Immunochemical detection of emerging organic contaminants in environmental waters

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Abstract

Immunochemical techniques exhibit great advantages of high efficiency, rapidness, reliability and low cost compared to instrumental methods for monitoring emerging organic contaminants (EOCs) in aquatic environments. This review covers recent advances in applying traditional and other antibody-like binders against these organic pollutants, and various antibody-based immunochemical methods such as enzyme-linked immunosorbent assays, time-resolved fluoroimmunoassay and immunosensors. Moreover, we also discuss the advantages and disadvantages of techniques for antibody production and analytical methods, and covers promising future prospects.

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1. Introduction

Emerging organic contaminants (EOCs) are unregulated organic pollutants, including antibiotics, personal care products, surfactants and surfactant residues, artificial sweeteners, pesticides, steroids and various industrial additives. These chemicals exist in the environment in unknown quantities and lack extensive eco-toxicological data, and they are also discharged into the aquatic environment that could lead to potential adverse ecological or human health effects [1–3].

Due to their low concentration in aquatic system and the presence of other contaminants, EOCs have to be enriched and cleaned from water samples prior to instrumental analysis. The most commonly used pretreatment method is solid phase extraction (SPE), which has been coupled with many instrumental analytical techniques such as liquid chromatography tandem mass spectrometry (LC-MS/MS) [4,5] and gas chromatography mass spectrometry (GC-MS) [6,7] for the detection of EOCs in environmental waters. Although these approaches possess the advantages of high sensitivity for both quantification and identification, they suffer from several limitations including (i) low throughput that cannot address the backlog of samples that need to be analyzed; (ii) large volume of each sample to be enriched and complicated pretreatment procedures to separate targets from matrices; (iii) expensive instrumentation that requires skilled analysts and is unsuitable for field studies and on-line monitoring of target pollutants [8].

Antibody-based immunochemical techniques provide alternative analytical methods for these organic chemicals in environmental waters, and they have already been demonstrated to be quick, simple, sensitive and reliable [9–11]. To date, many effective immunochemical methods for EOCs have been established, and some have been used to simultaneously analyze a large number of samples in a short time, such as enzyme-linked immunosorbent assays (ELISA) and time-resolved fluoroimmunoassays [12,13]. In contrast to these high-throughput immunoassays, immunosensor techniques, including electrochemical and optical sensors, use simple, portable and robust devices to carry out on-site analysis on single samples [14]. All of these techniques have been used to screen environmental EOCs, and share common features: miniaturization, automation, and sophisticated analysis without complex sample preparation [15].

In this review article, we describe some classical and novel techniques in the production of traditional antibodies and other binders against EOCs. Emerging technologies with new binders are
introduced as well. We also review EOC analysis in aquatic environments using high-throughput immunoassays and immunosensors.

2. Antibodies for EOCs

2.1. Traditional antibodies

Most antibodies against EOCs are traditional antibodies, including polyclonal antibodies (pAbs) and monoclonal antibodies (mAbs). pAbs are directly produced by hyperimmunized animals, while mAbs are obtained from hybridoma cell lines. mAbs are superior to pAbs in immunoassays because (i) antiserums (pAbs) are limited but mAbs can be cloned indefinitely; (ii) mAbs are from identical offspring of a single cell with the same affinity and specificity to antigens, while pAbs are from different B cells with diverse affinities that might result in undesirable matrix affects in immunoassay [16,17]. However, mAbs do not show better performance than pAbs in all aspects. For instance, mAbs require larger-scale screening strategies and are more expensive than pAbs, which is also the reason why pAbs are more widely used in bioanalytical applications for EOCs.

While some mAbs and pAbs are high-specific antibodies which show no or low cross-reactivities with similar-structure compounds, others are considered broad-specific antibodies or generic antibodies that can recognize multiple targets. The broad-specific antibodies are currently an area of active research because they can analyze several analytes in a single assay.

EOCs are small molecular weight compounds which are non-immunogenic and cannot be used to elicit an immune response by directly injecting animals. Thus, EOCs have to be conjugated with carrier proteins (e.g., bovine serum albumin, BSA; egg albumin, OVA; human serum albumin, HSA) to form artificial complete antigens. Considering that some target chemicals have no functional group (carboxy-, amino-, hydrosulfuryl-) in their molecular structures to bind proteins. They usually have to be modified in order to be coupled with carrier proteins called haptons. Therefore, appropriate design and synthesis of haptons is the key step in antibody production, which could influence the affinity and specificity of the antibody as well as its performance in an immunoassay [18]. In immunology it is widely accepted that antibodies preferentially recognize the part of molecule that is the farthest away from the hapten. Therefore, the main strategy is to make the group with common or similar molecular structure or spatial conformation furthest away from the hapten.

In order to get broad-specificity antibodies against structurally similar compounds, computer-assisted molecular modeling techniques have been used as a reliable tool to study steric and electronic characteristics of haptons, mimic the target molecule structures, and aid in the design of haptons [19]. For instance, based on molecular modeling, Wang et al. selected ciprofloxacin as an optimal hapten to synthesize an immunogen. After immunizing female BALB/c mice with the immunogen, mAbs with high cross-reactivity (35–100%) were obtained. Furthermore, they explained the mechanism of specificity of this mAb by evaluating its conformational and electronic properties [20]. Adrian et al. also synthesized haptons against the common aminobenzenesulfonfylalino moieties with the aid of theoretical calculations and molecular modeling tools, based on the haptons, they generated pAbs that showed high sensitivity and excellent cross-reactivity to recognize 10 sulfonamides directly [21]. Other selected examples of the antibodies against EOCs are listed in Table 1.

The generic antibodies focus on chemicals with closely-related molecular structures. In contrast to the approaches above, one approach to generate an antibody capable of simultaneously recognizing compounds with diverse molecular structures is to conjugate several haptons to the same carrier protein [43]. However, the challenge of this approach is how to control the number of each hapten derivative binding to the correspondent carrier protein, even the value of hapten to protein conjugates could be identified by MONID-TOF/MS through comparing the observed molecular weights of the prepared conjugates with the unreacted protein [44].

Table 1  Antibodies produced for EOCs with conventional methods.

| Class               | Organic pollutant             | Antibody | Ref   |
|---------------------|-------------------------------|----------|-------|
| Pharmaceutical      | Sulfonamides                  | mAb      | [22]  |
|                     | Carbamazepine                 | mAb      | [13]  |
|                     | Progesterone                  | mAb      | [23]  |
|                     | Quinolones                    | mAb      | [24]  |
|                     | Cephalos                      | pAb      | [25]  |
|                     | Indomethacin                  | pAb      | [26]  |
|                     | Tetracycline                  | pAb      | [27]  |
|                     | Nitrofurantoin                | pAb      | [28]  |
|                     | Progesterone                  | pAb      | [29]  |
| Pesticide           | Imidacloizit                  | mAb      | [30]  |
|                     | Deltamethrin                  | mAb      | [31]  |
|                     | Carbofuran                    | mAb      | [32]  |
|                     | Flucythrinate                 | mAb      | [33]  |
|                     | Methiocarb                    | mAb      | [34]  |
|                     | Fenarimol                     | pAb      | [35]  |
|                     | Imidaclophlorid               | pAb      | [36]  |
|                     | Chlorpyrifos                  | pAb      | [37]  |
| Industrial additive | Di-(2-ethylhexyl) phthalate    | mAb      | [38]  |
|                     | Bisphenol A                   | mAb      | [39]  |
|                     | Dimethyl phthalate            | pAb      | [40]  |
|                     | Nonylphenol                   | pAb      | [41]  |
| Personal care product | Triclosan                    | pAb      | [42]  |

2.2. Other antibody-like binders

Compared to traditional antibodies, other types of antibodies, or novel antibody-like biological and synthetic binders have been developed, such as molecularly imprinted polymers, nanobodies, aptamers, protein scaffolds and recombinant antibodies. Most of these techniques have been used in immunotherapeutic and immunodiagnostics applications because of their high affinity and specificity [45]. Fody et al. gave a comprehensive review of the progress and use of nanobodies, aptamers, and protein scaffolds in environmental detection. Although they stated that recombinant antibodies have achieved significant success in biomedicine, the defects of their biophysical properties and complicated molecular composition could hamper further development [17]. However, recombinant antibodies have characteristics that make them potentially useful for EOC analysis.

Recombinant antibodies are genetically engineered antibodies. The genes can be amplified by PCR and expressed through different expression systems to various formats, such as the antigen binding fragments (Fab), the bare variable fragment (Fv), the variable domain of heavy chain (VH) and the single chain variable fragment (scFv), as shown in Fig. 1. Different recombinant antibody fragments, like different formats of functional binding molecules, can effectively capture their corresponding targets similar to traditional antibodies. At the same time, recombinant antibody technology shows many advantages over traditional antibodies in the following aspects: (i) Recombinant antibodies can be adjusted through molecular biology techniques for expression, post-translational modification, optimization in affinity and specificity according to the application [45]; (ii) Recombinant antibodies can be generated in vitro via phage and bacterial display systems, making the selection and screening procedure flexibly [46].
Furthermore, recombinant antibodies can tolerate a range of conditions in solutions, such as acidic or basic pH, high levels of heavy metal ions, organic solvents at concentrations over 10%, all of which would denature conventional antibodies [47]. The robust nature of recombinant antibodies greatly broadens their application in immunoassays for EOCs analysis, because there might be some EOCs in industrial waste waters containing high concentration of heavy metals or organic wastes.

Some recombinant antibodies for EOCs have been manufactured that showed a remarkable range in characteristics. Kerrm et al. obtained a soluble Fab fragment against picloram (4-amino-3, 5, 6-trichloro-2-pyridinecarboxylic acid) that showed 34% cross-reactivity to the pyridine herbicide clopyralid [48]. Li et al. produced three scFv antibodies from a phage-display library of a hyperimmunized mouse against the organophosphate pesticide methamidophos, which displayed better binding properties than the corresponding Mab [49]. Other selected examples are shown in Table 2.

Recently another biological binder called a receptor protein was developed against low molecular weight compounds [57]. The pharmacological effects of some EOCs, especially some of antibiotics and pesticides, have been thoroughly studied. These drugs exert biological effects through binding to target protein. Interestingly, this kind of protein can recognize a family of compounds with similar structures, which could provide a new strategy for developing binders to EOCs. For example, β-lactam antibiotics act by covalently binding to transpeptidases, or penicillin binding proteins (PBPs), and inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls. A recombinant penicillin-binding protein (PBP) 2X* from Streptococcus pneumoniae R6 that is expressed in E. coli BL21 (DE3) and purified with His Bind® Columns was developed, and the assay based on PBP2X could detect all 16 β-lactams with a limit of detection (LOD) in the range of 0.1–810 ng/mL [58]. In addition to antibiotics, organophosphates (OP) have been widely used to kill insects and animals through inhibiting acetyl cholinesterase by forming stable covalent intermediates. Albareda-Sirvent et al. developed a biosensor based on enzyme (AChE and BChE) to detect paraoxon and carbofuran with a LOD of 0.165 and 0.047 ng/mL, respectively [59]. However, because the toxicological mechanisms of some EOCs are not yet clear, it is difficult to produce a corresponding targeting protein. With increasing discoveries of the toxicological mechanisms of EOCs, we believe it will be possible to find the receptor proteins against EOCs and use these proteins in analytical methods.

3. Immunochemical technique

EOCs usually occur in environmental waters at very low concentrations accompanied by various matrices, thus analytical methods with enough sensitivity and selectivity are required. With the help of suitable antibodies, or other antibody-like binders, effective immunoanalytical approaches with high sensitivity, selectivity and simple (or without any) sample pre-treatment could be developed. Among them, ELISA and TRFIA are considered as reliable high-throughput immunoassays, while the immunosensor is a sensitive approach to quickly measure signal with simple equipment and suitable for on-site analysis of EOCs in water samples [8].

3.1. ELISA

ELISA was first applied to environmental chemicals determination in 1971 by Ercegovich [45]. It is now one of the most effective tools for monitoring EOCs in water by measuring changes in color or emission of light. ELISAs can be classified into direct and indirect competitive immunoassays. In the direct format, targets (unlabeled antigens) and labeled antibodies, effective immunoanalytical approaches with high sensitivity, selectivity and simple (or without any) sample pre-treatment could be developed. Among them, ELISA and TRFIA are considered as reliable high-throughput immunoassays, while the immunosensor is a sensitive approach to quickly measure signal with simple equipment and suitable for on-site analysis of EOCs in water samples [8].

### Table 2

| Recombinant antibody fragment | Target                          | Ref  |
|-------------------------------|---------------------------------|------|
| scFv                          | Triazine herbicide              | [51] |
| scFv                          | Bisphenol A                     | [52] |
| Fab                           | 2,4-Dichlorophenoxyacetic acid  | [53] |
| scFv                          | Picloram                        | [54] |
| scFv                          | Chlorpyrifos-ethyl              | [55] |
| scFv                          | Chlorpyrifos                    | [56] |
matrices. The indirect mode was regarded as a suitable method for the detection of small molecular compounds in environmental water samples. As a result, ELISA has been the most popular immunoassay in the past decades for EOC determination in aquatic environments because of its low cost and simple procedure [11,12]. Assays based on traditional antibodies for EOCs are outlined in Table 3, and selected examples with receptor proteins and recombinant antibodies are shown in Table 4.

The selectivity and the sensitivity of ELISA are determined by antigen-antibody interaction. At the competitive reaction step, some matrices such as organic solvents [66,77–79] and other environmental factors [80] may interfere with the immunoreaction. To detect trace EOCs in aquatic environments, assays with more sensitive than ELISA have to be applied.

3.2. TRFIA

TRFIA is an ultrasensitive technology based on the unique fluorescence properties of the lanthanide ions such as europium (Eu³⁺), samarium (Sm³⁺), terbium (Tb³⁺), gadolinium (Gd³⁺) and dysprosium (Dy³⁺), which provide narrow-band emission lines, long Stokes shifts, high quantum yields, and long decay lifetimes [81,82]. These advantages can reduce background fluorescence inherent in the sample matrix [45] or scattering from nearby optics [83], and high sensitivity.

TRFIA has played an important role in determination of trace EOCs in water. It was reported that the detection limits of TRFIA against triazine herbicides was as low as 0.1 µg/L [84]. Using generic ScFv fragments, Korpimaki et al. established a TRFIA method for simultaneous determination of 18 different sulfonamides in meat, milk, and serum samples [85]. Our group also developed a TRFIA approach with commercially available Mab labeled Eu³⁺ chelates for trace analysis of sulfamethazine (SMZ), sulfamethoxazole (SMX) and sulfadiazine (SDZ) in environmental waters, and the results indicated satisfactory recoveries (accuracy) and good repeatability, as well as lower detection limits than the corresponding ELISA methods [86].

We also developed a class-specific mAb and established a TRFIA method to detect the total concentration of twelve fluoroquinolones in environmental waters. More importantly, this was the first report on the use of immunoassay–based total concentration for direct evaluation of the environmental risk of a class of compounds (Fig. 2). Traditionally, in evaluating environmental exposure and risk of a class of compounds with potential additive toxicity, the total concentration of target compounds is determined by the sum of each individual compound concentration that is usually determined by SPE coupled with LC-MS/MS. Unlike the traditional LC-MS/MS approach that is expensive and time-consuming, this TRFIA method is low-cost and efficient as it was performed without sample pre-treatment and consumed only microliters of samples [87].

Beyond TRFIA, radioimmunoassays (RIA) and chemiluminescence immunoassay (CI) are high-throughput immunoassays with high sensitivity, but both of these methods are mainly used in medical diagnosis because the techniques need specialized equipment and special labeling reagents. At the same time, in contrast with these methods, TRFIA can meet the requirement of multi-targets analysis using different lanthanide ions as tracers.

3.3. Superiority of high-throughput immunoassay for environmental samples

In food analysis, especially the detection of antibiotics residues in milk and animal tissues, high-throughput immunoassays (including ELISA, TRFIA, RIA and CI) are used as screening tools for large numbers of samples with LC-MS/MS or other chromatographic methods used for more quantitative results. In other words, immunoassays are just a kind of supplement for instrumental methods. But EOCs monitoring in environmental waters requires long-term investigation on the pattern, behavior or distribution and seasonal variation of these chemicals in an aquatic environment. The real trends which can be reflected only through the analysis of a great deal of samples. Therefore, high-throughput immunoassays that provide rapid answers at low cost are preferred, while expensive chromatographic methods with sophisticated pretreatment are unimaginable. It is noteworthy that although TRFIA is regarded as a more sensitive method than conventional ELISA, the cost of lanthanide ions chelates used in TRFIA system is much higher than that in ELISA. On the other hand, ELISA could meet the most of requirements for EOCs detections due to the high sensitivity of antibodies. As a consequence, ELISA was widely reported instead of TRFIA. Byer et al. [88] holds the similar view that ELISA could provide quicker answers at low cost, while expensive chromatographic methods with sophisticated pretreatment are unimaginable. In the meantime, ELISA is much more cost-effective alternative for improving temporal and/or spatial monitoring after they measured a total of 533 samples from more than 100 sites in Ontario, and they also think ELISA has great

Table 3

Selected examples of ELISA for EOCs based on traditional antibodies.

| Class               | EOC                                      | Sample | Detection limit (Ref) |
|---------------------|------------------------------------------|--------|-----------------------|
| Pharmaceutical-als  | Carbamazepine, caffeine cetirizine       | Water  | 0.025 ng/mL [60]      |
|                     | Sulfamethoxazole                          | Waste water | 0.001 ng/mL [61]     |
|                     | E2 and E2                                 | Aqueous sample | 0.02, 0.03 ng/L [62] |
|                     | Carbamazepine                             | Water  | 0.03 ng/mL [13]       |
|                     | Tylsoin                                   | Water water | 0.03 ng/L [42]        |
|                     | Sulfonamides                              | Water  | 0.04 ng/L [63]        |
|                     | Indomethacin                              | Water  | 0.01 ng/mL [26]       |
|                     | Nitrofurantoin                            | Water  | 0.2 ng/mL [28]        |
|                     | Carbofuran                                | Water, soil, vegetables | 0.11 ng/mL [32]        |
| Pesticides          | Diuron, monuron, linuron                  | Water  | 0.04–0.08 µg/L [64]   |
|                     | Malathion                                 | Water  | 0.11 ng/mL [10]       |
|                     | Carbofuran                                | Water  | 0.27 µg/L [65]        |
|                     | Triazophos                                | Water, soil | 0.02 µg/L [66]      |
|                     | Chlorpyrifos                              | Water  | 0.1 µg/L [37]         |
|                     | Malathion                                 | Water  | 0.11 ng/mL [10]       |
|                     | Deltamethrin                              | Water  | 1.1 ± 0.5 µg/L [67]   |
| Industrial additives| Bisphenol A                               | water  | 0.1 ng/mL [39]        |
|                     | Diethyl phthalate                         | water  | 0.096 ng/mL [68]      |
|                     | Di-(2-ethylhexyl)                         | Human urine | 0.56 ng/mL [38]      |
|                     | phthalate                                 |        |                       |
|                     | Caffeine and cotinine                     | Water  | 0.135, [69]           |
|                     | Dimethyl                                  | Water  | 0.047 ng/mL [30]      |
|                     | Phthalate                                 | Water  | 0.02 ng/mL [40]       |
| Personal care products| Alkylphenols                              | Soils and waters | 0.001 ng/mL [70]  |
|                     | Nonylphenol (NP)                          | Water  | 10 ng/mL [41]         |
|                     | Triclocarban                              | Body fluids | 0.13 ng/mL [71]      |
|                     | Triclosan                                 | Water  | 0.03 ng/L [42]        |

Table 4

Selected examples of ELISA for EOCs based on antibody-like binders.

| Binder | EOC                                      | Detection limit (Ref) |
|--------|------------------------------------------|-----------------------|
| ScFv   | Cyclohexanide                            | – [72]                |
| ScFv   | Organophosphorus pesticides              | – [56]                |
| Fab    | Picolorm                                 | 10 ng/mL [48]         |
| ScFv   | Cotinine                                 | 31 µg/mL [73]         |
| ScFv   | Methamidophos                            | 0.04–0.26 ng/mL [49]  |
| ScFv   | Clenbuterol                              | 0.78 ng/mL [1C0]      |
| ScFv   | Simetryn                                 | 1.1–70 ng/mL [51]     |
| Receptors | β-lactams          | 0.1–810 ng/mL [58]    |
| Receptors | Sulphonamides | 1.6–59 ng/mL [75]     |
potential applications because of the little to no sample pretreatment and rapidness. In the 2004 National Atrazine Occurrence Monitoring Program that was conducted under the joint sponsorship of the American Water Works Association’s (AWWA) Water Industry Technical Action Fund (WITAF) program and the American Water Works Association Research Foundation (AwwaRF), high-throughput immunoassay (ELISA kits) were selected as the most suitable method to investigate a pesticide in waters, and they also thought that frequent monitoring was costly and time-consuming with GC/MS in contrast with immunoassay [80].

3.4. Immunosensor

An immunosensor is an analytical device which incorporates antibodies to be responsible for recognizing the targets and contains a transducer to convert the recognition into a measurable signal for the rapid determination of analytes. An immunosensor is one type of biosensors that is based on biological or synthetic binders. The operating principle of immunosensors is that changes would begin in the physicochemical parameters of the detecting system when specific immunoreactions proportional to the concentration of targets happen, resulting in a measurable optical or electrical signal [89]. As an integrated device, immunosensors omit some complex steps such as washing and regent addition steps, and they could be automated to perform analysis without sample manipulation and in a miniaturized portable device [90].

In EOC analysis, immunosensors are indispensable supplements of high-throughput immunoassays and chromatographic methods. Immunosensors can achieve on-site analysis without the use of lab instruments. Concentrations of EOC contaminants in water could be obtained quickly without collection of a large number of water samples far away from the laboratory.

Many transducers have been used in immunosensors include piezoelectric, micromechanical, electrochemical and optical sensors. The latter two immunosensors are the most popular in EOC monitoring.

Optical immunosensors (shown in Fig. 3) exploit various optical properties based on fluorescence, light absorption, chemiluminescence or surface-plasmon resonance [91]. The reaction of an antibody capturing its corresponding antigens results in changes of absorption or emission of light in that can be measured [8]. In recent years optical immunosensors have found applications in detecting pollutants because the availability and range of optical transducers have grown, benefitting mainly from the development of fiber optic technology [92].

Electrochemical immunosensors (Fig. 4) measure signal responses through variation in ion concentration or electron density caused by antibody-antigen interaction. These sensors have high sensitivity, compatibility with modern microfabrication technologies and high tolerance [93], and have high tolerance against quenching, sample turbidity and interference from absorbing and fluorescing compounds (popular in biological samples) [94].

In order to increase signal to noise ratios and decrease time of response of immunosensors, nanoparticles have been introduced into this detection system and their main roles lie in: (i) improving the performance of immunosensors due to their unique physical and chemical properties; (ii) enlarging the surface area available to bind more antibodies and thus amplify the signals. In addition, for optical immunosensors, the application of nanoparticles contributes to the exploration of new optical phenomena and promotes the optical properties of materials [95]. As to electrochemical immunosensors, nanoparticles provide the ability to enhance selectivity and reduce the background current [90,96]. A disposable electrochemical immunosensor for the detection of diuron with laser ablated gold electrodes fabricated on polystyrene substrate was reported with a detection limit of 1 ng/L [97]. We have designed a novel electrochemical immunosensor for ultra-sensitive detection of dibutyl phthalate (DBP) in environmental waters, and the amplification of electrochemical signals was realized by the increase of the steric hindrance and electrostatic repulsion, which was achieved by optimizing the size of the gold nanoparticles and signal was amplified using AuNPs enlargement through NADH-
promoted catalytic precipitation. The approach showed one order of magnitude higher sensitivity than the conventional ELSIA that was carried out with same antibody (under revision). Table 5 lists optical immunosensors that have been used to sense EOCs in environmental waters.

4. Perspectives and conclusions

For EOC analysis, the first thing that analysts should take into account is cost, so among all kinds of biological and synthetic binders, pAbs are the first choice in immunoassays because of their low cost and simple production procedure. Being reliable, effective and well-established, traditional antibody production would still play an important role in immunochemical techniques. At the same time, in order to achieve multi-analyte determinations for EOCs, computer-assisted molecular modeling techniques are very useful in hapten-aided design for broad-spectrum antibodies. However, with the increasing development and maturity of other biological binders, such as aptamers, receptor and recombinant antibodies, the cost of production will decrease gradually, allowing them to have great potential in applications for detecting EOCs in waters instead of traditional antibiotics because of their unique properties.

| Organic contaminant | Sample          | Detector     | Detection limit       | Ref   |
|---------------------|-----------------|--------------|-----------------------|-------|
| Pesticides          | Water           | Fluorescence | 0.03–4.23 µg/L        | [100] |
| Estrone             | Water           | Fluorescence | <0.20 ng/L            | [101] |
| Propanil            | Water           | Fluorescence | 0.6 ng/L              | [102] |
| Bisphenol A         | Water           | Fluorescence | 0.014 µg/L            | [103] |
| TNT                 | Water           | Fluorescence | 0.05 µg/L             | [104] |
| Antibiotics         | Water           | Fluorescence | 2.4–6 ng/mL           | [105] |
| 17 Beta-estradiol   | Water           | Fluorescence | 0.6 ng/mL             | [106] |
| Bisphenol A         | Water           | Surface plasmon resonance | 0.08 ng/L    | [107] |
| Azinphos-methyl     | Water           | Amperometric | 0.6 nmol/L            | [108] |
| Sulfamethoxide      | Soil, water     | Piezoelectric quartz | 0.15 ng/L   | [109] |
In methodology, compared to the cumbersome, sophisticated and expensive chromatographic methods, we believe that high-throughput immunosensors show more advantages for studying the occurrence, temporal and spatial distribution, pattern and behavior of EOCs, and long-term influences caused by the organic pollutants because they are simple to operate, do not always require sample pretreatment, and possess high throughput. Furthermore, the real negative effects to the aquatic ecosystem by EOCs could remain in denatured environment, which will provide important background information of acidic, neutral and basic pharmaceuticals from aqueous samples at ambient (neutral) pH and their determination by gas chromatography-mass spectrometry for analysis of organic pollutants in the environment, Trac Trends Anal. Chem. 26 (2007) 1106–1122.

In addition, considering that EOCs could remain in denatured aquatic environments [high concentrations of heavy metals, organic solvents, salt ions], for instance, in industrial waste waters, molecularly imprinted polymers (MIPs) and recombinant antibody formats might be good choices because they possess high tolerance against various matrix effects, and biosensors based on these binders are able to quickly measure the concentration of EOCs in the waters. The sensitivity is the most important metric for all immunosensors, and the current trend for immunosensors is to achieve better sensitivity via nanomaterial properties. In the future nanoparticles may be used in immunosensor detection systems to improve their performance.

In summary, with developments in traditional antibodies, recombinant antibodies and other new binders, various effective, smart, and cheap immunochemical methods have been developed for the analysis of EOCs in aquatic environments. Using simple and suitable approaches, the pollution status of EOCs in environmental waters can be measured, which will provide important background data for risk assessment and contamination control of these contaminants in the aquatic environment.

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