Enzyme-linked immunosorbent assay for triclocarban in aquatic environments

Kun Zeng, Yanmin Zou, Jianxia Liu, Wei Wei, Meng Zhang, Jun Zhou, Zhen Zhang and Zikuan Gai

ABSTRACT

A sensitive, competitive indirect enzyme-linked immunosorbent assay (ELISA) was developed for the detection of triclocarban (TCC) in waters and sediments. Haptens were synthesized by derivatizing the paraposition of a phenyl moiety of TCC. The synthesized hapten was then coupled to bovine thyroglobulin to be used as an immunogen, based on which, a high affinity monoclonal antibody 4D5 was produced with the hybridoma technique. Under the optimized conditions, using the monoclonal antibody, excellent performances of the assay were obtained: satisfactory sensitivity (IC_{50} (50% inhibition concentration) value, 0.43 ng/mL; limit of detection, 0.05 ng/mL); good linear range (0.05–10 ng/mL); and satisfactory accuracy (recoveries 70.7–107% in waters; 74.8–98.3% in sediments). Furthermore, TCC was found with the concentration ranging from not detected to 422.12 ng/L in waters and from 6.68 ng/g to 78.67 ng/g in sediments in Yunliang River, Ancient Canal and Hongqiao Port in Zhenjiang City. In conclusion ELISA could be applied for monitoring TCC in aquatic environments.

Key words | ELISA, environmental samples, triclocarban

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INTRODUCTION

As a chlorinated diphenylurea antimicrobial agent, triclocarban (3,4,4′-trichlorocarbanilide, TCC) has been widely used in personal care products, including soaps, shampoos, toothpastes, mouthwashes, deodorants and detergents, and plastic kitchenware (Jones *et al.* 2000; Ahn *et al.* 2008). In the United States, TCC is predominantly used as an antibacterial active ingredient in bar soaps at a typical concentration of 0.6% (LANXESS Corporation & Henkel Consumer Goods, Inc. 2010), and the combined annual consumption of TCC usage was estimated to be 227–454 tons every year (Halden & Paull 2005). In China, the estimated quantity of antimicrobial agents was around 1,220 tons/yr based on the weight content of 0.1% and the collected statistical data of personal care products consumed (Zhao *et al.* 2013). Because of extensive usage of this compound, it has been found in many Chinese rivers: surface water and sediments from Liao River, Hai River, Yellow River, Zhujiang River and Dongjiang River. The concentrations of TCC ranged up to 338 ng/L in surface water and 2,723 ng/g in sediments (Zhao *et al.* 2010, 2013).

Recent reports indicated that TCC could act as an endocrine disruptor (Ahn *et al.* 2008; Chalew & Halden 2009; Christen *et al.* 2010). This organic pollutant could synergistically enhance hormone-dependent induction of estrogen- and androgen-dependent gene expression in nuclear-receptor-responsive bioassays at 1–10 μM (Ahn *et al.* 2008). The same action was observed whereby TCC could amplify the dihydrotestosterone response up to 130% at 0.01–5 μM (Christen *et al.* 2010). Acute and chronic toxicities of TCC also were observed in fish, noting that the chronic effect threshold was within the environmental concentrations in surface waters (Chalew & Halden 2009). Therefore, it is of great importance to monitor TCC in environmental waters.

Currently available methods for determination of TCC in environmental samples are mainly based on instrumental analytical methods, involving high-performance liquid chromatography–tandem mass spectrometry (LC-MS/MS) (Sapkota *et al.* 2007; Gonzalez-Marino *et al.* 2009; Zhou *et al.* 2012; Shi *et al.* 2013). Although these assays are sensitive and reliable, they require high-cost instruments and skilled analysts and involve time-consuming sample preparation steps.

As an alternative, the enzyme-linked immunosorbent assay (ELISA) was considered as a good choice depending on its significant properties of high specificity, sensitivity, simplicity and suitability for the high-throughput analysis in a

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short period, and it has been widely used in clinical diagnostics (Ishikawa 1987), food (Samarajeewa *et al.* 1991), agriculture (Kaufman & Clower 1995), and environmental analysis (Knopp 2006). However, so far, no immunochemical assay has been applied to detect TCC in aquatic systems; only Ahn *et al.* (2012) reported a competitive indirect ELISA for the detection of TCC in serum and urine.

The purpose of this study was to develop an indirect competitive immunoassay based on a monoclonal antibody to quantitatively detect TCC in aquatic environments. Parameters that could influence the performance of ELISA were optimized, the precision and accuracy was evaluated by spiked recoveries, and the established method was further applied for screening TCC at a river region located at Zhenjiang City, Jiangsu Province.

METHODS

Materials and reagents

3,4-Dichlorophenyl isocyanate, ethyl 4-aminophenylacetate, 4-chlorophenyl isocyanate, methyl 4-amino-2-chlorobenzoate, TCC and its analogs were purchased from J&K Scientific Ltd (Beijing, China). Bovine thyroglobulin (BTG), human serum albumin (HSA), 3,3′,5,5′-tetramethylbenzidine (TMB), hypoxanthine–aminopterin–thymidine, hypoxanthine–thymidine, Freund's complete adjuvant and Freund's incomplete adjuvant were obtained from Sigma Chemical Co. (St Louis, MO, USA). Fetal calf serum (HyClone) and peroxidase-conjugated goat anti-mouse IgG (IgG-HRP) (Jackson, West Grove, PA, USA) were used. All other reagents used were of analytical grade or better and obtained from standard sources. ELISA plates (96 wells) were obtained from Costar (Cambridge, MA, USA). Absorbance values were read with a microplate reader (Bio-Tek Instruments, Inc., USA).

Hapten synthesis

4-[3-(3,4-Dichlorophenyl)ureido]phenylacetic acid and 4-[3-(4-chlorophenyl)ureido]benzoic acid were synthesized according to Ahn *et al.* (2012) with minor modification.

4-[3-(3,4-Dichlorophenyl)ureido]phenylacetic acid was synthesized as shown in Figure 1(a). A mixture of 3,4-dichlorophenyl isocyanate (188 mg, 1 mmol) and ethyl 4-aminophenylacetate (179 mg, 1 mmol) in 40 mL of anhydrous tetrahydrofuran was stirred at room temperature for 4 h under an atmosphere of nitrogen. After evaporation, the crude product was washed with hexane–ethyl acetate (1:1) and purified by silica gel column chromatography eluting with hexane–ethyl acetate (1:1) to give 4,ethyl 4-[3-(3,4-dichlorophenyl)ureido]phenylacetate. The product (106 mg) was mixed with methanol (12 mL) and 1 N NaOH (12 mL) at 60 °C overnight. After being acidified to pH 4 with 6 N HCl, the mixture was extracted with hexane–ethyl acetate (1:1). 4-[3-(3,4-Dichlorophenyl)ureido]phenylacetic acid was obtained through recrystallization from the extraction mixture.

4-[3-(4-Chlorophenyl)ureido]benzoic acid was obtained as shown in Figure 1(b). 4,Methyl 4-[3-(4-chlorophenyl) ureido]benzoate was obtained through condensation of 4-chlorophenyl isocyanate and methyl 4-amino-2-chlorobenzoate as described above. After alkaline hydrolysis, 4-[3-(4-chlorophenyl)ureido]benzoic acid was developed.

Preparation of immunogen and coating antigens

Immunogens

4-[3-(3,4-Dichlorophenyl)ureido]phenylacetic acid (20 mg) dissolved in 0.5 mL dimethylformamide (DMF) was added into a mixture of 25 mg n-hydroxysuccinimide (NHS) and 30 mg 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) in 5 mL Tris-HCl (0.05 M, pH 7.8) and then stirred for 2 h at 4 °C. Twenty milligrams of BTG in 1.5 mL Tris-HCl (0.05 M, pH 7.8) was dropped into the compound above and the whole reaction solution was stirred at 4 °C overnight. The reaction solution was dialyzed against Tris-HCl (0.05 M, pH 7.8) for 48 h and stored at −20 °C until used.

Coating Antigens

4-[3-(4-chlorophenyl)ureido]benzoic acid (20 mg) in 0.5 mL DMF, 15 mg NHS and 15 mg EDC were mixed in 4 mL Tris-HCl (0.05 M, pH 7.8) and stirred for 2 h at 4 °C. And then 15 mg HSA in 1 mL Tris-HCl (0.05 M, pH 7.8) was added and stirred for 24 h at 4 °C. The dialysis and storage were detailed as above.

Monoclonal antibody production

BALB/c female mice (18–22 g) were immunized subcutaneously with artificial immunogen on days 1, 29, 43, 57 and 71. After the fifth boost, monoclonal antibody (MAb) was developed using the hybridoma technique as detailed in previously reports (Zeng *et al.* 2007). MAbs from ascetic fluid were purified by ammonium sulfate precipitation.

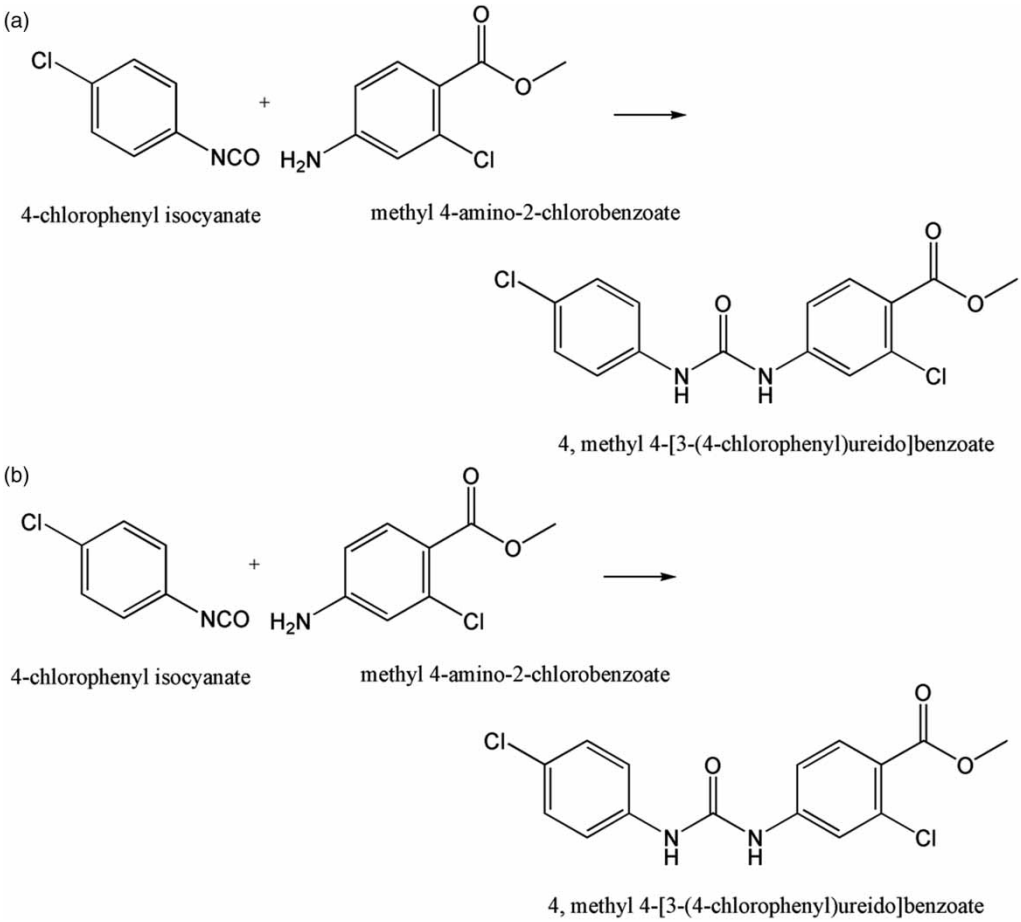


Figure 1 | The synthesis of 4-[3-(3,4-dichlorophenyl)ureido]phenylacetic acid for immunogen and 4-[3-(4-chlorophenyl)ureido]benzoic acid for coating antigen.

Competitive immunoassay to detect TCC

An indirect competitive ELISA format was utilized to measure TCC. The stock solution of TCC was developed in methyl alcohol at 1 mg/mL and stored at −20 °C. The working solution was prepared fresh before the experiment. Plates were incubated with TCC-HSA at 4 °C overnight. After washing with phosphate buffer solution (0.01 mol/L, pH 7.4) with 0.05% Tween-20 (PBST), the plate was blocked with 1% gelatin buffer at 37 °C for 1 h. An aliquot of 50 μL/well of TCC standard or related compounds and 50 μL/well of MAb solution were added into wells successively. After incubation for 30 min at 37 °C, the plate was washed as before. Afterwards, IgG-HRP in PBST with 0.1% gelatin was dropped with a volume of 100 μL/well and reacted for 30 min at 37 °C. After washing with PBST, the retained peroxidase activity was determined using TMB substrate buffer. The reaction was stopped with 50 μL/well H_2SO_4 (2 mol/L) and absorbance values were detected in a microplate reader at 450 nm. Fifty percent inhibition concentration (IC50) is defined as the concentration of inhibitor required to inhibit color development by 50% compared to control wells without competitor. The limit of detection (LOD) is the detectable concentration equivalent to 15% decline of zero binding. The linear range is the extent to which absorbance is proportional to the concentration of analyte.

Optimization of organic solution

Due to being hydrophobic and lipophilic in nature, an organic solution would be used in sample extraction for TCC. The influence of several organic solutions was evaluated, including methyl alcohol, acetonitrile, acetone and dimethyl sulfoxide (DMSO). Standard curves of TCC were prepared in dilution buffer containing different amounts of organic solutions (0%, 10%, 20%, 30% and 50% in PBST). Every standard curve was developed three times and standard errors were calculated. IC_{50} and OD_{max} (maximum absorbance) of competitive curves were evaluated under different conditions on three different days.

Analysis of environmental samples

Sediment and water samples were collected from Yunliang River, Ancient Canal and Hongqiao Port in Zhenjiang City (Jiangsu Province), at 15 sites shown in Figure 2. Samples were collected quarterly, on February 2 and November 2 in the dry season and on May 2 and August 2 in the wet season respectively. Before collecting the samples, samplers and sample bottles were rinsed three times with acetone and once with sterile deionized water, and dried. At each site, three water samples from 0.5 to 1 m below the surface were collected at 3 h intervals and each 250 mL sample was combined to form one composite sample with the total volume of 750 mL, which was then stored in 500 mL amber polypropylene bottles. Concurrent with the collection of water samples, three sediment samples near one site were collected from the 0–10 cm depth of river bottom using a grab sampler and each 500 g sample was pooled to form one composite sample, which was stored in sterilized amber plastic bags. All samples were immediately stored in a cooler box until they were returned to the laboratory for immediate processing (<12 h) or storing at −20 °C.

Sediment

The samples were dried at 60 °C for 24 h and pulverized with a mortar and pestle. The dried samples (2 g) were shaken with 2 mL organic solutions for 1 h at room temperature and then centrifuged for 10 min at 4,000 rpm to remove the solids. The supernatants were diluted four fold with PBS buffer and used in microwell plate analysis. For some samples with high concentration of TCC, more dilution was needed to ensure the data were in the linear range of ELISA. One millilitre of the organic layer was dried under high purity nitrogen at 50 °C and the dry residue was dissolved in 200 μL of methanol–water (80:20, v/v) for LC-MS/MS analyses. Each sample was processed in triplicate.

River water

The water samples were used in microwell plate analysis without preparation. For LC-MS/MS analyses, 2 mL of dichloromethane was added to 2 mL of water samples and shaken for 2 h at room temperature. The organic phase solutions were collected, dried and resuspended as described above. Each sample was processed in triplicate.

Here the organic solution above was used on the basis of 'optimization of organic solution'.

LC-MS/MS analyses

The LC-MS/MS system used was a modification of the method by Shi *et al.* (2013). LC separation was performed with a Waters Acquity UPLC™ system (Waters Corp., Milford, MA, USA) equipped with a Waters Acquity UPLC™ HSS T3 column (2.1 × 100 mm, 1.8 μm). The gradient elution was set as follows: methanol–water (75:25, v/v) for 3–4 min, held at 100% methanol for 2 min, equilibrated at methanol–water (75:25, v/v) for 4 min. The flow rate of the mobile phase was kept at 0.2 mL/min and the injection volume was 15 μL.

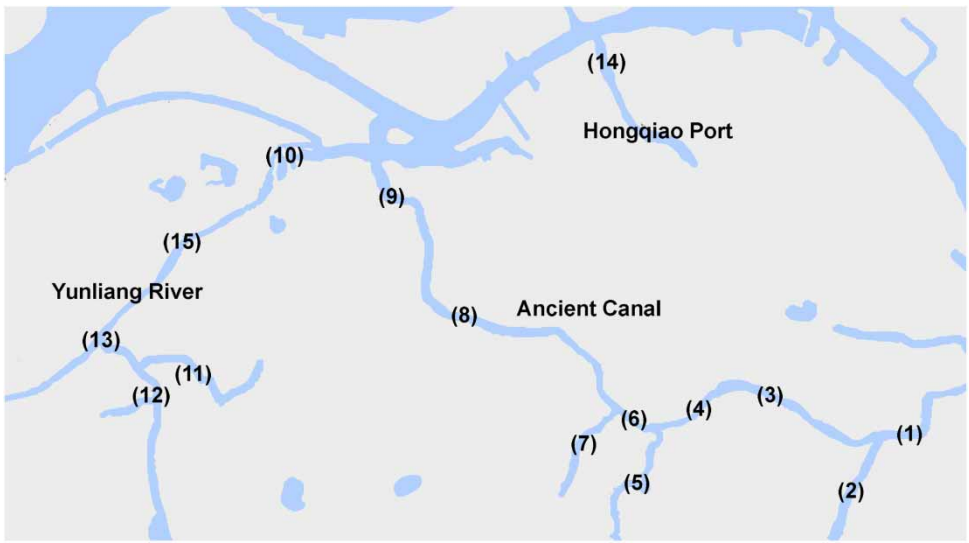


Figure 2 | Location of sampling sites of rivers in Zhenjiang City.

MS/MS acquisition was operated on a Waters Xevo™ TQ MS triple-quadrupole mass spectrometer (Waters Corp., Milford, MA, USA) with an electrospray ionization interface operating in negative ion mode. The main mass spectrometric conditions were as follows: source gas temperature, 135 °C; desolvation gas temperature, 300 °C; pressure of the collision cell, 1.2×10^{-3} mbar; flow rate of the desolvation gas (nitrogen gas, 99%), 650 L/h; capillary voltage, 3.0 kV. Ion transition (m/z) 313–160 was selected for quantification and ion transition (m/z) 313–126 was used for confirmation.

Quantification was performed using external standard method. LOD and the limit of quantitation (LOQ) were calculated based on the standard deviation of six replicates of the spiked samples at the concentration of 10 ng/L for water and 10 ng/g for sediment, and LOD and LOQ were defined as three and 10 times the standard deviation for TCC measurements, respectively. The LODs and LOQs of TCC are 1.5 ng/L and 4.9 ng/L in surface water, and 0.9 ng/g and 2.9 ng/g in sediments. The linear range was 4.9–870 ng/L. During each set of samples analyzed, a reagent blank, method blank and spiked matrix (10 ng/L for surface water, and 10 ng/g for sediment) were analyzed simultaneously with samples. None of the target analytes were detected in the blank controls.

Statistical analyses

The statistics were performed using SPSS software, version 17.0 (Chicago, IL, USA). The correlation between two parameters (nonparametric distributions) was analyzed by Spearman's rank coefficient of correlation. The Mann–Whitney U and Kruskall–Wallis tests were used for comparison between groups. The differences were considered significant with $P < 0.05$.

RESULTS AND DISCUSSION

Synthesis of haptens

Because of a small nonimmunogenic molecule, TCC must be conjugated to carrier proteins for stimulating the immune response of animals to produce antibody. However, there are no functional groups (amino, carboxy group or others) in the TCC molecule structure; so this compound has to be modified to obtain some analogues.

In the study by Ahn *et al.* (2012) for immunizing haptens, saturated hydrocarbon linkers or isosteric analogue, S, were introduced at the 4 or 4′ position of the structures, while coating antigens were designed through altering substitutions in the handle or adjusting the parent structure. It was found that the lowest IC_{50} values, and largest ratios of signal to noise were obtained when 3-((2-chloro-4-(3-(4-chlorophenyl) ureido)phenyl)thio)propanoic acid was used as immunizing haptens, and 3-(4-(3-(4-(trifluoromethoxy)phenyl)ureido) piperidin-1-ylsulfinyl)propanoic acid as coating haptens, respectively. However, the synthetic method was complex and time-consuming. To simplify steps and improve efficiency, in our study haptens were designed by condensation of isocyanate, amine group and alkaline hydrolysis, which are shown in Figure 1. At the same time, to retain the whole structure of TCC, 4-[3-(3,4-dichlorophenyl)ureido]phenyl moiety was used for antigen epitope. It was confirmed that the antibody obtained through this immunogen had good reorganization with free TCC in the following experiments.

Characterization of MAbs to TCC

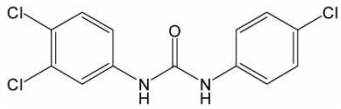
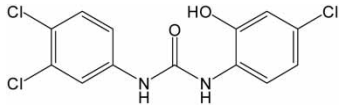
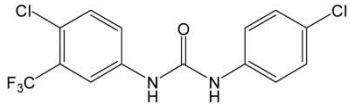
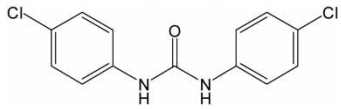
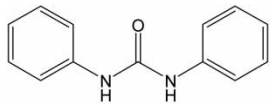
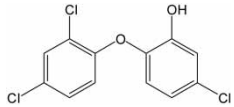
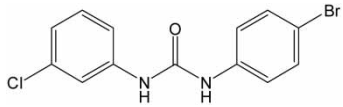
Through cell fusion experiments, nine clones were generated with the highest titer and affinity, all of which were cultured under good cell growth status. Among the nine clones, 4D5 showed the best sensitivity (IC_{50}, 0.43 ng/mL) and was selected for further study.

The cross-reactivity (CR) of clone 4D5 was evaluated by determining the respective IC_{50} values with TCC-related compounds using indirect competitive ELISA. The results (seen in Table 1) showed that 4D5 had high specificity with low CR (<20%) to TCC metabolites and analogues, which meant this antibody had the tendency for the recognition of compounds with the structure of 4,4′-dichlorocarbanilide, such as 2′-OH TCC (CR 19%), 3-trifluoromethyl-4,4-dichlorocarbanilide (CR 13%) and 4,4′-dichlorocarbanilide (CR 9%). And it showed that the substituents in phenyl moieties may be the important antigenic determinant because only one or two different substituents in phenyl moieties led to lower CR (shown in Table 1 except triclosan). For triclosan, the CR was <0.1%, which showed that the urea bridge participated in the identification between antigen and antibody.

Optimization of competitive ELISA

To improve the assay performance, parameters that affect the assay were studied, including concentration of coating antigen and antibody. At the appropriate situation (coating antigen, 0.25 μg/mL; MAb 4D5, 1:5,000 dilution), the optimum value that gave the highest OD_{max}/IC_{50} was observed (Degelmann *et al.* 2004; Liang *et al.* 2007; Zhang *et al.* 2010), From the representative standard curve for TCC in Figure 3, the calculated IC_{50} value was 0.43 ng/mL,

Table 1 | Cross-reactivity of MAb (4D5) to TCC and related compounds

Compounds	Structure	Cross-reactivity (%)
TCC		100
2′-OH TCC		19
3-Trifluoromethyl-4,4′-dichlorocarbanilide		13
4,4′-Dichlorocarbanilide		9
Carbanilide		<0.1
Triclosan		<0.1
N-(4-Bromophenyl)-*N*′-(3-chlorophenyl)-urea		<0.1

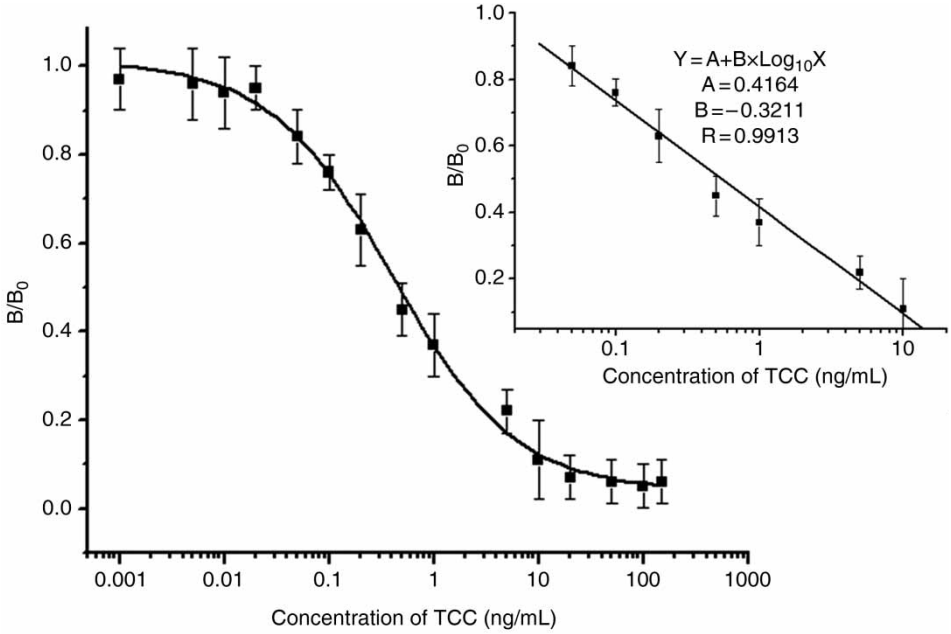


Figure 3 | The standard curve of TCC in indirect competitive ELISA. Values were determined in triplicate experiment (B: measured absorbance with different concentrations of TCC; B_0: absorbance without TCC).

which was more sensitive than the previous results (Ahn *et al.* 2012). LOD was 0.05 ng/mL and the linear range was from 0.05 ng/mL to 10 ng/mL.

Effect of organic solvents

In sample preparation, especially solid sample, the chemical and physical properties of the target, such as n-octanol–water partition coefficient (K_{OW}) and organic carbon normalized sediment/water partition coefficient (K_{OC}), should be taken into consideration. It was reported that log K_{OW} and log K_{OC} value of TCC were 4.9 and 4.5 respectively, which showed moderate lipophilicity (Heidler & Halden 2008). Therefore organic solvents are commonly adopted to extract the analyte from environmental samples. It is necessary to evaluate the influences of organic solvents to antigen–antibody reaction. Various ratios of organic solvents/assay buffer (0–50%) (v/v) were used for the preparation of working solutions. As shown in Figure 4, the higher tolerance to acetonitrile (≤30%, v/v) and methyl alcohol (≤20%; v/v) exhibited in this study may be partly attributed to excellent performance of antibodies. In contrast, acetone and DMSO at the same concentration could significantly affect OD_{max} and slope of the assay. Therefore acetonitrile was chosen to extract TCC from environment samples.

Evaluation of the assay

The accuracy and precision of the established method were evaluated through analysis of some environmental waters (sample analysis < LOD) fortified with a variety of TCC with six replicates. Because TCC is widely used in personal care products, it is difficult to obtain blank environmental

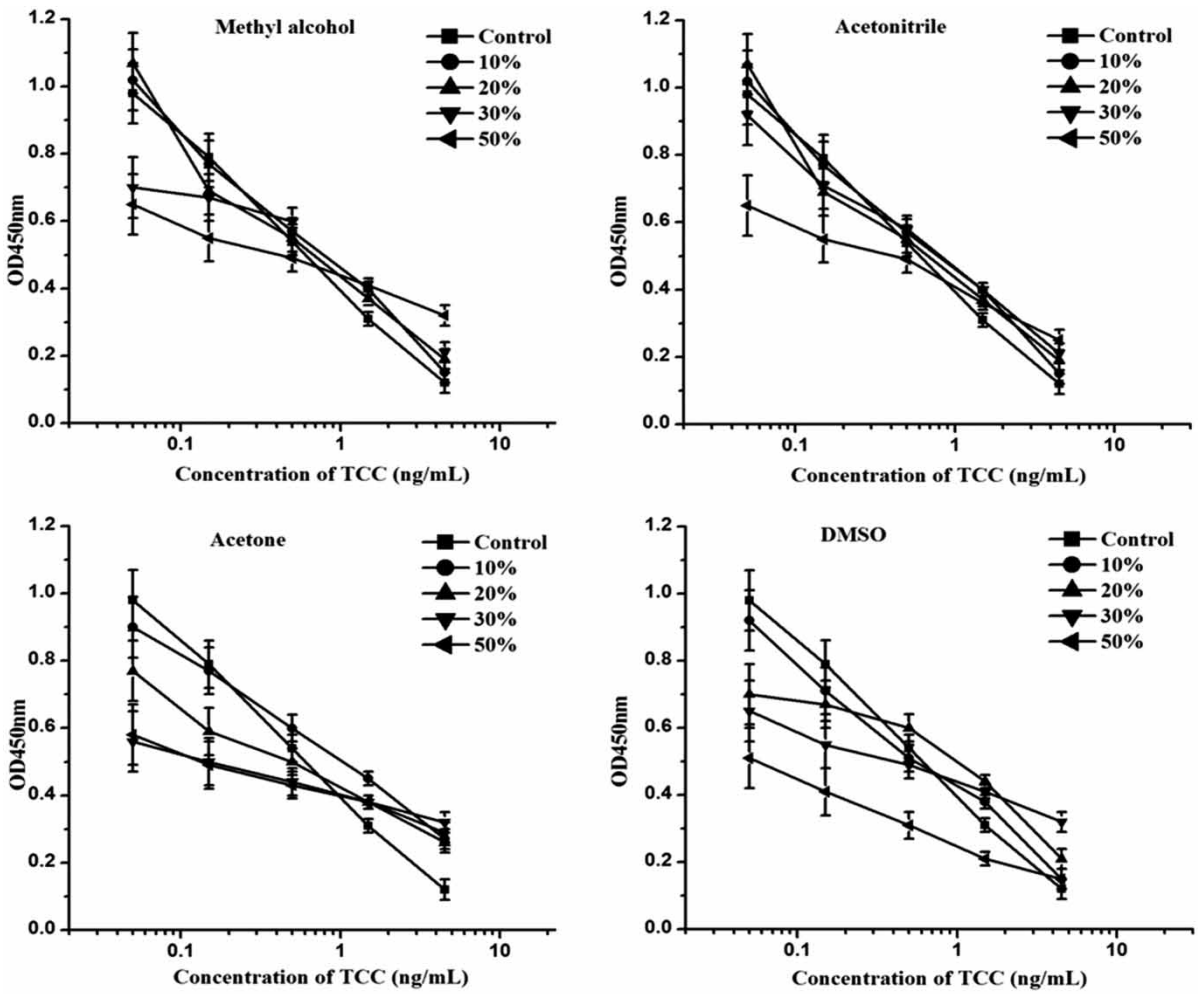


Figure 4 | Influence of different organic solution, methyl alcohol, acetonitrile, acetone and DMSO, on the performance of assay. Results are the means of three independent experiments.

Table 2 | Recoveries of TCC in spiked samples of waters and sediments measured by immunoassay

Water			Sediment		
Spiked concn (ng/mL)	Detected concn (ng/mL)	Recovery (%)	Spiked concn (ng/g)	Detected concn (ng/g)	Recovery (%)
0.1	0.09 ± 0.02	70.7–106.1	5	4.25 ± 0.51	74.8–95.2
1	0.96 ± 0.11	85.2–107.4	20	17.6 ± 2.05	77.8–98.3
10	8.92 ± 0.81	81.1–97.3	100	82.1 ± 7.06	75.1–89.2
Mean		70.7–107.4			74.8–98.3

samples, especially of sediments. For the spiked assay, samples with relatively low concentration confirmed by LC-MS/MS were adopted, which were W03 for water sample and S03 for sediment samples. Meanwhile non-spiked sample controls were set in each test and used to eliminate the background. As listed in Table 2, the average recoveries of TCC in spiked waters and sediments were 70.7–107% and 74.8–98.3%, respectively, which was satisfactory when applied for monitoring TCC in a real aquatic system.

To further assess the developed method, randomly selected river water samples and sediments were directly measured in parallel by ELISA and LC-MS/MS. The results (seen in Table 3) indicated that the two methods generally agreed well, noting that TCC concentrations detected by ELISA were higher than that by LC-MS/MS. As a rapid screening method, the performance of ELISA was determined by the antibody used. The antibody developed here had CR with three compounds with the structure of 4,4′-dichlorocarbanilide. 2′-OH TCC is one of the oxidative metabolites in urine after human exposure to TCC (Zhou *et al.* 2012); 3-trifluoromethyl-4,4′-dichlorocarbanilide, like TCC, has been used as an antibacterial agent in personal care products (Nolen & Dierckman 1979); 4,4′-dichlorocarbanilide was found to be a co-contaminant, up to 30 μg/kg, with TCC in primary sludge from some wastewater treatment plants in the USA (Sapkota *et al.* 2007). So it is speculated that the samples collected may contain these three compounds, which led to higher detection concentration by ELISA. The speculation will be confirmed in further experiments.

Table 3 | Detection of TCC in river water and sediment by ELISA and LC-MS/MS

		Concentration of TCC		
Samples		ELISA (A)	LC-MS/MS (B)	Ratio (A/B)
Water ng/L	W-a	61.41 ± 5.45	42.74 ± 3.87	1.4
	W-b	155.42 ± 17.5	122.31 ± 12.7	1.3
Sediment ng/g	S-a	78.27 ± 5.41	62.48 ± 7.12	1.3
	S-b	35.54 ± 2.97	31.56 ± 3.02	1.6

All indications were that the developed method was reliable and could be used as an effective tool in detection of TCC in environments.

Monitoring of TCC in environmental samples

Ancient Canal and Yunliang River are branches of Yangtze River in east China, and are important rivers in Zhenjiang City. Because the target compounds are widely used as personal care products and discharged to aquatic environments (such as the tributary and trunk river), TCC residues were determined in the stream and adjacent areas by the developed ELISA method and confirmed with LC-MS/MS, shown in Table 4. After analysis by SPSS, the concentrations detected by ELISA correlated strongly with those by LC-MS/MS ($r = 0.98$, $P < 0.01$), which shows the feasibility of ELISA again.

It was reported that the detection frequencies for TCC in surface water and sediments were 100% or close to 100% in the Liao River, Hai River, Yellow River, Zhujiang River and Dongjiang River (Zhao *et al.* 2013). Here in our research, TCC was found in Yunliang River, Ancient Canal and Hongqiao Port in Zhenjiang City and the detection frequency was 80% (12/15) in river water and 100% (15/15) in sediments. TCC was detected up to 422.12 ng/L in river water and in the range from 6.68 ng/g to 78.67 ng/g in sediment samples.

During the analysis, it was observed that the concentration of TCC was up to 26.8 ng/mL in water in the first batch of samples (data were not listed), which was far beyond the data reported in literature (up to 338 ng/L in surface water, Zhao *et al.* 2013). But then four batches of samples were collected in various months over a year and the average of the results are listed in Table 4, which shows TCC in river waters was up to 422 ± 25.8 ng/L. It was speculated that two reasons led to this state. Firstly, the first batch of samples was collected in February, as this organic pollutant content was higher in the dry season. Secondly, a more important point was that sample bottles were cleaned with detergents and rewashed with waters only once originally, and TCC in

Table 4 | TCC concentrations in environmental samples by immunoassay and LC-MS/MS

	Concentration of TCC (ng/L)				Concentration of TCC (ng/g)		
Water	**ELISA (A)**	**LC-MS/MS (B)**	**Ratio (A/B)**	**Sediment**	**ELISA (A)**	**LC-MS/MS (B)**	**Ratio (A/B)**
W01	62.04 ± 9.11	51.77 ± 5.88	1.20	S01	8.75 ± 1.12	4.14 ± 1.25	2.11
W02	-[a]	30.68 ± 3.75	\[b]	S02	11.45 ± 1.42	7.78 ± 1.08	1.47
W03	-[a]	ND	\[b]	S03	6.68 ± 0.74	3.25 ± 0.75	2.06
W04	94.71 ± 12.3	72.71 ± 8.45	1.30	S04	12.55 ± 1.64	8.77 ± 0.74	1.43
W05	-[a]	17.56 ± 1.15	\[b]	S05	24.35 ± 3.74	15.66 ± 2.02	1.55
W06	165.71 ± 23.14	121.24 ± 9.74	1.37	S06	29.24 ± 2.33	21.46 ± 2.58	1.36
W07	107.45 ± 10.95	75.24 ± 6.45	1.43	S07	14.78 ± 1.87	9.54 ± 0.87	1.55
W08	422.12 ± 25.84	342.21 ± 24.51	1.23	S08	78.67 ± 8.46	52.45 ± 5.45	1.50
W09	220.72 ± 18.77	180.44 ± 15.68	1.22	S09	58.45 ± 4.56	32.78 ± 7.32	1.78
W10	270.75 ± 24.41	227.24 ± 19.51	1.19	S10	45.67 ± 3.68	27.89 ± 4.71	1.64
W11	80.72 ± 6.57	57.81 ± 4.78	1.40	S11	17.35 ± 1.12	11.54 ± 1.23	1.50
W12	130.12 ± 10.94	108.47 ± 8.45	1.20	S12	18.56 ± 1.92	12.83 ± 1.22	1.45
W13	-[a]	ND	\[b]	S13	28.35 ± 2.74	17.74 ± 1.89	1.60
W14	-[a]	ND	\[b]	S14	15.78 ± 1.67	9.54 ± 1.15	1.65
W15	370.14 ± 28.94	290.24 ± 19.41	1.28	S15	46.78 ± 5.89	34.23 ± 4.78	1.37

[a]Below the LOD of ELISA.
[b]Data could not be calculated.
ND: Not detected.

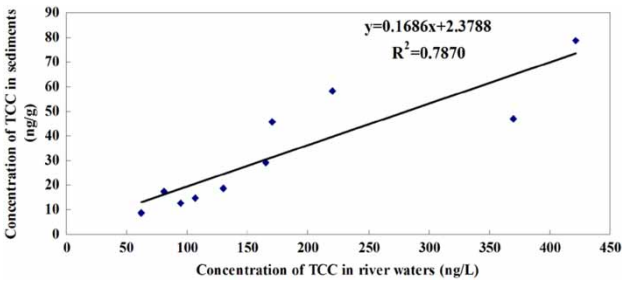


Figure 5 | The relationship between the TCC levels in river waters and sediments.

detergents may have contaminated the river samples. So in the following sampling, cleaned sample bottles were rinsed with acetone and sterile deionized water to avoid the pollution of TCC from detergents.

As shown in Figure 5, the concentration of TCC in sediments had good relationship with that in waters ($R^2 = 0.7870$): the higher the TCC in water, the more TCC in sediment. And it was observed that the concentration of TCC residues in some sampling sites (No. 8, 9, 10, 15) were higher than those in other sites. According to the sampling sites, it was speculated that there are many high density areas of people around the four sites, which were polluted seriously by personal care products' TCC.

CONCLUSIONS

To develop a sensitive immunoassay for TCC in environmental samples, haptens were synthesized through condensation of the isocyanate and amine group. High affinity monoclonal antibody 4D5 was developed through the hybridoma technique. An indirect competitive immunoassay was developed for the detection of TCC. The effect of organic solvents on the performance of ELISA was evaluated, which showed acetonitrile had the best tolerance to antigen–antibody reactivity and was used in further study. The IC_{50} value of this assay was 0.43 ng/mL, the LOD was 0.05 ng/mL and the linear detection range was 0.05–10 ng/mL. The average recoveries of TCC in spiked water and sediment were 70.7–107% and 74.8–98.3%, respectively. In Yunliang River, Ancient Canal and Hongqiao Port in Zhenjiang City, TCC was found from not detected (ND) to 422.12 ng/L in water and 6.68 ng/g to 78.67 ng/g in sediment samples. There was relatively good agreement between ELISA and LC-MS/MS data for water and sediment, which means that this method provides a simple and rapid screening tool to monitor TCC in environmental samples.

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