at a flow rate of 1 mL/min. The sample injection volume was 1 μL. The temperatures of injector port and detector were 220 and 320 °C, respectively. The oven temperature was initially at 150 °C for 2 min, increased to 280 °C at a rate of 6 °C/min, and held for 10 min.

Molecular Computations. Global energy minimum structures were obtained by CaChe Worksystem Pro (Fujitsu Computer Systems Co., Sunnyvale, CA). Energy minimization was performed with PM3/water semiempirical force field. Electrostatic potential was mapped over electron density isosurface, where the electron density was 0.1 eV.

Hapten Synthesis. A solution of 3-bromopropionic acid (1.14 g, 7.5 mmol) was added, under argon, into a suspension of PCP (2 g, 7.52 mmol) and potassium carbonate (1.03 g, 7.5 mmol) in acetone (30 mL) (**Figure 1**). The solution was heated in a status of circumfluence reaction for 2 h followed by the addition of a second portion of bromopropionic acid (0.5 g, 3.7 mmol). The mixture was kept in circumfluence reaction overnight, and the gas produced was led to a solution of sodium hydroxide. After removal of the solvent, the residue was partitioned between HCl (aq) and CH_2Cl_2. The organic phase was collected and dried over anhydrate sodium sulfate and the solvent removed with a rotary evaporator. The final compound (pentachlorophenoxypropionic acid, hapten) was obtained by purification of the crude residue with flash chromatography: yield, 50%; ^{1}H NMR ($CDCl_3$) δ 4.35 (t, 2H, J = 7.3 Hz), 2.95 (t, 2H, J = 7.3 Hz); ^{13}C NMR (DMSO) δ 171.9, 151.7, 131.0, 128.2, 128.0, 70.3, 35.0.

Hapten Conjugation. The hapten was conjugated to carrier protein, BSA and OVA, via its carboxylic group by the *N*-hydroxysuccinimide active ester method (*17*). Conjugate formation was confirmed with Spectrophotometer 4060 (Pharmacia LKB Biochrom, Uppsala, Sweden). UV−vis spectra showed qualitative differences between carrier proteins and conjugates in the region of maximum absorbance of the hapten (298 nm). The molar ratios of hapten to BSA and OVA were approximately 31:1 and 12:1, respectively. The conjugates were frozen at −20 °C.

Immunization Procedure and Antiserum Preparation. BSA−hapten conjugate (1 mg) was dissolved in 1 mL of PBS (10 mM phosphate buffer, 137 mM NaCl, 2.7 mM KCl, pH 7.4). The resulting solution was emulsified with Freund's complete adjuvant (1 mL) followed by intramuscular injection into three female New Zealand white rabbits. The animals were immunized and boosted at 15 day intervals with the same immunogen (BSA−hapten conjugate) in Freund's incomplete adjuvant. The rabbits were bled from the ear vein 10 days after each injection except for the initial injection. BSA−hapten conjugates were injected five times into each rabbit. After whole blood was obtained, it was allowed to coagulate overnight at 4 °C and centrifuged to separate the serum. Aliquots of the sera were frozen at −20 °C.

ELISA Development. Optimal concentrations for coating conjugates (OVA−hapten) and antiserum dilution were chosen to give absorbance from 0.5 to 1.2 in the absence of analyte. For this purpose, a checkerboard titration assay was done with different amounts of the coating conjugate (from 0.02 to 10 μg/mL) and the diluted antiserum (from 1:2500 to 1:160,000) in PBS containing 0.05% Tween-20 (PBST). After the screening of antiserum and coating antigen, an indirect competitive ELISA was developed as follows: a microplate was coated with OVA−hapten (5 ng in 100 μL per well of 0.05 M carbonate−bicarbonate buffer, pH 9.6) by incubation at 4 °C overnight. The plate was washed four times with PBST and blocked with 5% skimmed milk powder in PBS (150 μL/well) by incubation at room temperature for 2 h. The plate was washed and incubated with a mixture of a constant concentration of antiserum with various concentrations of analytes at room temperature for 1 h. The plate was washed again and further incubated with goat anti-rabbit IgG−HRP (1:10000 in PBST, 100 μL/well) at room temperature for 1 h. After another washing, 100 μL/well of OPD (1.0 mg/mL in 0.05 M citrate−phosphate with 0.03% sodium perborate, pH 5.0) was added. The reaction was stopped with 2 M sulfuric acid solution (50 μL/well) after an incubation of 15−20 min at room temperature. The absorbance at 490 nm was read immediately, and the data were fitted with the four-parameter logistic equation using SigmaPlot 2000 (version 6.0).

Effects of Buffer pH, Salt Concentrations, and Solvents. The effects of these variables were examined by running a standard curve in media of various pH values, salt concentrations, and percentages of organic solvents. The experiments included multiple levels of the variables and were repeated on three different days. The effects of the variables on A_{max} (the absorbance of zero concentration of PCNB) and the half-maximum inhibition concentrations (IC_{50}, the value comes from the parameter determined by the least-squares fit of the four-parameter equation) were evaluated. To determine the effects of pH, PCNB was diluted in PBST having pH values of 4, 5, 6, 7, 8, and 9. To determine the effects of salt concentrations, PCNB was diluted in 10 mM phosphate buffer (pH 7.4) and NaCl was added to give concentrations of 0.05, 0.1, 0.2, 0.3, 0.5, and 1.0 M. To evaluate solvent effects, methanol, acetonitrile, acetone, or dimethyl sulfoxide (DMSO) were diluted in PBST to yield final solvent compositions of 0, 5, 10, and 20% (v/v).

Fortification and Recovery of Water and Soil Samples. Well water and river water were collected from the campus of China Agricultural University and Xiao Qing He River, respectively. Soil samples were collected from the test field in China Agricultural University. PCNB was fortified in these samples in four levels of 10, 50, 200, and 500 ppb. Water samples were filtered through a 0.45 μm syringe filter (Gelman Sciences, Ann Arbor, MI) and then analyzed by ELISA directly. For the GC method, a Florisil solid-phase extraction (SPE) column was conditioned with 6.0 mL of a mixture of hexane/acetone (v/v, 4:1) and 5.0 mL of hexane. The fortified water sample (2 mL) was mixed with 8 mL of acetonitrile followed by the addition of 0.5 g of NaCl. The sample was shaken vigorously to make sure all NaCl was dissolved, and the supernatant was collected. The remaining sample was further extracted with 8 mL of acetonitrile. The combined supernatant was concentrated to 2 mL and loaded on the top of the cartridge followed by elution with 6.0 mL of a mixture of hexane/acetone (v/v, 4:1). The eluent was collected and evaporated to nearly dryness under a nitrogen stream at 45 °C. The residue was reconstituted in 2 mL of hexane and determined on GC.

A soil sample (10 g) was weighed in a 100 mL beaker followed by the addition of 40 mL of a mixture of distilled water and acetonitrile (v/v, 1:3). PCNB was extracted ultrasonically for 10 min. The solution was transferred to a 50 mL centrifuge tube. After 5 g of NaCl had been added, it was shaken vigorously for 2 min and then centrifuged for 20 min at 6000 rpm. An aliquot (20 mL) was taken from the supernatant. For ELISA, the extraction was dried in a water bath (45 °C) under a nitrogen stream, and the residues were reconstituted with 10 mL of distilled water containing 5% methanol. Before GC analysis, the extraction was cleaned with a Florisil column as mentioned above.

RESULTS AND DISCUSSION

Antisera. All of the antisera showed the highest titer after the final boosting. The titer of rabbit 3 antisera is lower than those of the other two rabbits' (**Figure 2**). The antisera from rabbits 1 and 2 were pooled together and used for the indirect competitive ELISA of PCNB. **Figure 3** shows the standard curve of inhibition by PCNB dissolved in PBST. At the optimal

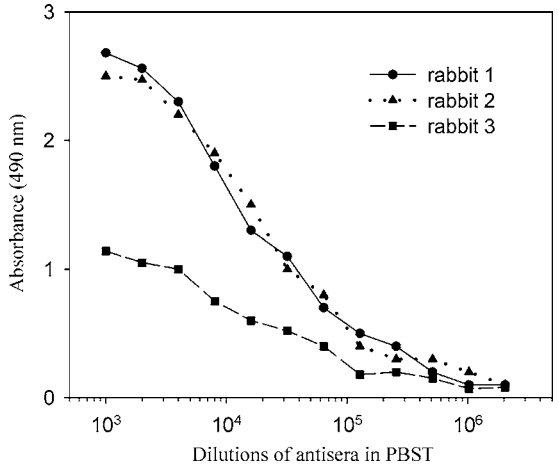


Figure 2. Titration of antisera after final injection by indirect ELISA. A microplate was coated with 0.1 μg/mL OVA–hapten conjugate.

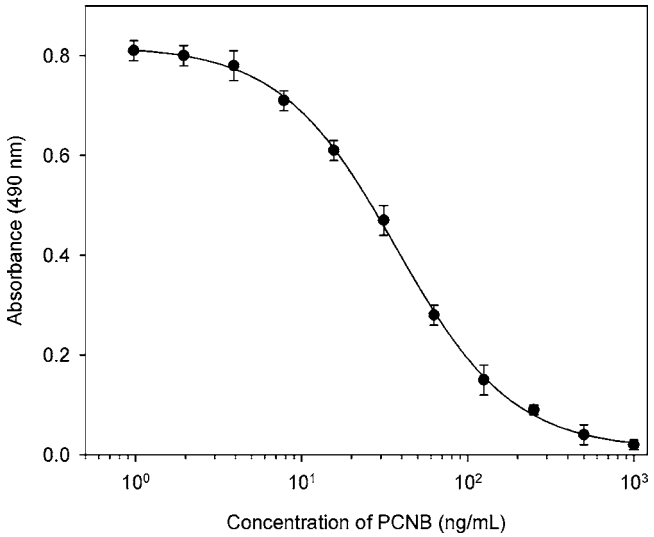


Figure 3. Standard inhibition curves of PCNB by indirect ELISA. Plates were coated with 5 ng of OVA–hapten per well. The pooled antiserum of rabbits 1 and 2 was used and diluted 5000-fold in PBST. Data are the average of four replicates.

concentrations of OVA−hapten (0.05 μg/mL) and antisera (1:5000), the IC_{50} was 37 ppb and the LOD (calculated as IC_{10} value) was 7 ppb, which indicates the antisera have high affinity to PCNB.

Chemical Effects on Assay Performance. Immunoassay performance is often affected by chemical parameters such as ionic strength, pH, surfactant, organic solvent, and other substances in the sample matrix. In practice, these parameters always vary in environmental samples. The effects of these parameters were estimated by comparing IC_{50} values obtained under various conditions with that of a control. Variation of assay pH from 5 to 8 caused little fluctuation in the IC_{50} values (**Figure 4A**). The A_{max}, reflecting maximal binding to the competitive coating hapten in the absence of analyte, fluctuated slightly between pH 6 and 9. When the assay was performed between pH 6 and 8, both the IC_{50} and A_{max} changed a little. This assay is fairly sensitive to pH changes. Thus, the pH value of the sample usually was kept close to 7.0.

The salt effect on ELISA was dramatic when the concentration of NaCl was >0.3 M (**Figure 4B**). In many ELISAs, the IC_{50} value usually decreased with the increase of NaCl concentration in assay buffer (*18*−*20*). This sensitivity improvement may be due to dispersion and weakening of the nonspecific

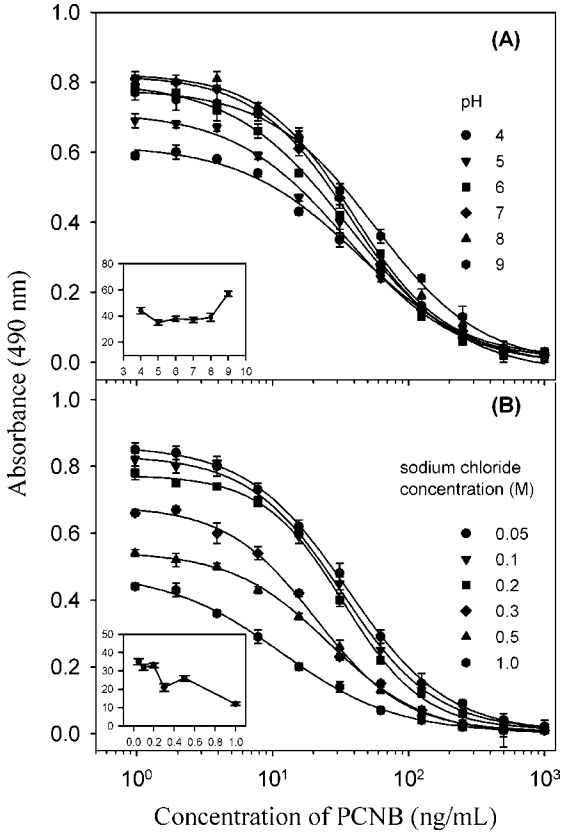


Figure 4. Effects of pH (**A**) and NaCl concentration (**B**) of assay buffer on ELISA. (Insets) Fluctuations of IC_{50} (*Y*-axis) as a function of pH or NaCl concentration (*X*-axis). Each value represents the mean of four replicates.

binding derived from antibodies. Although the IC_{50} decreased, the A_{max} also went down in high NaCl concentrations. In this study, the A_{max} was <0.6 when the NaCl concentration was >0.3 M. It was better to keep A_{max} close to 0.8, so the salt concentration in sample was kept between 0.05 and 0.2 M. However, if a lower LOD is required, the NaCl concentration in the assay buffer can be prepared at 0.3 M, because the IC_{50} was 21 ppb and the A_{max} was approximately 0.65 at this concentration. This condition is also good for ELISA. In practice, the ionic strength of environmental samples can be adjusted by simple dilution with water or concentrated buffer.

In addition, the effects of methanol, acetonitrile, acetone, and DMSO on ELISA were studied because these solvents are water miscible and are commonly used in sample extractions. **Figure 5** shows normalized dose−response curves at various solvent compositions. In general, methanol showed less effect on A_{max} and IC_{50} than other tested solvents. IC_{50} was negligibly affected by methanol up to 10%, whereas it increased at the same concentrations of acetonitrile or acetone. Small effects on A_{max} were observed when the assay was run in concentrations of methanol, acetonitrile, and acetone up to 20, 10, and 5%, respectively. This assay was very sensitive to DMSO as the presence of 5% DMSO resulted in a 15% drop in the A_{max} and a 14% increase in IC_{50}. Thus, to accurately determine the concentration of PCNB, the concentrations of solvents in the assay buffer need to be minimized. A highly sensitive assay would allow reducing the solvent presence by dilution. Alternatively, if higher solvent concentrations are necessary, consistent results could be obtained by maintaining constant concentrations of solvent in both the standards and samples. The sensitivity of the assay might decrease slightly (<20%)

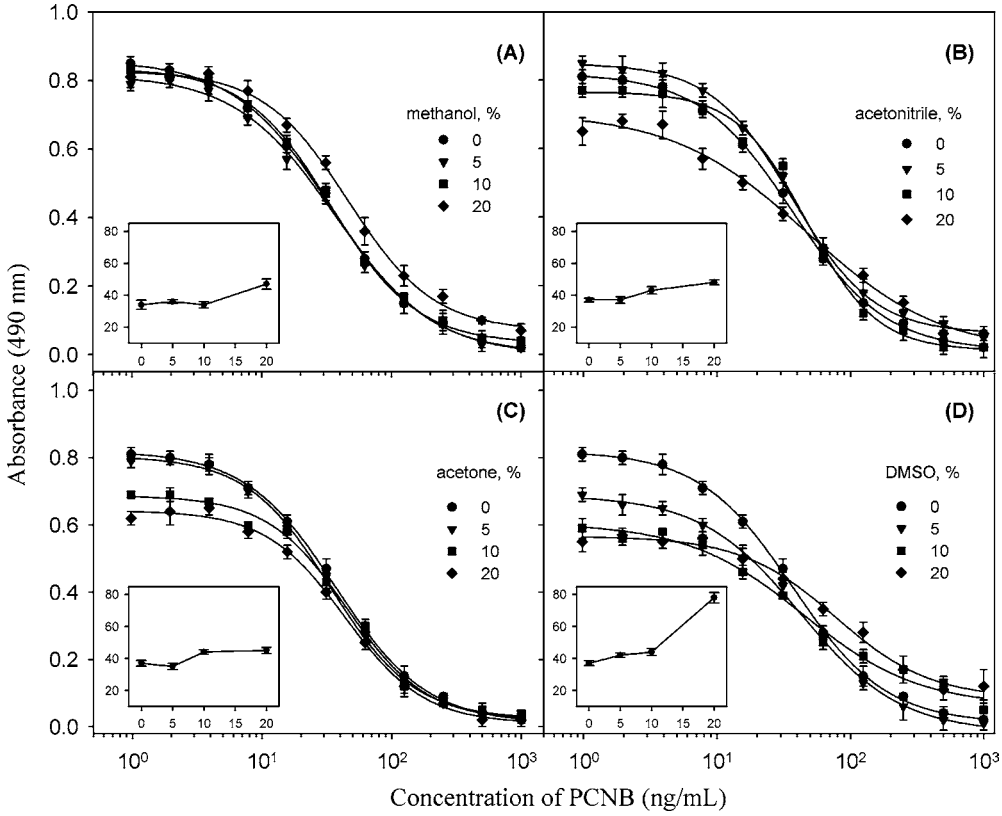


Figure 5. Effects of methanol (**A**), acetonitrile (**B**), acetone (**C**), and DMSO (**D**) on ELISA. Values refer to the final solvent compositions (v/v) in assay buffer. (Insets) Fluctuations of IC_{50} (*Y*-axis) as a function of solvent compositions (*X*-axis). Each value represents the mean of four replicates.

when the concentrations of methanol, acetonitrile, and acetone were kept at ≤10%.

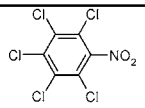
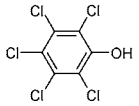
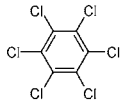
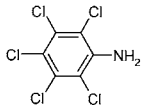
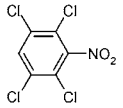
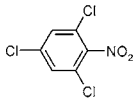
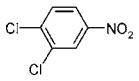
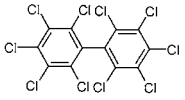
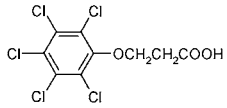
Antisera Specificity. The hapten was originally designed to generate a specific antiserum against PCNB. To test this design we evaluated antiserum recognition to some chemicals structurally resembling portions of PCNB structure (**Table 1**). Cross-reactivity values were calculated as percentages of the IC_{50} of the standard PCNB to the IC_{50} of the test compounds. The most sensitive assay was obtained for PCNB, and its cross-reactivity was considered as 100%. For all of the other compounds tested, the cross-reactivities achieved were very low (≤3%) except that for HCB (12%) and the hapten (238%) for the antiserum tested.

In this study, the result of cross-reactivity for PCP is quite different from that of Noguera et al. (*21*), who obtained the polyclonal antibody against PCP based on the same hapten as used in this study. The PCP assay had an average IC_{50} value of 2.9 ppb in the antibody-coated ELISA (*21*). Generally, the sensitivity and cross-reactivity of antibodies are dependent on several factors such as the structure of immunizing haptens and the physicochemical properties of the test chemicals (i.e., ligand). Ligands should have a proper three-dimensional shape to fit into the recognition site. Electrostatic interaction can determine the strength of binding and cross-reactivity through the difference of electron distribution between ligands. Hydrogen bonds can be an additional factor for various ligands, having atoms with nonbonding valence electron pairs (e.g., oxygen, nitrogen). Hydrophobic interaction is also an important factor to determine the binding affinity. According to computational molecular modeling, the electrostatic properties of PCNB (**Figure 6B**) and un-ionized PCP (**Figure 6C**) were more similar to the hapten (**Figure 6A**) than any other ligands. It is well-known that analytical properties of ELISAs for halogenated phenols are highly dependent on the pH or ionic strength of sample solutions (*22*). For example, PCP is very easily dissociated into its anionic form ($pK_a = 4.71$). The shapes of electrostatic potential isosurfaces of both forms were quite different (**Figure 6C**,**D**). Because of the strong electron negativity of chlorines and aromatic resonance, large portions of negative charges on the phenolate oxygen atom were dispersed into the entire molecule. Similar discrepancies were also found in partial atomic charges (**Figure 7**). Such differences may explain the limited cross-reactivities of PCP in this study. The high-affinity antibodies of PCP were developed with haptens of (2,3,5,6-hydroxyphenoxy)acetic acid (*23*) or pentachlorothiophenol acetic acid (*24*).

In comparison with PCNB, other chlorinated nitrobenzenes (**Figure 6G−I**) can be characterized with their less positive electrostatic potentials over chlorine/hydrogen atoms. HCB (**Figure 6E**) also showed similar differences, which may indicate the strong contribution of electrostatic potentials on the antibody−antigen recognition. In consideration of appreciable cross-reactivity of HCB, the limited or nonexistent cross-reactivities of lower chlorinated nitrobenzene may be explained in part by hydrophobicity. The three-dimensional shape of PCB 209 (**Figure 6J**) is very different from that of the hapten and the other ligands. Because of the steric hindrance of four chlorine atoms in the ortho position, both phenyl rings are positioned perpendicular to each other, which may inhibit proper binding. The electrostatic potentials on the phenyl ring also showed a noticeable difference. Because of the weak electron-withdrawing properties of the amine group, the electrostatic properties of PCA (**Figure 6F**) were largely different from those of the other ligands with a nitro group (**Figure 6B**,**G−I**), which may also explain the limited cross-reactivity.

Recovery of PCNB from Spiked Samples. As is well-known, immunochemical methods have many advantages. On the other hand, these methods are susceptible to matrix interference; however, they can be minimized by simple dilution

Table 1. Assay of Cross-Reactivity among Compounds Structurally Related to PCNB

Compounds	Chemical structure	Cross reactivity (%)
PCNB		100
PCP		3
HCB		12
PCA		2
2,3,5,6-tetrachloronitrobenzene		0.5
2,4,6-trichloronitrobenzene		<0.1
3,4-dichloronitrobenzene		<0.01
PCB 209		<0.01
pentachlorophenoxypropionic acid (hapten)		238

with water or appropriate buffer as long as the analyte concentration is above the LOD. In this study, the suspension was removed from water samples by passing them through the filter. Matrix and ionic strength of the water samples were adjusted by diluting with PBST and the pH was adjusted to around 7.0 before ELISA. The soil extract was dissolved in 10 mL of 5% aqueous methanol, and then the solution was diluted for ELISA. With these simple pretreatments, matrix effects were minimized effectively for all of the samples. As seen in **Table 2**, the recoveries of PCNB from fortified well water, river water, and soil are in ranges of 88−94, 80−91, and 70−81%, respectively. The recovered concentrations of PCNB by ELISA correlated well with the fortified concentrations. These results show that the ELISA is suitable for the analysis of PCNB in water and soil samples.

Comparison between GC and ELISA. To validate the ELISA, correlation studies were performed by analyzing PCNB in fortified samples with both ELISA and GC. Water and soil extracts were too complicated for direct analysis by GC; therefore, a cleanup procedure was necessary. In the present study, a Florisil column was effectively applied to clean up the extracts. **Figure 8** shows the excellent correlations between the results obtained by ELISA and those by GC analyses. The slopes of the correlation curves between the two methods for well water, river water, and soil samples were 1.04, 1.10, and 0.86, respectively. Both methods are sensitive enough to detect 10 ppb of PCNB in the environmental samples. However, there are some differences of pretreatment between these two methods. Compared to ELISA, sample cleanup procedures are required for conventional GC analysis. On the other hand, the ELISA analysis has practically no burden to the environment or health hazards to analysts because of its consumption of a trifle of organic solvent. Because ELISA has by far a higher sample throughput than GC analysis and can fulfill the require-

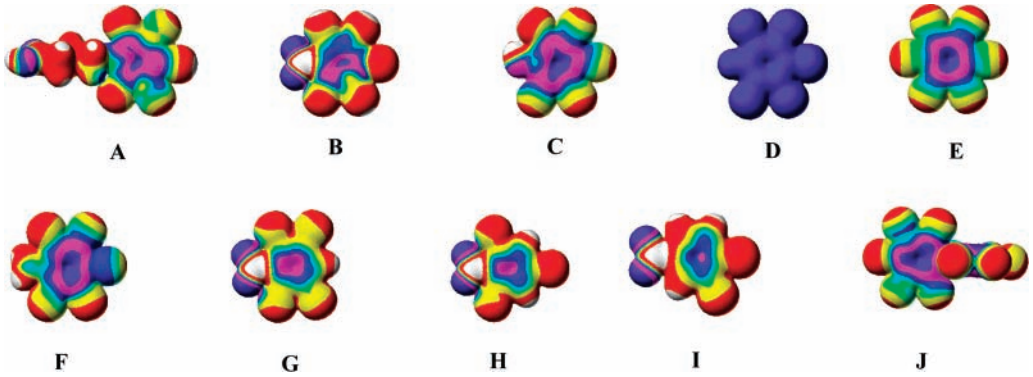


Figure 6. Electrostatic potential maps on electron density isosurface of hapten (**A**), PCNB (**B**), PCP (**C**), PCP anion (**D**), HCB (**E**), PCA (**F**), 2,3,5,6-tetrachloronitrobenzene (**G**), 2,4,6-trichloronitrobenzene (**H**), 3,4-dichloronitrobenzene (**I**), and PCB-209 (**J**). Color code for electrostatic potential: white > 0.090 > red > 0.030 > yellow > 0.010 > green > 0.000 > pale blue > –0.010 > blue > –0.030 > pink > –0.06 > violet.

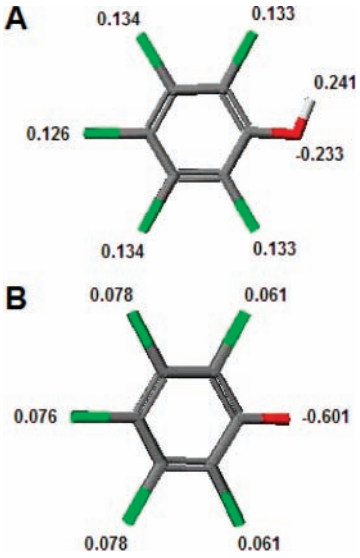


Figure 7. Partial charges of atoms of pentachlorophenol (**A**) and pentachlorophenolate anion (**B**).

Table 2. Recovery of PCNB in Fortified Samples by ELISA

	concentration of PCNB (ppb)		
sample	fortified	detected, $n = 4$	average recovery (%)
well water	0	ND[a]	
	10	9 ± 0.7	90
	50	47 ± 3	94
	200	175 ± 12	88
	500	442 ± 9	88
river water	0	ND	
	10	8 ± 0.5	80
	50	40 ± 1	80
	200	182 ± 9	91
	500	427 ± 13	85
soil	0	ND	
	10	7 ± 0.8	70
	50	38 ± 2	76
	200	158 ± 6	79
	500	407 ± 16	81

[a] Not detected.

ments for monitoring PCNB in the environmental samples, this ELISA can be a valuable screening tool on site or in the laboratory.

Conclusion. We have developed a fast and reliable method based on indirect competitive ELISA format to determine PCNB in water and soil samples. The ELISA results correlated very well with the GC results. The ELISA also presented some advantages over the conventional chromatographic techniques and allowed sensitive, quick, and simple analysis of PCNB. This immunoassay can complement chromatographic techniques in field assay conditions or screening procedures. This method is applicable to water and soil samples; nevertheless, further studies should be performed for its application to more environmental samples and agricultural products.

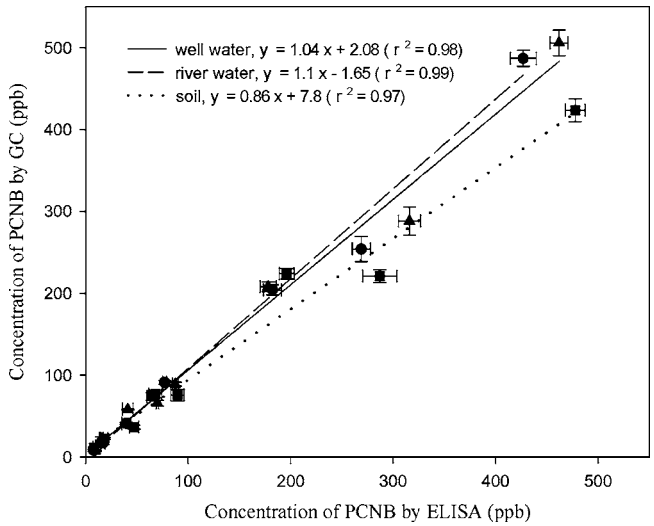


Figure 8. Correlation between ELISA and GC results of PCNB concentrations in well water (▲), river water (●), and soil (■) samples. Error bars are standard deviations.

LITERATURE CITED

(1) Katan, J.; Lockwood, J. L. Effect of pentachloronitrobenzene on colonization on alfalfa residues by fungi and *Streptomyces in* soil. *Phytopathology* **1970**, *60*, 1578−1582.
(2) Ingham, E. R. Review of the effects of 12 selected biocides on target and non-target soil organisms. *Crop Prot.* **1985**, *4*, 3−32.
(3) Carling, D. E.; Helm, D. J.; Leiner, R. H. In vitro sensitivity of *Rhizoctonia solani* and other multinueleate and binucleate *Rhizoctonia* to selected fungicides. *Plant Dis.* **1990**, *74*, 860−863.
(4) http://www.itmonline.org/arts/quintozene.htm.
(5) Zou, E.; Hatakeyama, M.; Matsumra, F. Foci-formation of MCF7 cells as an in vitro screening method for estrogenic chemicals. *Environ. Toxicol. Pharmacol.* **2002**, *11*, 71−77.
(6) Rennet, G.; Nguyen, P. T. Acute toxicities of the fungicide pentachloronitrobenzene and of some of its sulfur-containing metabolites administered to mice. *Arch. Toxicol.* **1982**, *51*, 329−331.

(7) Fushiwaki, Y.; Tase, N.; Saeki, A.; Urano, K. Pollution by the fungicide pentachloronitrobenzene in an intensive farming area in Japan. *Sci. Total Environ.* **1990**, *92*, 55−67.
(8) http://www.scorecard.org/chemical-profiles/html/pentachloronitrobenzene.html.
(9) Borzelleca, J. F.; Larson, P. S.; Crawford, E. M.; Hennigar, G. R. J.; Kuchar, E. J.; Klein, H. H. Toxicologic and metabolic studies on pentachloronitrobenzene. *Toxicol. Appl. Pharmacol.* **1971**, *18*, 522−534.
(10) Shin, S. K.; Kim, J. E.; Kwon, G. S.; Kwon, J. W.; Oh, E. T.; So, J. S.; Koh, S. C. Isolation and identification of a pentachloronitrobenzene (PCNB) degrading bacterium *Alcaligenes xylosoxidans* PCNB-2 from agricultural Soil. *J. Microbiol. (Korea)* **2003**, *41*, 165−168.
(11) Quan, L.; Li, S. F.; Tian, S. J.; Xu, H.; Lin, A. Q.; Gu, L. Determination of organochlorine pesticides residue in ginseng root by orthogonal array design soxhlet extraction and gas chromatography. *Chromatographia* **2004**, *59*, 89−93.
(12) Bierman, R. E.; Riechers, D. E.; Sprague, C. L.; Bollero, G.; Pedersen, W. L. Fungicide−herbicide interaction in soybean (*Glycine max*). *Crop Prot.* **2006**, *25*, 134−139.
(13) Rabie, C. J.; Liibben, A.; Marais, G. J.; Vuuren, H. J. V. Enumeration of fungi in barley. *Int. J. Food Microbiol*. **1997**, *35*, 1l7−127.
(14) Lievremont, D.; Murandia, S. F.; Guyod, J. L. B. Effects of culture parameters on pentachloronitrobenzene removal by *Sporothrix cyanescens*. *Chemosphere* **1996**, *32*, 361−375.
(15) Heikes, D. L. Residues of pentachloronitrobenzene and related compounds in peanut butter. *Bull. Environ. Contam. Toxicol*. **1980**, *24*, 338−343.
(16) http://www.aiha.org/1documents/committees/Meth-Method8270d.pdf.
(17) Lee, J. K.; Ahn, K. C.; Park, O. S.; Kang, S. Y.; Hammock, B. D. Development of an ELISA for the detection of the residues of the insecticide imidacloprid in agricultural and environmental samples. *J. Agric. Food Chem.* **2001**, *49*, 2159−2167.
(18) Li, Q. X.; Zhao, M. S.; Gee, S. J.; Kurth, M. J.; Seiber, J. N.; Hammock, B. D. Development of enzyme-linked immunosorbent assays for 4-nitrophenol and substituted 4-nitrophenols. *J. Agric. Food Chem.* **1991**, *39*, 1685−1692.
(19) Shelver, W. L.; Kim, H. J.; Li, Q. X. Development of a monoclonal antibody-based enzyme-linked immuosorbent assay for the β-adrenergic agonist zilpaterol. *J. Agric. Food Chem.* **2005**, *53*, 3273−3280.
(20) Kim, H. J.; Shelver, W. L.; Li, Q. X. Monoclonal antibody-based enzyme-linked immunosorbent assay for the insecticide imidacloprid. *Anal. Chim. Acta* **2004**, *509*, 111−118.
(21) Noguera, P.; Maquieira, A.; Puchades, R.; Brunet, E.; Carramolino, M. M.; Rodryguez-Ubis, J. C. Development of an enzyme-linked immunosorbent assay for pentachlorophenol. *Anal. Chim. Acta* **2002**, *460*, 279−288.
(22) Galve, R.; Camps, F.; Sanchez-Baeza, F.; Marco, M. Development of an immunochemical technique for the analysis of trichlorophenols using theoretical models. *Anal. Chem.* **2000**, *72*, 2237−2246.
(23) Li, M.; Tsai, S. F.; Rosen, S. M.; Wu, R. S.; Bal Reddy, K.; DiCesare, J.; Salamone, S. J. Preparation of pentachlorophenol derivatives and development of a microparticle-based on-site immunoassay for the detection of PCP in soil samples. *J. Agric. Food Chem.* **2001**, *49*, 1287−1292.
(24) Abuknesha, R. A.; Griffith, H. M. T. Evaluation of a polyclonal antiserum to pentachlorothiophenol-acetic acid-KLH immunogen: binding properties and use with heterologous PCP derivatives in ELISA for pentachlorophenol. *Anal. Bioanal. Chem.* **2004**, *379*, 411−418.

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