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An indirect competitive enzyme-linked immunosorbent assay for the determination of 3, 4-dichlorobiphenyl in sediment using a specific polyclonal antibody

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ABSTRACT: A specific polyclonal antibody targeting non-dioxin-like PCB 3, 4-dichlorobiphenyl (PCB12) was obtained, and a sensitive indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) was developed for the determination of PCB12 in sediment samples. Under optimal conditions, good linearity was achieved within a range of 0.06 to 6 μg L^{-1}. The observed half-maximal inhibition concentration (IC_{50}) was 2.37 μg L^{-1}, and the limit of detection (LOD) was 0.021 μg L^{-1}. This method was used for the detection of PCB12 in the sediment samples collected from the East China Sea adjacent to Shanghai, China. The concentrations of PCB12 in the samples ranged from 0.21 μg kg^{-1} to 8.59 μg kg^{-1}. The recovery was from 81% to105% and the CV values were from 2.8% to 8.4%. The consistency between the results obtained from ic-ELISA and GC-ECD was 98%. It further confirmed the reliability and accuracy of the ic-ELISA for rapid detection of PCB12 in the environment.

Key words: Polychlorinated biphenyls; PCB12; ic-ELISA; Polyclonal antibody; Sediment

1. Introduction

Polychlorinated biphenyls (PCBs) are a class of anthropogenic chlorinated organic compounds comprised of 209 congeners. Because of the PCBs desirable physical and chemical properties such as a low vapour pressure, non-flammability, heat-resistance, dielectric, and good thermal and chemical stability, they were used as the dielectric fluid in capacitors and transformers in the electric power industry[1]. PCBs have also been used in other products, such as microscope immersion oils, carbonless copy paper, inks, cutting oils, adhesives, waxes and as an inert ingredient in pesticides[2, 3]. PCBs were globally produced decades before they were banned, there were approximately 1.3 million tons of PCBs produced during 1929 to 1993[4]. Without exception, approximately 10 thousand tons of PCBs were produced from 1965 to 1974 in China, most of which were used in power capacitors and used as paint additives[5]. Even today, a large proportion of the PCBs are still present in old transformers and power capacitors, which have the potential to be released into the environment. Although the production of PCBs was banned in 1974 in China, they remain ubiquitous in the environment, even in the Tibetan Plateau[6].

PCBs were listed as one of the dozen persistent organic pollutants (POPs) in the Stockholm Convention for their ability to bio-accumulate in food chains, their long term stability, and high toxicity to human beings and the natural environment. PCB exposure routes include the following: inhalation of contaminated air (both outdoor and indoor), dermal contact with contaminated surfaces, and particularly from the ingestion of contaminated food[7]. It has been shown that PCBs can have hazardous

effects on human beings, including hepatotoxicity, developmental neurotoxicity[8], endocrine system disruption, and carcinogenicity. Four non-ortho and eight mono-ortho PCB congeners (CB-81, 77, 126, 169, 105, 114, 118, 123, 156, 157, 167, and 189) are recognised by the World Health Organization (WHO) as "dioxin-like" in reference to their toxic effects similar to dioxins[1].

The determination of PCBs in various environmental matrixes including sedimen ts has been based mostly on gas chromatographic methods, which were coupled with different detector types such as an electron capture detector (ECD)[9, 10], a low resolution mass spectrometer (LRMS)[11, 12], or a high resolution mass spectrometer (HRMS)[13, 14]. Although these techniques are certainly suitable for PCB analysis for various samples as proven by their widespread use in the last dec ades, they have two main drawbacks, time-consuming and expensive. Time-consuming is from the sample processing protocols and the high cost is mainly from the detection system (instrumental analysis itself). Various sampling and processing techniques are well developed for PCB determinations to shorten the total analysis time, but it's hard to reduce the high cost of the traditional analysis methods. Therefore, a fast, cost-effective and reliable screening tool is needed for determination of the PCBs in environmental samples.

Recently, there has been an increasing use of immunoassays for the detection of environmental contaminants because of their reliability, rapid detection, ease-of-operation, and relatively low cost[15]. During the past two decades, several immunoassays, including the radioimmunoassay[16, 17], ELISA[18-20], the

fluoroimmunoassay[21], immunosensor assay[22-24], bioelectrochemical immunoassay[25, 26], real-time quantitative fluorescence immuno PCR[27-29], and commercial PCB test kits, have been developed for PCB detection in the environment. Immunoassays are also capable of detecting a wide variety of PCB congeners at sub-microgram levels.

Indeed, a large number of studies using immunoassays for the determination of PCB concentrations (individual congeners or sums of various congeners) have been performed in the past few decades; however, most of the studies were focused on the "dioxin-like" PCBs[21, 28-32], the indicator PCBs (PCB-28, 52, 101, 138, 153, 180 and occasionally PCB-118)[23], and the mixture PCBs such as Aroclor[19, 25]. Only a few studies focused on the detection of other single PCB congeners. Although these single PCB congeners may not be as dioxin-like as the other PCBs, they are nonetheless persistent organic pollutants that are potentially hazardous to humans and ecosystems.

Although PCB12 is not one of the dioxin-like or indicator PCBs, its developmental toxicity has the potential to adversely affect a developing baby: adverse health effects include low birth weight, birth defects, behavioural and psychological problems, and foetal death[33]. Meanwhile, di-PCB is the major PCB homologue group in Chinese background and rural soil[34]; therefore, we developed a rapid, reliable, and sensitive method for the detection of 3, 4-dichlorobiphenyl in sediment. A specific polyclonal antibody targeting 3, 4-dichlorobiphenyl was obtained and a sensitive indirect competitive ELISA (ic-ELISA) method was subsequently developed. The experimental conditions of the ic-ELISA method were optimised including the concentration of the coating antigen, the dilution factor for

the antibody, the incubation time, and the blocking buffer, the solvent, the pH of the assay buffer and the ionic strength. This optimised method was implemented to determine PCB12 in sediment sampled from the East China Sea adjacent to Shanghai, China. The ic-ELISA results were further compared with those by GC-ECD analysis.

2. Materials and methods

2.1. Chemicals and solutions

The standards for PCB12, 37, and 77, and Aroclor 1242, 1248, 1254, 1260 were purchased from Accustandard, Inc (New Haven, CT, USA). Dimethylsulfoxide (DMSO), ethanol and dimethylformamide (DMF) were purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd (Shanghai, China). $NaHCO_3$, Na_2CO_3, KCl, NaCl, Na_2HPO_4, KH_2PO_4 ·$12H_2O$, gelatin, pesticide-grade hexane, *N*-hydroxysuccinimide (NHS), n-butylamine, isobutyl chloroformate, dicyclohexylcarbodiimide (DCC), 3, 3', 5, 5'-tetramethylbenzidine (TMB), Bovine Serum Albumin (BSA) and hydrogen peroxide (H_2O_2) were all purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Ovalbumin (OVA) was purchased from Sango Biotech Co., Ltd (Shanghi, China). Horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG was purchased from Solarbio (Shanghai, China). Freund's complete adjuvant (cFA) and incomplete adjuvant (iFA) were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Hapten PCB12 was directly from our lab. The chemical structure of the hapten PCB12 is shown in Fig. 1. The details of the buffers and solutions were described in the electronic supplementary information (ESI). All animal studies performed complied with the

institutional guidelines.

Fig. 1

2.2. Materials and Instruments

Microtitre plates were purchased from Sango Biotech Co., Ltd (Shanghai, China). Immunoassay absorbance was measured with a Multiskan photometer in dual wavelength mode (450-630 nm) purchased from Thermo Labsystems (Vantaa, Finland). Ultraviolet-visible (UV-VIS) spectra were obtained on a DU-800 spectrophotometer (Beckman Coulter, Inc., Brea, CA).

2.3. Preparation of protein-hapten conjugates

As a small molecule, PCB12 is not capable of initiating an immune response unless conjugated with a protein to form a complete antigen; therefore, the hapten was used for the preparation of the immunogen and the coating antigen conjugates with BSA and OVA, respectively (see ESI). The UV spectra showed qualitative differences between carrier proteins and conjugates in the region of maximum absorbance of hapten (see ESI, Fig. S1 and Fig. S2).

2.4. Immunisation and antibody production

Two female New Zealand white rabbits were immunised by subcutaneous and intramuscular injections with the immunogen. The initial immunisation was performed by injecting 1 mg of hapten-BSA dissolved in 0.5 mL normal saline and emulsified with 0.5 mL of CFA. Twenty days after the injections, the rabbits were

boosted five times at two week intervals by injecting a solution of 1 mg of the immunogen dissolved in 0.5 mL normal saline and emulsified with 0.5 mL of IFA. The last booster (1 mg hapten-BSA dissolved in 1 mL normal saline) was performed ten days later. From the third booster onward, each rabbit was bled from the ear vein seven days after each immunisation. Serum titres were determined by ELISA to monitor the quality of the antisera from the immunised rabbits. Seven days after the last booster, the blood was collected from the jugular vein of each rabbit and the serum was separated by the caprylic acid/ammonium sulfate precipitation method[35]. The obtained antiserum was freeze-dried and stored at -20 ℃. And the titre of the final purified antibody was 1:204800.

2.5. Indirect competitive ELISA

Indirect competitive ELISA, based on the immobilisation of coating antigens, was performed as follows: the microwell plates were coated with the coating antigen (4.58 μg mL^{-1}) in 100 μL of coating buffer (pH 9.6) overnight at 4 ℃. The plates were then washed three times with PBST and blocked with 1% gelatin (200 μL/well) for 1h at 37 ℃. After three times wash, 50 μL of the PCB12 standard solution or the sample solutions (diluted in PBS with 5% DMSO), combined with 50 μL of the diluted antibody (1:6000) solution, were added to the allocated wells. A total of 100 μL of PBS was added to the blank wells. The plates were then incubated at 37 ℃ for 1 h. After another wash, 100μL of HRP-conjugated goat anti-rabbit IgG was added to the plates and incubated for 45 min. After an additional five times wash, 100 μL of the TMB substrate solution was added. The reaction was stopped by adding 50 μL of

2 mol L^{-1} sulphuric acid after 15 min. The absorbance was immediately recorded by the microplate reader in dualwavelength mode (450 nm as test and 630 nm as reference).

The results were represented as inhibition (%) = $(1- B/B_0) \times 100$, where B is the absorbance of the well with the competitor and B_0 was the absorbance of the well without the competitor. The competitive inhibitory curves were plotted as inhibition versus Log C (concentrations of PCB12).

2.6. Cross-reactivity

The assay specificity was evaluated by testing the cross-reactivity (CR) of the antibody with other analogues and stereoisomers. The CR values were calculated according to the following formula: cross-reactivity (%) = (IC_{50} of PCB12) / (IC_{50} of other compounds) $\times 100$.

2.7. Sample preparation

Eight sediment samples collected from the East China Sea, adjacent to Shanghai, were dried in the shade, filtered through a 60 mesh sieve, and stored at 4 ℃. Aliquots of the samples for the recovery test were spiked with known amounts of the PCB12 standard solution within the quantitative working range. An ultrasonic extraction method (see ESI) was used to extract PCB12 from the un-spiked and spiked samples. The treated sample was divided into two fractions: one for the ELISA detection and the other for GC- ECD analysis (see ESI).

2.8. Recovery tests

Recovery tests were performed by spiking sediment samples with a series of

known PCB12 concentrations to determine the efficiency of the ic-ELISA assay. The spiked samples were prepared by the aforementioned sample preparation procedure. Recoveries were calculated using the following formula: Recovery (%) = 100 × (Css–Cus) /Cs. Where Css and Cus are concentrations measured in the spiked and unspiked samples, respectively, and Cs is the spiked concentration.

3. Results and discussion

3.1. Optimisation of ELISA

To develop a sensitive method for the detection of PCB12, several parameters such as the concentration of the coating antigen, the dilution of the antibody, the blocking buffer, the incubation time, the solvent, the pH of the assay buffer and the ionic strength were optimised. The IC_{50} and the maximum absorbance (A_{max}) were used to assess the optimum conditions for the assays[36].

Accordingly, the concentrations of the immobilised antigen and the dilution factor of the antibody were optimised using a checkerboard procedure. The concentration of the coating antigen ranged from 9.17 μg mL^{-1} to 1.14 μg mL^{-1} and the dilution of the antibody ranged from 1:1000 to 1:8000. An optimal combination for the reagents was 4.58 μg mL^{-1} of the coating antigen combined with dilutions of 1:6000 for the antibody, producing an absorbance around 1 in the absence of analytes (data not shown).

The blocking buffer was used to prevent non-specific binding in the ELISA analysis. Without the blocking buffer, unoccupied sites in the plates may absorb components such as antibody or HRP-conjugated goat anti-rabbit IgG during the

incubation steps, which may cause high background signals. Three blocking buffers (OVA, gelatin, and skim milk powder) prepared with PBS at a concentration of 1% were tested for their blocking capacity. The gelatin showed a better result because of the lower background value (0.05) than that of 1% OVA (0.13) or 1% skim milk powder (0.09), thus, it was chosen as the blocking buffer in this study.

The optimal incubation periods for the coating antigen (first incubation period) and the immunoreactions (second incubation period) were evaluated according to the A_{max} and IC_{50} values. The first incubation periods were overnight at 4 ℃, for 60, 90, and 120 min at 37 ℃, when the coating antigen was coated overnight at 4 ℃, the A_{max} was the highest and the IC_{50} was the lowest (see ESI, Table S1). So the plates were coated overnight at 4 ℃. The second incubation periods were 30, 60, 90, and 120 min at 37 ℃, when the time was increased, the A_{max} was increased. However, the IC_{50} was lowest when the the incubation time was 60 min (see ESI, Table S2). So the immunoreactions were incubated for 60 min at 37 ℃. Because of the lipophilic character of PCB12, a water-miscible organic cosolvent is needed to ensure solubility. DMSO is a common solvent used in immunoassays and has proven to be an effective solubiliser for hydrophobic PCBs[37]; therefore, we used DMSO as the water-miscible organic cosolvent and investigated the effects of various concentrations (5%, 10%, 15%, 20%) of these solvents on the assay. The maximum absorbance decreased with increasing concentrations of DMSO, and the IC_{50} values calculated from the standard curves increased slightly (see ESI, Table S3). So, the PBS solution containing 5% DMSO (v/v) was used to improve the analyte solubility in this study.

The effects of pH values were evaluated using different PBS solutions ranging from pH 5.5 to 9.0. It was found that the pH had an insignificant effect on the sensitivity of the assay (see ESI, Table S4). When the pH was increased from 7.4 to 9.0, the IC_{50} value was slightly increased from 2.51 to 3.01 μg L^{-1}, and pH 7.4 was selected with a lowest IC_{50} value of 2.51 μg L^{-1}. PBS buffers with different ionic strength (from 0.1 mol L^{-1} to 0.4 mol L^{-1}) were tested to determine the effects of ionic strength. When the ionic strength was increased, the IC_{50} was increased and the A_{max} was decreased (see ESI, Table S5). So the salt concentration of 0.14 mol L^{-1} was selected because of the lowest IC_{50}.

3.2. Sensitivity and stability of ic-ELISA

Under optimum conditions, series of diluted concentrations of PCB12 standard sample (0.01 μg L^{-1}, 0.06 μg L^{-1}, 0.1 μg L^{-1}, 0.2 μg L^{-1}, 0.6 μg L^{-1}, 1 μg L^{-1}, 2 μg L^{-1}, 6 μg L^{-1}, 10 μg L^{-1}, 60 μg L^{-1}, 100 μg L^{-1}) were reacted in the method to construct standard curves. Sixteen independent assays were performed over a forty five days period, each concentration had six reactions in an independent run, and the mean values of the sixteen assays were used to plot the standard curves (Fig 2). The linear range was from 0.06 to 6 μg L^{-1} and the linear equation was $y=0.16x+0.44$ ($r^2=0.99$, Fig 2). The $IC_{50,}$ which is a key criterion for evaluating the sensitivity of ELISA, was 2.37 μg L^{-1}, the IC_{15}, which was calculated as the concentration that gave 15% inhibition of the maximal signal[38, 39], was 0.015 μg L^{-1}, suggesting that the established ic-ELISA was highly sensitive. The determination of limit of quantitation (LOQ) was base on 20 blank samples accepting no false positive rates, and the result, which was

obtained by adding 10 times the standard deviation of the 20 blank samples to the mean blank value[40], was 0.065 μg L^{-1}. The limit of detection, which was calculated by adding 3 times the standard deviation of the 20 blank samples to the mean blank value[40], was 0.021 μg L^{-1}.

The stability of the method was tested by running the ic-ELISA procedures for eight individual times with five concentrations of PCB77 (0.06 μg L^{-1}, 0.1 μg L^{-1}, 0.6 μg L^{-1}, 1 μg L^{-1}, 6 μg L^{-1}). Each concentration had six reactions in an independent run. Two results were obtained from the six reactions for each concentration in an independent run. So, sixteen results for each concentration were obtained from the eight independent runs. The relative standard deviation (RSD) of the sixteen results at each standard concentration was from 2% to 6.3%, indicating the good stability and reproducibility of the method.

Fig. 2

3.3. Specificity of ic-ELISA

The cross-reactivity of the ic-ELISA was evaluated using benzene, chlorobenzene, dichlorobenzene, six PCB congeners (PCB8, PCB15, PCB28, PCB29, PCB37, PCB77) and four mixture PCBs (Aroclors 1242, 1248, 1254, 1260). Cross-reactivity values and general structures of the compounds are presented in Table 3. In all cases, there was a low cross-reaction between PCB12 and other structurally similar compounds, whereas PCB8, PCB15, Aroclors 1242 and 1248 showed slightly higher cross-reaction values, which were 8.96%, 8.27%, 9.55% and 9.1%, respectively. Benzene, chlorobenzene, dichlorobenzene can be used as the

materials to synthesise PCB congeners, even more, chlorobenzene and dichlorobenzene have chloride substituent on the benzene ring, but their structures only have one benzene ring, which were different with PCB12. This may explain their low cross-reactivity (<0.3). The cross-reactions of the four PCB congeners were a little higher because their structures are much similar with PCB12, and the same structure is that all of them have a biphenyl ring. The poor affinity (Table 1) of the produced anti-PCB12 antibody toward PCB28, PCB29 and PCB77 seems reasonable due to the different quantity and substituent positions of the chloride substituent on the biphenyl ring of these compounds from those of PCB12. It is hard to explain the low recognition of PCB37 (4.34%) because PCB37 is quite structurally similar to PCB12, the only difference between the molecular structures of PCB37 and PCB12 is the substituent at position 4' of the biphenyl ring, which is a chloride substituent for PCB37 but no substituent for PCB12. Aroclors 1242 and 1248 are PCB mixtures mainly composed of low chloro-substituted biphenyls, containing little PCB12; therefore, Aroclors 1242 and 1248 showed slightly higher cross-reaction values. Cross-reactions of Aroclors1254 and 1260 had the lowest values, as they were mainly composed of highly chlorinated biphenyls, which may be indicative of the relatively large structural differences between the Aroclors and PCB12. The low cross-reaction between PCB12 and other structurally related compounds suggests that the antibody is very specific for the PCB12.

Table 1

3.4. Analysis of sediment samples

The ic-ELISA method has been used to detect the presence of PCB12 in eight sediment samples collected from the East China Sea. PCB12 was found in all the samples, and the concentrations ranged from 0.21 ± 0.02 μg kg^{-1} to 8.59 ± 0.22 μg kg^{-1} (table 2). The concentrations of PCB12 in sample 2 and sample 3 were much higher than other samples. This is because sample 2 and sample 3 were collected close to petrochemical industrial parks where may be polluted by PCBs, and PCB12 may be the intermediate of dechlorination process of some trichlorodiphenyls in the environment[41]. As the sampling site of sample 1 was far from the land, it had the lowest concentration of PCB12. The classical GC-ECD method was used to confirm the accuracy of ic-ELISA: consistency ($y=0.91x-0.14$, $R^2=0.98$, Fig. 3) was observed between the two methods. This indicated that the ic-ELISA could offer a practical approach for screening of PCB12 in real samples. The p-value from the paired sample t-test for the comparisons of the two methods was 0.034. That is mean, at the 0.05 level, the difference was statistically significant. In a general, the ic-ELISA results were higher than the GC-ECD results across all the samples. This difference may be caused by the non-specific absorbance of reagents used in the method, including polyclonal antibody and HRP-conjugated goat anti-rabbit IgG. In addition, polyclonal antibody had cross-reactivity for other PCBs present in the samples, which were not measured by the GC-ECD method and contribute to the ic-ELISA-derived concentrations.

Table 2

Fig. 3

310 *3.5. Recovery*

311 The recovery of the spiked samples and the CV were calculated to evaluate the
312 accuracy and precision of the ELISA. Four samples (samples 1, 2, 4, and 7) were
313 spiked with PCB12 standard concentrations ranging from 0.05-20 μg kg^{-1}. Table 3
314 shows that the recoveries of the PCB12 from the spiked samples were ranged from 81%
315 to 105%, the CV were below 9%.

316 Table 3

317 **4. Conclusions**

318 A sensitive ic-ELISA assay for the determination of non-dioxin-like PCB12 in
319 sediment samples has been developed on the basis of specific polyclonal antibodies.
320 Under optimised conditions, the IC_{50} value and the LOD of the assay were 2.37 μg L^{-1}
321 and 0.021 μg L^{-1}, respectively. The ic-ELISA was used to detect the presence of
322 PCB12 in samples obtained from the environment, and satisfactory recoveries were
323 achieved for PCB12 from the spiked samples. Consistent results were observed from
324 ic-ELISA and GC-ECD. The results showed that this method would be a useful option
325 for screening PCB12 in real environmental samples. Furthermore, the microplate used
326 for this method contained 96 wells that allow for a higher throughput analysis (HTA),
327 thus the method will be useful for the preliminary screening of large numbers of real
328 samples before GC-ECD analysis.

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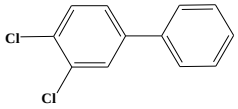
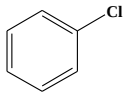
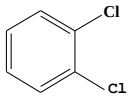
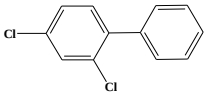
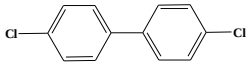
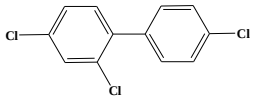
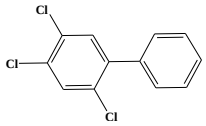
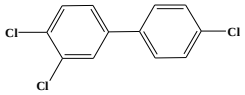
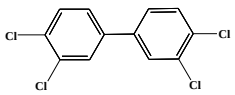
397 Table 1. Cross-reactivity of the antibody against PCB12 and other PCB compounds.

398 Table 2. Concentration of PCB12 in sediment samples determined by ic-ELISA and GC-ECD.

399 Table 3. Recovery of PCB12 detected by ic-ELISA in spiked sediment sample.

400

401 Table 1. Cross-reactivity of the antibody against PCB12 and other PCB compounds.

Compound	Structure	IC_{50} ($\mu g\ L^{-1}$)	Cross reaction (%)
PCB12		2.39	100
benzene		>1000	<0.3
chlorobenzene		>1000	<0.3
dichlorobenzene		>1000	<0.3
PCB8		21.42	8.96
PCB15		28.89	8.27
PCB28		68.43	3.49
PCB29		57.39	4.16
PCB37		54.98	4.34
PCB77		69.55	3.43
Aroclors 1242	Mainly include trichlorinated biphenyls	25.02	9.55
Aroclors 1248	Mainly include tetrachlorinated biphenyls	26.25	9.10
Aroclors 1254	Mainly include pentachlorinated biphenyls	>1000	<0.3
Aroclors 1260	Mainly include hexachlorinated biphenyls	>1000	<0.3

402

403

404 Table 2. Concentration of PCB12 in sediment samples determined by ic-ELISA and GC-ECD

405 (n=6) (μg Kg^{-1}, mean ±SD).

Sediment samples	Concentration (μg Kg^{-1})	
	ic-ELISA (n=6)	GC-ECD (n=6)
Sample 1	0.21 ±0.02	0.12 ±0.031
Sample 2	8.59 ±0.22	8.14 ±0.12
Sample 3	6.56 ±0.16	5.33 ±0.24
Sample4	3.31 ±0.12	3.21 ±0.066
Sample5	2.10 ±0.048	1.48 ±0.031
Sample6	0.80 ±0.09	0.52 ±0.035
Sample7	0.79 ±0.086	0.64 ±0.041
Sample8	0.74 ±0.027	0.69 ±0.039

406

407 Table 3. Recovery of PCB12 detected by ic-ELISA in spiked sediment sample.

Sediment samples	spiked concentration ($\mu g\ Kg^{-1}$)	recovery (%)	CV (CV%, n=6)
Sample1	0.05	81	4.9%
	0.1	88	5.7%
	0.5	86	4.6%
Sample2	5	105	2.8%
	10	98	7.1%
	20	92	6.5%
Sample4	1	87	3.4%
	5	92	6.5%
	10	95	8.4%
Sample7	0.1	86	3.5%
	0.5	102	6.9%
	1	97	6.2%

408

409

410 **Figure Captions**

411

412 Fig. 1.The structure of hapten PCB12.

413 Fig. 2. Standard curve for PCB12 analyzed by ic-ELISA. The concentrations of PCB12 were

414 0.01μg L^{-1}, 0.06 μg L^{-1}, 0.1μg L^{-1}, 0.2μg L^{-1}, 0.6 μg L^{-1}, 1μg L^{-1}, 2μg L^{-1}, 6 μg L^{-1}, 10 μg L^{-1}, 60

415 μg L^{-1}, 100 μg L^{-1}. The linear range was from 0.06 μg L^{-1} to 6 μg L^{-1}. The linear equation was

416 $y=0.16x+0.44$ ($r^2=0.99$, n=16).

417 Fig. 3. Comparison of data from the ic-ELISA and GC-ECD analysis in the sediment samples. The

418 regression equations was $y=0.91x-0.14$ ($R^2=0.98$).

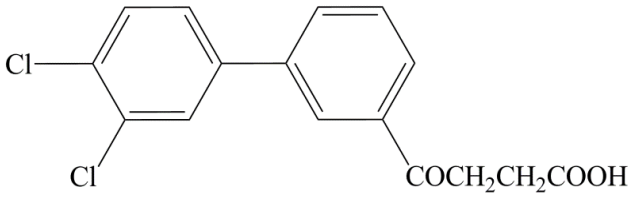


Fig. 1.The structure of hapten PCB12.

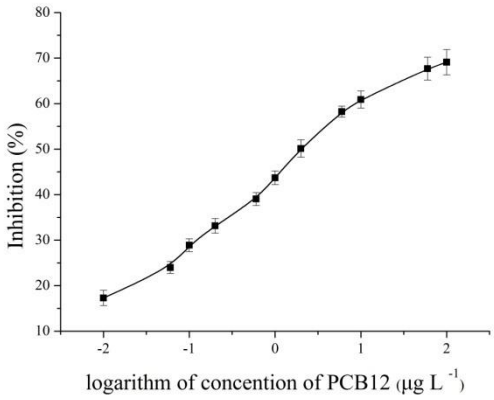


Fig. 2. Standard curve for PCB12 analyzed by ic-ELISA. The concentrations of PCB12 were 0.01μg L^{-1}, 0.06 μg L^{-1}, 0.1μg L^{-1}, 0.2 μg L^{-1}, 0.6 μg L^{-1}, 1μg L^{-1}, 2 μg L^{-1}, 6 μg L^{-1}, 10 μg L^{-1}, 60 μg L^{-1}, 100 μg L^{-1}. The linear range was from 0.06 μg L^{-1} to 6 μg L^{-1} with a linear equation $y=0.16x+0.44$ ($r^2=0.99$, $n=16$).

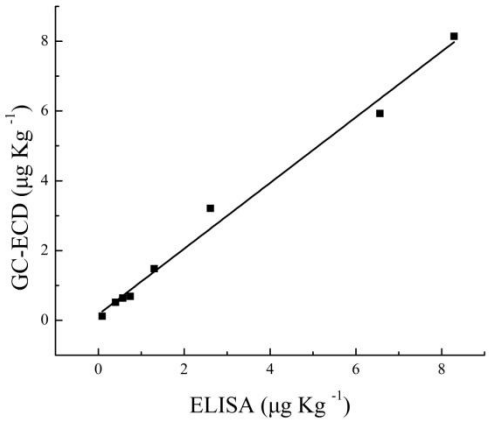


Fig. 3. Comparison of data from the ic-ELISA and GC-ECD analysis in the sediment samples. The regression equations was y=0.91x-0.14 (R^2=0.98).