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Development of a Multianalyte Enzyme-Linked Immunosorbent Assay for Permethrin and Aroclors and Its Implementation for Analysis of Soil/Sediment and House Dust Extracts

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ABSTRACT: Development of a multianalyte enzyme-linked immunosorbent assay (ELISA) for detection of permethrin and aroclors 1248 or 1254 and implementation of the assay for analysis of soil/sediment samples are described. The feasibility of using the multianalyte ELISA to monitor aroclors 1254 and permethrin simultaneously was tested with permethrin and aroclor standards and with aroclor- and permethrin-containing soil/sediment and house dust samples. Comparison of the I_{50} and I_{20} values of the multianalyte with those of a single-analyte assay revealed similar results, and multianalyte ELISA determination of analyte amounts in soil/sediment dust samples yielded similar results to those of a single-analyte assay. A single-analyte assay of permethrin content in permethrin-containing dust samples showed that the ELISA can determine the analyte accurately in samples with dust matrix contents ranging from 6.25 to 100 mg as indicated by the good correlation between the results of the immunoassay and those of the gas chromatography analysis.

KEYWORDS: *aroclors, permethrin, multianalyte ELISA, residue analysis, soil/sediment residue analysis*

INTRODUCTION

Polychlorinated biphenyls (PCBs) comprise 209 different compounds that share a common structure but vary in the number and position(s) of attached chlorine atoms. Aroclors are specific mixtures of PCB congeners. PCBs were widely used in many applications, especially as dielectric fluids in transformers and capacitors and as coolants, but their manufacture was banned in the United States in the late 1970s and by the Stockholm Convention on Persistent Organic Pollutants in 2001 because of their persistent accumulation in the environment and harmful effects on humans. Although the manufacture of PCBs was stopped over 30 years ago, they are still being detected in various environments (e.g., air, soil, dust, sediments, and food).[1] Because of the ubiquitous presence of PCBs in the environment, humans can be exposed to PCBs through several routes: inhalation of contaminated air (both outdoors and indoors), ingestion of contaminated food, and dermal contact with contaminated surfaces. Studies have shown that dietary ingestion, for example, through the consumption of contaminated fish or oil, is the primary route of exposure to PCBs, and adverse health effects in both children and adults have been linked to PCB exposure. In addition, PCBs have been found in structural caulking materials used in schools and other public buildings, where they present widespread exposure hazards.[2]

Synthetic pyrethroid insecticides have been used in agricultural, domestic, and veterinary applications for more than four decades, and they account for approximately 25% of the worldwide insecticide market.[3] The growth in the use of synthetic pyrethroids, relative to other classes of insecticides, is attributed to their remarkably high insecticidal activity and their generally assumed low acute toxicity to mammals. Although these compounds are widely considered safe for mammals, recent studies have shown that short-term and long-term neonatal and subsequent adult exposure to synthetic pyrethroids may cause developmental neurotoxic and immunotoxic effects that may lead to spontaneous behavioral aberrations; exposure may also cause changes in the muscarinic cholinergic system, impairment of memory and learning, lymph node and spleen damage, and carcinogenesis.[4] The widespread use of pyrethroids in agriculture, horticulture, and forestry increases human exposure via the diet and via occupational and domestic routes and presents potential risks to mammals, nontarget invertebrates, and aquatic organisms[5] that may be exposed to field runoff or drift from aerial and ground-based spraying. All of the above raise an urgent need for large-scale monitoring of PCBs and pyrethroids in agricultural produce and in environmental, domestic, and biological samples.

Currently, high-resolution gas chromatography−mass spectrometry (GC-MS) is the widely accepted and reliable technique for quantitation of PCB contaminants. Receptor gene assays such as the chemically activated luciferase gene expression assay (termed CALUX[6]) and a wide variety of other bioanalytical screening methods are also used for monitoring PCBs and other dioxin-like compounds (for example, see ref 7). Many methods have also been developed and employed for the detection of pyrethroids (for example, see refs 8 and 9). Although the above methods are reliable, sensitive, precise, and reproducible, they are time-consuming and expensive, must be

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performed by highly trained personnel, involve the use of large volumes of toxic solvents, and typically cannot be applied on-site. These methods are, therefore, unsuitable for quick or high-throughput screening tests. In attempts to develop highly sensitive methods and to meet the increasingly stringent regulations and demands for continuous monitoring of PCB and pyrethroid residues in food and in the environment, alternative methods were sought, among them immunoassays (for example, see refs 10−16).

Although the application of immunoassays for individual compound screening is now well established and despite the relatively large number of immunoassays that have been developed for residue monitoring, high-volume application of these methods is still limited. One major reason is the inability of the immunoassay to detect several analytes in a given sample, unlike chemical analytical methods, which can accommodate multiresidue samples. As a result, a main trend that focuses on the development of multianalyte immunoassays has emerged recently. Indeed, in the past few years, multiresidue immunoassays have been developed for a variety of agricultural, industrial, environmental, and medical applications in many different formats (see refs 17and 18).

In the present study, we developed a multianalyte enzyme-linked immunosorbent assay (ELISA) for the detection of permethrin and aroclors 1248 or 1254 simultaneously and implemented the assay for analysis of soil/sediment and house dust samples. The feasibility of using the multianalyte ELISA to monitor aroclor 1254 and permethrin simultaneously was tested with permethrin and aroclor standards as well as with aroclor- and permethrin-containing soil/sediment samples.

MATERIALS AND METHODS

Materials. Aroclors 1016, 1221, 1242, 1254, 1262, and 1268 and PCBs 77, 126, and 169 were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Aroclors 1232, 1248, and 1260 were purchased from Sigma-Aldrich Corp. (St. Louis, MO). Aroclors 1232, 1248, and 1260 and all PCB stock solutions were prepared in Ultra Resi-Analyzed absolute methanol (J. T. Baker, Phillipsburg, NJ). Aroclors 1016, 1221, 1242, 1254, and 1262 were provided in cyclohexane and aroclor 1268 in iso-octane.

Pyrethroids permethrin, tetramethrin, imiprothrin, allethrin, prallethrin, cyphenothrin, cyfluthrin, phenothrin (summithrin), deltamethrin, esfenvalerate, bifenthrin, λ-cyhalothrin, resmethrin, tralomethrin, γ-cyhalothrin, tefluthrin, τ-fluvalinate, and fenvalerate were purchased from Dr. Ehrenstorfer GmbH. Cypermethrin and fenpropathrin were purchased from Riedel-de-Haen (Buchs SG, Switzerland). All pyrethroid stock solutions were prepared in absolute methanol (J. T. Baker).

Immunochemical Methods. *Antisera and Coating Antigens.* Anti-PCB polyclonal antiserum was produced against a 4′-hydroxy analogue of 2,2′,4,5-tetrachlorobiphenyl, namely, 6-[(2,2′,4′,5′-tetrachloro-4-biphenylyl)-oxy]hexanoic acid, which was linked covalently to keyhole limpet hemocyanin (KLH) (Sigma, Rehovot, Israel). The PCB−BSA (bovine serum albumin) coating conjugate (CoAg 560-52) was generated by using 4-(2,4,5-trichlorophenoxy)butyric acid conjugated to BSA as previously described.[15] The protein content of the conjugate was determined with Bradford (Bio-Rad Laboratories GmbH, Munich, Germany) by comparison with a BSA standard curve and was found to be 4 mg/mL. Antipermethrin polyclonal antiserum, generated in rabbits (termed 549), and a coating antigen, permethrin−BSA conjugate (termed *cis*-4-BSA) (10.3 mg/mL), were generated as previously described[19] and kindly provided by Prof. Bruce Hammock of University of California, Davis, United States.

Single-Analyte Assays. Aroclor 1248 and 1254 (Figure 1A) ELISA. The assay was used to determine both the cross-reactivity

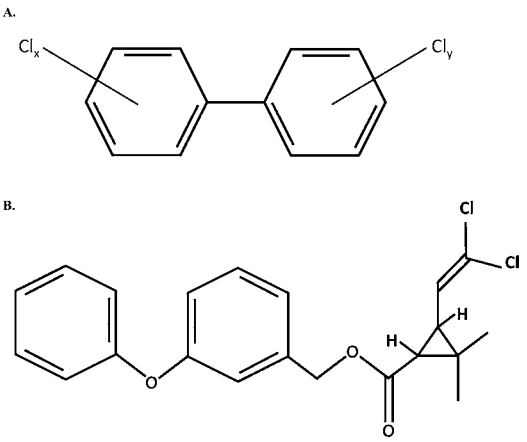

Figure 1. Structure of Aroclors 1248 and 1254 (**A**) and the Pyrethroid Permethrin (**B**). Aroclor 1248 and 1254 are PCB mixture with 48% and 54% chlorine, respectively. "x" and "y" denote the number of chlorine; x + y ranges from 1 to 7 for Aroclor 1248 and from 1 to 9 for Aroclor 1254.

(CR) of the antiaroclor antiserum with a variety of aroclors, PCBs, and pyrethroids and the content of aroclor in soil/sediment samples (see Determination of Aroclor 1254 in Soil/Sediment Samples) and served as a basis for the development of the multianalyte ELISA. MaxiSorp ELISA plates (NUNC Roskilde, Denmark) were coated with 100 μL of PCB−BSA conjugate and diluted 1:40000 (containing 10 ng per 100 μL) in 0.5 M carbonate buffer, pH 9.6. After an overnight incubation at 4 °C, the wells were washed three times with phosphate-buffered saline (PBS) that comprised 0.15 M NaCl in 50 mM sodium phosphate, pH 7.2, containing 0.1% (v/v) Tween-20 (PBST), and 50 μL of aroclor standards (1248 or 1254, in 12 serial dilutions ranging from 0.0048 to 10 ng per 50 μL of PBST containing 40% methanol (hereafter "PBST/methanol") were added to the wells in duplicate, together with 50 μL of anti-PCB antiserum diluted 1:3000 in PBST (final dilution 1:6000). Four wells received a 10-fold excess of each aroclor (i.e., 100 ng per 50 μL of PBST/methanol) and served to determine the background of the reaction, and four additional wells received just PBST/methanol, instead of the aroclors, and served to determine maximal binding in the absence of competing analyte, which was designated as 100%. The plates were incubated for 3 h at room temperature and washed as above with PBST, and 100 μL of secondary antibody conjugated to horseradish peroxidase (HRP) (antirabbit HRP conjugated, Sigma), diluted 1:30000 in PBST, was added to the plates. The plates were incubated for 2 h at room temperature, rinsed with PBST, and tested for HRP activity by addition of 100 μL of substrate solution—3,3′,5,5′-tetramethyl benzidine (TMB) (Thermo Scientific, Rockford, IL). The reaction was stopped after 10 min by addition of 50 μL of 4 N sulfuric acid, and the absorbance at 450 nm was measured with a Lucy 2 microplate reader (Anthos, Eugendorf, Austria).

The CR of the antibodies was tested with a variety of aroclors, PCBs, and pyrethroids. The reaction was carried out as described above (Single-Analyte Assays) by adding these compounds (instead of aroclors 1248 or 1254 in the respective assays) at 12 serial dilutions, using the same concentration range (0.0048−10 ng per 50 μL) and testing their ability to compete with the PCB−BSA conjugate coated on the microplate in binding the anti-PCB antiserum.

Permethrin (Figure 1B) ELISA. The permethrin competitive ELISA assay was carried out as described above for aroclors 1248 or 1254, except for the following modifications: microtiter plate wells were

coated with 100 μL of permethrin−BSA conjugate and diluted 1:2000 (containing 515 ng per 100 μL of protein) in 0.5 M carbonate buffer, pH 9.6, and 50 μL of each of 12 serial dilutions of permethrin standard (a 58/42% mixture of *trans* and *cis*, respectively) was added, together with 50 μL of antipermethrin antiserum diluted 1:1250 in PBST (final dilution, 1:2500). All other details were identical to those described above.

The CR of the antibodies was tested with a variety of aroclors, PCBs, and pyrethroids. The reaction was carried out as indicated above for permethrin competitive ELISA but with these compounds added instead of permethrin, at 12 serial dilutions, covering the same concentration range (0.0048−10 ng per 50 μL) and testing their ability to compete with the permethrin−BSA conjugate coated on the microplate in binding the antipermethrin antiserum.

Multianalyte Assay. MaxiSorp ELISA plates (NUNC) were coated with either 100 μL of PCB−BSA conjugate, diluted 1:40000 (containing 10 ng per 100 μL), or with permethrin−BSA conjugate, diluted 1:2000 (containing conjugate at 515 ng per 100 μL), in 0.5 M carbonate buffer, pH 9.6. After incubation overnight at 4 °C, the wells were washed three times with PBST, and 50 μL of a mixture of aroclor (1248 or 1254) and permethrin standard (12 serial dilutions of each standard compound, ranging from 0.0048 to 10 ng per 50 μL of PBST/methanol) was added, in duplicate, to all the wells, together with 50 μL of a mixture of anti-PCB and antipermethrin antiserum diluted 1:3000 and 1:1250, respectively, in PBST (i.e., final dilutions of 1:6000 and 1:2500, respectively). Four wells received a 10-fold excess of a mixture of each aroclor with permethrin (i.e., each at 100 ng per 50 μL of PBST/methanol) and served to determine the background of the reaction, and four additional wells received just PBST/methanol and served to determine maximal binding in the absence of competing analyte (designated as 100%). The plates were incubated for 3 h at room temperature and washed as above with PBST, and 100 μL of secondary Ab conjugated to HRP, diluted 1:30000 in PBST, was added to the plates. The plates were incubated for 2 h at room temperature, rinsed with PBST, and tested for HRP activity by the addition of 100 μL of TMB substrate solution. The reaction was stopped after 10 min by addition of 50 μL of 4 N sulfuric acid, and the absorbance at 450 nm was measured with a Lucy 2 microplate reader (Anthos).

Analysis of Analytes in Soil/Sediment Samples. *Determination of Aroclor 1254 in Soil/Sediment Samples.* Soil/sediment samples ("aroclor- containing soil/sediment samples") were prepared by a procedure based on the extraction method developed for dioxins and furans in sediment/soil.[20] In brief, an aliquot (4 g) of sediment/soil sample was mixed with Hydromatrix (3 g), alumina (3 g), 10% $AgNO_3$ in silica (1 g), and acid silica (6 g) and extracted with dichloromethane using Dionex Accelerated Solvent Extraction 200 system (Sunnyvale, CA). The extraction was carried out at 100 °C, with a purge time of 60 s, a flush volume of 100%, and an extraction time of 10 min and three cycles. The resulting extracts were concentrated and divided into two portions: one for GC and one for ELISA. The sample extract for ELISA portion was solvent exchanged to methanol for subsequent analysis.

GC Analysis. The samples and standard solutions were analyzed by GC with electron capture detection for aroclor concentrations based on EPA Method 8082.[21] The GC column used was a DB-5 fused silica capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness), and hydrogen was used as the carrier gas. Identification and quantitation were accomplished by integrating representative major peaks in the aroclor standard and identifying and integrating those same peaks (by retention time and pattern matching) in the samples.

ELISA Analysis. Single- and multianalyte aroclor ELISA was used to analyze aroclor 1254 content in soil/sediment samples as described above. The soil/sediment samples were diluted 1:2.5 with PBST to bring the methanol concentration in the sample to 40%. Samples 1 and 2 were then diluted 1:30, and samples 3, 4, and 5 were diluted 1:150, all in PBST/methanol.

In the single-analyte aroclor, ELISA soil/sediment samples were added (in a volume of 25 μL) together with 25 μL of PBST/methanol to wells coated with PCB−BSA to reach final dilution ranges of 1:150 to 1:2400 for samples 1 and 2 (equivalent to 6.7 to 0.42 mg of sediment/soil) and 1:750 to 1:12000 for samples 3, 4, and 5 (equivalent to 1.3 to 0.083 mg of sediment/soil). Fifty microliters of PCB antiserum that had been diluted 1:3000 in PBST (final dilution of 1:6000) was added to the plates, and the reaction mixture was incubated for 3 h at room temperature and processed as above (see Single-Analyte Assays).

In the multianalyte ELISA format, aroclor soil/sediment samples at the same initial dilution (1:30 for samples 1 and 2; 1:150 for samples 3, 4, and 5), together with 25 μL of standard permethrin (at 2.5 ng per 25 μL) instead of the PBST/methanol were added to wells coated with either PCB−BSA or permethrin−BSA. Fifty microliters of a mixture of anti-PCB and antipermethrin antiserum diluted 1:3000 and 1:1250 in PBST (final dilutions 1:6000 and 1:2500, respectively) was added to the PCB−BSA- and permethrin−BSA-coated wells, and the reaction mixture was incubated as indicated above.

To exclude the possibility that the aroclor soil/sediment samples interfered with the permethrin ELISA and might thereby have affected the values obtained in the multianalyte assay, aroclor soil/sediment samples were tested for their interference with a single-analyte permethrin assay. Twenty-five microliter samples (diluted 1:2.5 with PBST as above), together with 25 μL of PBST/methanol, were added to wells coated with permethrin−BSA conjugate (diluted 1:2000) to reach final sample dilution ranges of 1:150 to 1:2400 (samples 1 and 2) or 1:750 to 1:12000 (samples 3, 4, and 5). Fifty microliter aliquots of permethrin antiserum, diluted 1:1250 in PBST (to a final dilution of 1:2500), were added to the wells, and the reaction mixture was incubated for 3 h at room temperature and processed as above.

Five serial dilutions (ranging from 5.0 to 0.312 ng per 50 μL) of standard aroclor 1254 in PBST/methanol served as a quality control to determine the assay accuracy. The aroclor content in the single-aroclor format was calculated from a calibration curve of aroclor 1254, and that in the multiaroclor format was calculated from a calibration curve of a mixture of aroclor 1254 and permethrin diluted in PBST/methanol (each analyte in the range of 0.0048 to 10 ng/50 μL, diluted in PBST/methanol), after linearization of the data by transformation to a logit-log plot by means of Microcal Origin software, Version 7.5 (Microcal Software, Northampton, MA). All aroclor soil/sediment samples were tested in duplicate at dilutions within the range of the standard curve. The slopes of all samples were tested for parallelism with the standard curve, by examining the homogeneity of regression slopes, and only samples whose regression lines were parallel to the standard curve were considered.

Determination of Permethrin in House Dust Samples. House dust samples (permethrin-containing dust samples) were prepared for analysis as described previously.[9] In brief, the dust sample was extracted with dichloromethane using sonication and solvent exchanged into acetonitrile for subsequent solid-phase extraction (SPE) cleanup prior to GC-MS analysis. For ELISA analysis, the dust sample (0.5 g) together with neutral silica (5 g), acid silica (0.5 g), and Hydromatrix (1 g) was extracted with dichloromethane. The extraction was performed at 2000 psi and 100 °C for three cycles of 5 min. The dichloromethane extract was concentrated and solvent exchanged into methanol prior to ELISA.

GC-MS Analysis. The GC-MS procedure for analyzing the permethrin-containing dust sample extracts was described previously.[9] Briefly, a Hewlett-Packard GC-MS was operated in the SIM mode. The GC column was a DB35MS fused silica capillary (30 m × 0.25 mm i.d., 0.25 μm film thickness). Helium was used as the carrier gas. Data acquisition and processing were performed with a ChemStation data system.

ELISA Analysis. Single-analyte permethrin ELISA was applied to the permethrin-containing dust samples as described above. The samples were diluted 1:2.5 with PBST, to bring the methanol concentration in the sample to 40%, and then serially diluted four times in PBST/methanol, to final dilutions ranging from 1:5 to 1:80 (equivalent to 100 to 6.25 mg of house dust). Fifty microliter samples were added to

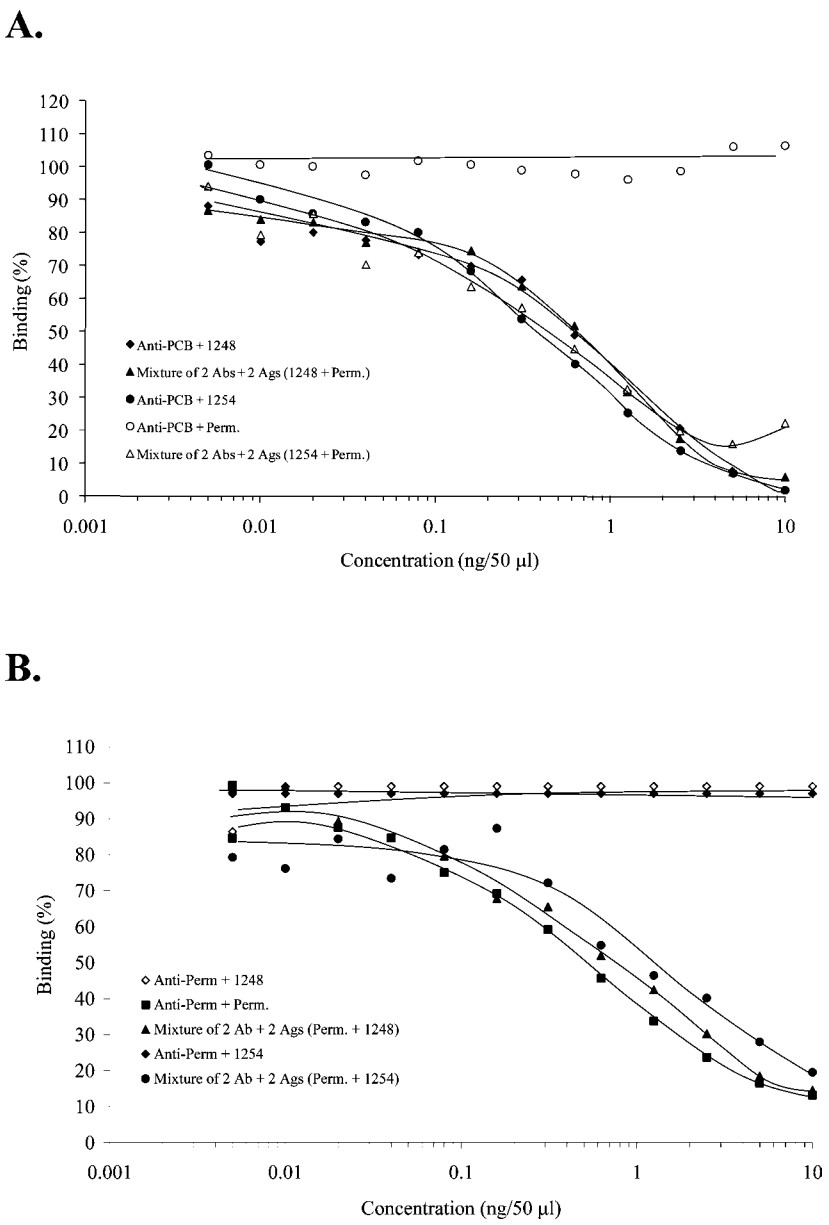

Figure 2. Representative standard curves of a single- and a multianalyte aroclors 1248 or 1254 ELISA in the presence and absence of permethrin (A) and in the presence and absence of aroclors 1248 or 1254 (B).

permethrin–BSA conjugate-coated wells together with 50 μL of antipermethrin antiserum diluted 1:1250 in PBST, to a final dilution of 1:2500. Five serial dilutions, ranging from 10 to 0.625 ng of standard permethrin per 50 μL of PBST/methanol, served as a quality control to determine the assay accuracy. Permethrin concentrations were calculated from a calibration curve (in the range of 0.0048–10 ng per 50 μL diluted in PBST containing 40% methanol), after linearization of the data as described above. All permethrin dust samples were tested in duplicate at dilutions within the range of the standard curve. All other details were as described above for single-analyte assay.

Statistics. Results were subjected to one-way analysis of variance (ANOVA). The significance of differences among means was evaluated with the Tukey–Kramer HSD (honestly significant difference) test at $p < 0.05$.

RESULTS AND DISCUSSION

Single-Analyte ELISA. The first part of the study focused on optimization of two single-analyte ELISAs: permethrin and aroclors (1248 and 1254). Although the antisera that were used in the study have been previously described and employed to develop permethrin and aroclor ELISAs,[15,19] the assay conditions in this study had to be re-evaluated and modified to fulfill the basic requirement of a multianalyte ELISA: employment of the very same reaction conditions (i.e., buffers, incubation times, secondary Ab dilution, etc.) with all antibodies and analytes. As a consequence, optimization of each ELISA was carried out separately for each analyte. The optimization involved two sets of experiments: the first set was intended to determine the optimal concentrations of the coating antigen conjugates permethrin–BSA or PCB–BSA, the antiserum and the secondary Ab (checkerboard tests); the second set was intended to generate a standard curve, to determine the I_{50} value and the limit of detection (LOD, I_{20}) of the assay, CR, and the tolerance of the antibodies to organic solvents.

The first set of experiments revealed that for the permethrin ELISA dilutions of 1:2000 for the permethrin–BSA conjugate

Table 1. Comparison of I_{50} and LOD (I_{20}) Values of Single- and Multianalyte ELISAs[a]

	I_{50} (ng/mL)		I_{20} (ng/mL)	
analyte	single-analyte	multianalyte	single-analyte	multianalyte
aroclor 1248	16.0 ± 2.8 (n = 2)	16.0 ± 2.8 (n = 2)	1.6 ± 2.0 (n = 2)	1.6 ± 2.0 (n = 2)
aroclor 1254	7.5 ± 1.0 (n = 8)	8.9 ± 2.9 (n = 7)	1.8 ± 0.8 (n = 8)	1.5 ± 0.7 (n = 7)
permethrin	35.8 ± 26.7 (n = 6)	48.0 ± 29.0 (n = 4)	6.2 ± 7.1 (n = 6)	14.3 ± 18.1 (n = 4)

[a]Each value represents the mean ± SEM (standard error mean) of n measurements (as indicated in the table). I_{50} represents the concentration of the analyte required to displace 50% of the analyte bound to the Ab. Statistical analysis compared values obtained in the single- and the multianalyte formats for each analyte independently. Values for all tested samples did not differ significantly at $p < 0.05$.

and 1:2500 (final) of the antipermethrin antiserum resulted in high binding and a low background, that is, nonspecific binding. In the aroclor ELISA, dilution of 1:40000 for the coating antigen and 1:6000 for the anti-PCB antiserum resulted in a good signal-to-background ratio for both aroclors (1248 and 1254; data not shown). The second set of experiments determined the working ranges of both assays (0.06−2.5 ng per 50 μL) (Figure 2A,B) and their I_{50} and I_{20} values (Table 1). Basically, there were no marked differences in the working ranges of the two ELISAs, and the I_{50} and I_{20} values were very similar. Because of the very low solubility of permethrin and the aroclors in neutral aqueous buffers, both assays were carried out in the presence of methanol at a final concentration of 20%.

CR. Once the assays had been optimized and the sensitivities determined, the ELISAs were used to characterize the antiserum for specificity and for CR with other pyrethroids, aroclors, and PCBs (Table 2). Although both antisera have been previously tested for their CR,[15,19] they were not tested under the same reaction conditions (as specified in this study) nor were they examined for their ability to react with analytes of the other group (namely, anti-PCB antisera with pyrethroids and antipermethrin antisera with aroclors and PCBs). Because CR may differ as a function of reaction conditions and because it was most important to prove lack of CR between the antisera and the other group of analytes, CR had to be re-evaluated under the tested experimental conditions. The CR of the PCB-antiserum was tested with aroclor and PCB compounds (Table 2) as well as with pyrethroid compounds (listed in Table 3). A similar analysis was carried out with the permethrin antibodies. The CR was determined by means of the single-analyte ELISA format. As indicated in Table 2, the PCB antiserum did cross-react with a few aroclors. When aroclor 1248 was used as a reference, the reactivity of the PCB antiserum with 1254, 1016, and 1242 was higher than that with 1248, and the antiserum also showed high CR with aroclors 1262 and 1260; CR with 1232 was low and no CR with aroclors 1221 and 1268, and the three tested PCBs could be detected. Examination of CR with 1254 as a reference revealed lower values (76, 76, 47, 41, and 35% with aroclors 1016, 1242, 1248, 1262, and 1260, respectively) and 13% with 1232. No CR was detected with any of the other aroclors or PCBs. Under the conditions of the ELISA experiments, the PCB antiserum did not cross-react with any of the tested pyrethroid compounds (Table 3) at concentrations up to 0.2 ppm (10 ng per 50 μL) (data not shown). CR analysis of the antipermethrin antiserum revealed very high values toward tetramethrin, imiprothrin, allethrin, and prallethrin (Table 3). The antiserum also recognized cypermethrin and cyphenothrin, cyfluthrin, and phenothrin to a lesser extent. No CR was monitored with the other pyrethroids or with any of the tested aroclors or PCBs (listed in Table 2, data not shown). The CR of the anti-PCB antiserum with several aroclors and that of the antipermethrin antiserum with several pyrethoids necessitate to term the assays developed herein "single-group" and "multigroup" ELISAs. For simplicity

Table 2. Cross-Reactivity of AC-3 Antiaroclor Antiserum with Various Aroclors and PCBs[a]

N	compd	CR (%)	compd	CR (%)
1	aroclor 1248	100	aroclor 1254	100
2	aroclor 1254	163	aroclor 1248	47
3	aroclor 1016	125	aroclor 1016	76
4	aroclor 1242	125	aroclor 1242	76
5	aroclor 1262	67	aroclor 1262	41
6	aroclor 1260	57	aroclor 1260	35
7	aroclor 1232	22	aroclor 1232	13
8	aroclor 1221	0	aroclor 1221	0
9	aroclor 1268	0	aroclor 1268	0
10	PCB-77	0	PCB-77	0
11	PCB-126	0	PCB-126	0
12	PCB-169	0	PCB-169	0

[a]Reactivity with aroclor 1248 was designated as 100% in the left-hand column and that with aroclor 1254 as 100% in the right-hand column. CR was calculated as the ratio (as percentage) between the I_{50} value of aroclor 1248 or 1254 and that of the tested compounds.

Table 3. Summary of the CR of Antipermethrin Antibodies with Various Pyrethroids[a]

N	compd	CR (%)
1	permethrin	100
2	tetramethrin	5242
3	imiprothrin	4325
4	allethrin	2883
5	prallethrin	2163
6	cypermethrin	100
7	cyphenothrin	47
8	cyfluthrin	29
9	phenothrin (sumithrin)	12
10	deltamethrin	0
11	esfenvalerate	0
12	bifenthrin	0
13	λ-cyhalothrin	0
14	resmethrin	0
15	tralomethrin	0
16	fenpropathrin	0
17	γ-cyhalothrin	0
18	tefluthrin	0
19	τ-fluvalinate	0
20	fenvalerate	0

[a]Reactivity with permethrin was designated as 100%. CR was calculated as the ratio (as percentage) between the I_{50} value of permethrin and that of all other tested compounds.

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reasons, we chose to term the assays "single-analyte" and "multianalyte" ELISAs, respectively, despite the fact that each assay can monitor more than one analyte of a given group.

Multianalyte ELISA. The absence of CR of the PCB antiserum with the tested pyrethroids and of the permethrin antiserum with the aroclors and PCBs enabled us to develop a multianalyte assay format in which the plates were coated with either PCB or permethrin−BSA conjugates, and the PCB or permethrin antisera were tested for their ability to recognize their specific analytes (aroclors 1248 or 1254 or permethrin, respectively) in the presence of a mixture of two antibodies and two analytes (one of the aroclors and permethrin). The assay was performed in the presence of both antibodies to investigate that other components present in the reaction mixture (e.g., other antibodies or analytes) do not cause interference. Each experiment included, in addition to the above, a positive control in which the assay was carried out with a single Ab and its respective single analyte (i.e., PCB antiserum and aroclor 1248 or 1254 and permethrin antiserum and permethrin) and a negative control in which each Ab was tested for its ability to recognize the other analyte (i.e., PCB antiserum and permethrin and permethrin antiserum and aroclor 1248 or 1254). The data presented in Figure 2A and Table 1 clearly indicate that the presence of the permethrin antiserum and permethrin analyte did not interfere with the ability of the antiaroclor antiserum to recognize the aroclors, and the I_{50} values that were obtained in their presence and absence did not differ significantly from those obtained in the single-analyte aroclor 1248 assay [16.0 ± 2.8 (n = 2) and 16.0 ± 2.8 (n = 2), respectively]. Similar data were obtained with the aroclor 1254 multianalyte ELISA format [I_{50} values of 7.5 ± 1.0 (n = 8) and 8.9 ± 2.9 (n = 7) in the single- and multianalyte formats, respectively]. PCB antiserum did not react with permethrin under the tested conditions.

The data presented in Figure 2B and Table 1 indicate similar results for the permethrin antiserum. The presence of the PCB antiserum and aroclors 1248 or 1254 did not interfere with the ability of the permethrin antiserum to recognize the permethrin, and the I_{50} values that were obtained in their presence and absence did not differ significantly from those obtained in the single-analyte permethrin assay [48.0 ± 29.0 (n = 4) and 35.8 ± 26.7 (n = 6), respectively]. Antipermethrin by itself did not react with any of the aroclors.

Various approaches have been reported to the simultaneous determination of several analytes using immunochemical assays indicating that the concept "multiresidue" immunoassay refers to a large variety of formats.[18,22] To date, the most commonly used method for multianalyte analysis is the bead-based Luminex flow cytometric system.[23] As in the case of the multianalyte ELISA, the absence of CR of the antibodies is a prerequisite for the development of a reliable Luminex assay. The current assay format (which employs several antibodies with high affinity toward a given class of analytes and no CR with the other class of compounds) enables quantitation of the concentrations of the individual tested analytes in a simple, fast, and direct manner using identical assay conditions (i.e., same buffers, reporting enzyme substrates, incubation times, etc.) for both analytes. An assay of such a format can be used for a large variety of analytes and therefore introduces many advantages over current published methods. A similar approach has been reported for the detection of pharmaceutical residues in porcine kidneys,[24] although it is difficult to determine from the report whether quantitation of a given analyte was determined in the presence of all other analytes and antibodies.

Application of Single- and Multianalyte ELISA for Analysis of Aroclor 1254 and Permethrin Content in Aroclor Soil/Sediment Samples. We examined the ability of the above ELISAs to determine the content of aroclor 1254 in soil/sediment extracts and compared the values obtained in single and multiple ELISAs. In the single-analyte aroclor assay, samples were applied onto PCB−BSA-coated plates and tested with anti-PCB antiserum; this assay was designed to determine the amount of aroclor 1254 in the soil/sediment samples in standard aroclor assay format. In the multianalyte assay, soil/sediment samples spiked with permethrin at 100 ng/mL were tested in the presence of both PCB and permethrin antiserum in plates that were coated with PCB−BSA conjugate. This assay was designed to determine the amount of aroclor 1254 in the tested soil/sediment samples in an assay format in which another analyte (permethrin) and another Ab (antipermethrin) were present in the reaction mixture. In another multianalyte assay that was carried out in a similar manner, the aroclor soil/sediment samples, spiked with permethrin at 100 ng/mL, were tested with plates coated with permethrin−BSA conjugate and a mixture of the two antibodies. This assay was designed to determine whether the aroclor soil/sediment samples interfered in any manner with the ability to determine permethrin in a multianalyte ELISA format.

In parallel, a single-analyte permethrin assay was carried out on these aroclor soil/sediment samples (in the absence of spiked permethrin); plates coated with permethrin−BSA were used, which were tested just with antipermethrin antiserum, to determine the degree of interference of the aroclor soil/sediment samples themselves with the ELISA and to exclude the possibility that the samples interfered with the permethrin ELISA and thereby affected the values obtained in the multianalyte assay. Comparison of the amount of aroclor 1254 in the aroclor soil/sediment samples obtained in the single aroclor 1254 with that obtained in the multianalyte ELISA format (Figure 3) revealed no significant differences, indicating that the presence of an unrelated analyte (permethrin) and antiserum (antipermethrin) in the reaction mixture did not have a significant effect on the ability to evaluate aroclor 1254 content in the samples. The values

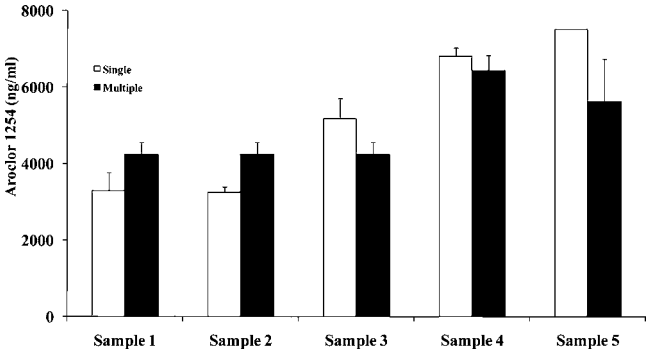

Figure 3. Aroclor 1254 content in aroclor soil/sediment samples: comparison of values obtained in single- and multianalyte aroclor 1254 ELISA formats. Each bar represents the mean ± SEM (standard error mean) of three or four measurements. Statistical analysis compared values obtained in the single vs multiple assay formats for each sample independently. Values of all tested samples did not differ significantly at $p < 0.05$.

obtained were in good agreement with those obtained by GC-MS analysis (data not shown).

Application of a Multianalyte ELISA for Analysis of Spiked Permethrin Content in Aroclor Soil/Sediment Samples. Examination of permethrin content in the aroclor soil/sediment samples spiked with permethrin at 100 ng/mL, which were tested in a multianalyte ELISA format, revealed that the values obtained did not differ significantly from those expected (Table 4), which confirmed once again that the presence of an unrelated analyte (aroclor) and its antiserum (anti-PCB) did not affect the ability to quantitate permethrin accurately. The aroclor soil/sediment extracts themselves did not interfere with the permethrin ELISA, and the percentage of binding of the permethrin antiserum to the coating antigen (permethrin−BSA) in the presence of the aroclor soil/sediment samples yielded binding values (84, 87, 81, 80, and 82% for samples 1−5, respectively) that did not differ markedly from those obtained in the absence of the soil/sediment sample (designated as 100%).

Table 4. Amount of Spiked Permethrin Detected in Aroclor Soil/Sediment Samples in a Multianalyte ELISA Format[a]

sample	permethrin content (ng/mL) ± SEM
standard	100[b]
sample 1	78 ± 11 (n = 5)
sample 2	109 ± 20 (n = 2)
sample 3	78 ± 16 (n = 3)
sample 4	130 ± 15 (n = 3)
sample 5	110 ± 10 (n = 2)

[a]The permethrin content was determined from a mixed aroclor/permethrin standard curve in wells coated with permethrin−BSA conjugate. Each value represents the mean ± SEM (standard error mean) of n measurements (as indicated in the table). n represents the number of repetitions of each sample whose results fell within the linear range of the standard curve and were used to calculate the amount of aroclor in the sample. The significance of differences among means was evaluated with the Tukey−Kramer HSD (honestly significant difference) test at $p < 0.05$. No differences were observed between the permethrin contents in the presence and absence of the soil/sediment samples. [b]Expected value based on the amount of spiked permethrin (100 ng/mL). Results were subjected to one-way ANOVA.

Application of a Single-Analyte Permethrin ELISA for Analysis of Permethrin Content in Permethrin Dust Samples. The permethrin ELISA was also used to determine the content of permethrin in the permethrin dust samples, and the values obtained in the ELISA were compared with those obtained by GC-MS. The data in Table 5 show a good correlation in six out of the 10 samples, revealing that the values obtained in the immunoassay did not differ significantly from those obtained for *trans*-permethrin by the chemical analytical method. Four samples (nos. 1, 3, 7, and 9) yielded significantly lower values in the immunoassay than those obtained in the GC-MS analysis. The ELISA and GC-MS values were compared only for *trans*-permethrin, because the antiserum recognizes *trans*-permethrin and has low CR (13%) toward *cis*-permethrin. It is interesting to note that lack of correlation between the values obtained by the two respective methods was evident mainly for the samples that contained high levels of *cis*-permethrin, which may have resulted from interference of the *cis*-isoform with the accurate determination in the immunochemical assay. Lack of correlation was also noticed in sample no. 1, in which the level of *cis*-permethrin was not that high. It

Table 5. Permethrin Content in Permethrin Dust Samples[a]

	experimental value (ng/mL) GC-MS		experimental value (ng/mL) ELISA
sample	*cis*-permethrin	*trans*-permethrin	*trans*-permethrin
sample 1	21	25	7 ± 2 (n = 6)*
sample 2	26	22	22 ± 3 (n = 9)
sample 3	95	106	33 ± 7 (n = 12)*
sample 4	47	46	24 ± 3 (n = 8)
sample 5	5	6	0 ± 0 (n = 5)
sample 6	27	26	12 ± 2 (n = 5)
sample 7	157	168	30 ± 3 (n = 18)*
sample 8	6	6	5 ± 1 (n = 5)
sample 9	267	243	66 ± 4 (n = 20)*
sample 10	8	7	0 ± 0 (n = 5)

[a]The experimental value represents *trans*-permethrin content in permethrin dust samples as obtained with a single-analyte permethrin ELISA format or GC-MS. The amount of *trans*-permethrin (obtained by ELISA) was calculated by multiplication of the value obtained from the standard curve by 0.58 to correct for the 58% of *trans*-permethrin in the standard (which was composed of 58 and 42% *trans*- and *cis*-permethrin, respectively). Each value obtained by ELISA represents the mean ± SEM (standard error mean) of n measurements (as indicated in the table). Statistical analysis compared the *trans*-permethrin values obtained by GC-MS with those obtained by ELISA. An asterisk indicates a significant difference at $p < 0.05$.

may very well be that this sample as well the other samples in which no correlation was observed contained ingredients that might have interfered with the assay. It is important to indicate that in contrast to the aroclor soil/sediment samples, which contained relatively large amounts of analyte and had to be diluted by at least 150-fold (equivalent to 6.7 mg of sediment/soil) prior to ELISA analysis, the permethrin samples were diluted only 1:5 (equivalent to 100 mg of house dust) and may thus contain interfering components. The difference in dilutions employed in the aroclor- and permethrin-containing samples is mostly due to the difference in sample size, sample matrix, and analyte concentrations. Nonetheless, it seems that in most of the samples, matrix interference did not have a major effect: the curve generated by the different sample dilutions in the ELISA fully paralleled the standard curve, and the data, in most samples, correlated well with the GC-MS analysis. In the past few years, we developed highly efficient sol−gel immunoaffinity purification methods for pyrethoids and PCBs, which eliminate matrix interference.[10,16] Such methods can be used in further studies, in combination with ELISAs, to overcome this problem.

Most studies on the presence of pyrethroids in soil and dust samples were based on chemical instrumental analysis. Only a few immunoassays were employed for analysis of pyrethroids in soil and dust samples. In a study by Nakata,[13] methanol-extracted spiked soil samples were analyzed by means of an immobilized hapten-conjugate competitive ELISA. Although the recoveries obtained were above 95%, the study did not examine the correlation of the immunoassay results with those of GC-MS. In another study, high recoveries (nearly 100%) of type II synthetic pyrethroids and an excellent correlation coefficient (0.99) with an immobilized Ab competitive ELISA were reported,[11] and recently, the permethrin content in soil and dust samples was evaluated with a magnetic particle format immunoassay.[25] As in the present study, the ELISA-derived permethrin concentrations were highly correlated with the GC-

MS-derived sums of *cis*/*trans* permethrin concentrations, with a high correlation coefficient.

In summary, the ELISAs that were developed in the course of the present study can serve as an excellent tool for further studies toward the development of similar ELISA formats with many more analytes and be easily adjusted to a high-throughput automated format, which will allow to widen the application of immunoassays for screening of agricultural, environmental, and medical samples; to reduce costs; to increase precision, through elimination of variations between individual experiments; and to further shorten analysis time and thereby overcome one of the major obstacles in the way of further implementation of such simple, cost-effective, and sensitive assays for analysis of "real world" samples.

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Notes

The authors declare no competing financial interest.

[‖]Retired.

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