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Perspective—Electrochemical Sensors to Monitor Endocrine Disrupting Pollutants

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Endocrine disrupting compounds (EDCs) interfere with natural hormonal feedback loops that control many aspects of development, often at sub-nanomolar concentrations. Numerous synthetic chemicals present as trace environmental contaminants are classified as EDCs, including xenoestrogens, polychlorobiphenyls (PCBs), phthalates, and perfluoroalkyl substances (PFAS) (Figure 1a). Importantly, these compounds are defined by their biological activity, not their specific chemical structure. EDCs have been implicated in many diseases, namely cancer, metabolic disorders (including obesity), and reproductive disorders. These chemicals are known to similarly affect wildlife, causing lasting damage to reproductive systems upon exposure. Consequently, regulatory agencies including the United States Environmental Protection Agency (US EPA) and the World Health Organization (WHO) have set exposure limits and minimum allowable contaminant levels (MCL), which are often very low due to the sensitivity of native hormone systems to these compounds. Enforcement of MCL standards and elucidation of the biological effects of known endocrine disruptors in addition to newly-introduced chemicals require accurate and sensitive detection at biologically relevant concentrations.

Rapid, sensitive EDC detection is essential for environmental monitoring. To date, the majority of detection efforts have focused on biological and chemical assays for pollutant sensing, many of which require laboratory settings. As the number of common chemicals that are found to interfere with endocrine signaling increases, there is an urgent need for improved detection methods that are portable, rapid, and inexpensive, and that elucidate modes of action. Electrochemical sensors exhibit high sensitivity and are amenable to low-cost, miniaturized designs (Table I) and wearable sensors for real-time human health monitoring. Importantly, electrochemical sensors are sufficiently flexible for both periodic field testing and continuous, remote monitoring.

Current Status

Non-electrochemical detection strategies.—Many analytical methods exist to monitor EDCs, including colorimetric, fluorescent, and chemiluminescent assays, mass spectrometry, surface-enhanced Raman spectroscopy, and immunoassays, many of which require pre-treatment to concentrate the analyte prior to detection. Mass spectrometry is most frequently used to measure EDCs and is often combined with chromatographic separation to enable monitoring from complex solutions. Bisphenol A (BPA), PCBs, and phthalates can be directly monitored, but polar PFAS compounds require derivatization for some chromatography methods.

These techniques are sensitive but often require centralized facilities and trained personnel to run. Fluorescence detection, in contrast, is portable and has been successfully used to detect EDCs by native emission (phenolic xenoestrogens including BPA), by derivatization with a fluorescent label (BPA, PFAS), or by energy transfer to fluorogenic substrates (PCBs). Derivatization and coupling with chromatographic methods improve sensitivity but impose cost and equipment constraints that limit the point-of-exposure applicability of these methods. Immunoassays are well-suited for EDC detection because EDCs naturally interact with biomolecules, but these assays are often relatively slow and expensive. Kits using competitive enzyme-linked immunosorbent assays (ELISAs) are commercially available for PCBs, phthalates, and BPA. These kits involve an immobilized antigen bound to an antibody against the target. The target is quantified by a signal decrease upon competition with the labeled analyte. Immunoassays are sensitive but require multiple steps and longer incubation times than are practical for real-time monitoring. The majority of analytical methods are impractical for real-time monitoring of trace concentrations in the field, and few provide biological insight into the modes of action of these chemicals.

Electrochemical detection of endocrine disruptors.—Electrochemical detection methods are rapid, sensitive, and require small sample volumes. Additionally, electrochemical sensors are readily integrated into small, portable devices, enabling their point-of-exposure deployment. Many are compatible with complex background matrices, reducing the need for sample pre-treatment and simplifying sampling protocols. Because of the versatility of such sensors, many strategies have been adopted for electrochemical EDC detection. Electroactive EDCs, including xenoestrogens and phthalates, can be detected directly (Figure 1b). Electrochemically-inactive EDCs, including PCBs and PFAS, can be monitored by changes to electroactive species that are affected by their presence. Biorecognition with an antibody, aptamer, or hormone receptor integrated into an electrochemical sensor facilitates specific detection and can provide insight into biological modes of action of the EDC. The majority of recent advances in electrochemical detection of EDCs have relied on novel nanostructuring of materials to enhance selectivity for specific analytes and enable lower detection limits at times approaching biological relevance. A lesser-used strategy is to use the bioactivity of endocrine disrupting compounds to monitor the total biological activity of a sample, the readout of interest for most point-of-care monitoring. Below we discuss analyte-specific and activity-based approaches for the most prevalent EDCs and argue that the field is poised to further develop the latter technologies with the potential to vastly improve public health monitoring.
BPA and other phenolic xenoestrogens.—BPA is a component of many plastics and resins often used for food storage, making it especially problematic as an EDC. It is also electroactive, undergoing a 2-electron, 2-proton oxidation, which can be directly monitored. However, direct detection of BPA by oxidation is limited by electrode fouling and a high overpotential. Consequently, many sensors incorporate carbon nanotubes (CNTs), graphene, or metal nanoparticles (NPs) to increase the electroactive surface area and lower the overpotential. Selectivity is improved by attaching a recognition element to the electrode, most often molecularly imprinted polymers (MIP) or aptamers. Fouling can also be reduced by simply adding surfactants to the electrode, most often molecularly imprinted polymers (MIP) or potential. Selectivity is improved by attaching a recognition element (NPs) to increase the electroactive surface area and lower the overpotential. We developed an electrochemical aptasensor based on a BPA-specific DNA aptamer. This sensor’s linear range (0.1 to 100 ppm) fails to meet the guidelines established by regulatory agencies.

Table I. Sensitivities of electrochemical endocrine disruptor detection strategies as compared to minimum allowable containment levels by regulatory agencies.

| Sensor Design | Analyte | Linear range | Detection limit |
|---------------|---------|--------------|----------------|
| Minn. EPA drinking water standard<sup>38</sup> | BPA | 20 ppb = 87 nM |
| Aptamer/Au<sup>32</sup> | BPA | 10 nM – 5 μM | 10 nM |
| Tyr/PVA-SbQ/CFP<sup>35</sup> | BPA | 0.1 – 1 μM | 5 nM |
| Laccase/thionine/CB/SPCE<sup>36</sup> | BPA | 0.5 – 50 μM | 0.2 μM |
| CYP2C9/PA/GCE<sup>37</sup> | BPA | 1.25 – 10 μM | 0.58 μM |
| Biosensor<sup>46</sup> | Total estrogenic activity | 500 pM – 10 μM | NR* |
| US EPA MCR<sup>49</sup> | DEHP | 0.006 ppm |
| MIP-Pa/Au<sup>49</sup> | DEHP | 0.1 – 100 ppm |
| US EPA MCR<sup>49</sup> | PCBs | 0.5 ppb ~ 1–2 nM |
| βCDP/thGO/PP/PGIE<sup>43</sup> | PCBs | 1 pM – 10 μM | 0.5 pM |
| DNA/Cds/TSQ, PECS<sup>44</sup> | PCB77 | 0.3 – 340 pM | 0.3 pM |
| Ab/graphite/SPE<sup>45</sup> | PCBs | 0.1 – 50 ppm | 200 ppb |
| US EPA health advisory limit<sup>40</sup> | PFAS | 70 ng/L |
| Bubble nucleation<sup>47</sup> | PFOS, PFOA | 0.1 mg/L – 10 g/L | 30 μg/L |

See text for full description of sensors designs.

*NR – not reported.

Phthalates.—Phthalates are broadly-used small-molecule plasticizers. These compounds are so pervasive that the Centers for Disease Control (CDC) found phthalate metabolic products in urine from the majority of Americans tested, making detection and monitoring of these compounds vital. Phthalates can be directly reduced at negative potentials, which necessitates the use of mercury or silver amalgam electrodes. The application of these electrode materials can be impractical for real-world detection. Thus, more practical platforms have been developed, especially for the most common phthalate plasticizer (di(2-ethylhexyl)phthalate (DEHP)). DEHP was detected by frequency response analysis (FRA) using a thin film interdigitated electrode. Selectivity was imparted by molecularly imprinting DEHP in a polycrylamide coating. Despite this selective design, the sensor’s linear range (0.1 to 100 ppm) fails to meet the guidelines for real-world detection.
Contaminants, and animal studies increasingly implicate them in the formation of perfluorooctanoic acid (PFOA) and Perfluorooctane sulfonic acid (PFOS) on the EPA list of emerging pollutants. This area is ripe for the development of portable, specific, and sensitive sensors. Electroanalytical applications. Recent work by Luo et al. exploited the surfactant properties of these compounds to detect PFOS and PFOA at concentrations of 0.01 μg/L. The presence of a surfactant affects electrochemical bubble nucleation by reducing the surface tension of the gas-liquid interface, thereby decreasing the gas supersaturation level required for a bubble to form. PFAS concentrations were determined by a decrease in the bubble nucleation barrier, as measured by changes in peak current in HClO₄ solution. This method was robust to the presence of PEG at 1000-fold excess, lysozyme, and humic acid, and the platform exhibited a wide linear range of 0.1 ng/L–10 g/L. However, the system required pre-concentration to meet the EPA drinking water guideline of 70 ng/L. Measurements depend on the surfactant activity of each compound, yielding native LODs of 72 and 160 nM for PFOA and PFOS, respectively. Because the dangers of PFAS have only recently emerged, effective point-of-exposure detection strategies remain extremely limited. Additionally, the mechanism of action and biological target of these compounds remain unclear, emphasizing the importance of platforms that can elucidate these pathways by developing sensors based on the likely human hormone receptor targets themselves. The unreliability of these compounds demands creative strategies for detection at trace levels.

Figure 1. Electrochemical detection strategies for endocrine disrupting compounds (EDCs). a) Common EDCs. b) BPA can be detected through the direct electrochemical oxidation of its phenolic groups. c) A competition-based immunoassay for PCBs. An enzyme-linked PCB (green with gold star) is pre-bound to surface. Following sample exposure, the enzyme-linked signal decreases based upon the PCB concentration in the analyte solution. d) A sandwich assay based on bacterial binding to an electrode is used to detect EDCs. E. coli modified with a mammalian estrogen receptor only bind to gold electrodes modified with a monobody in the presence of xenoestrogen pollutants. This assay is a measure of the bioactivity of an analyte solution rather than the concentration of a particular EDC.

Polychlorinated biphenyls (PCBs) are used in many items with which we come into regular contact, including lubricants, plasticizers, and components of electrical systems. PCBs are not inherently electrochemically active. Thus, detection often relies on using these chemicals to perturb the interaction of an electroactive molecule with an electrode. In a targeted approach, a photoelectrochemical (PEC) aptasensor was developed for one member of the PCB family, PCB77, using an aptamer attached to N-doped TiO₂ nanotubes (NT). Single-stranded DNA modified with CdS quantum dots (DNA-CdS) was initially hybridized to the aptamer. Upon addition of PCB77, the DNA-CdS was displaced, resulting in a signal decrease. Though this aptasensor is sensitive, the ability to detect only a single compound can be limiting for environmental monitoring where bioactivity may be more relevant than targeted analyte detection. As an alternative strategy, several methods have been developed to detect PCBs as a class of chemicals without differentiating between specific PCBs. In one example, PCBs displaced a guest molecule in a host-guest complex. An electroactive ferrocene acted as the guest molecule to be displaced in a host-guest complex with beta-cyclodextrin that was attached to a modified pyrolytic graphite electrode. PCBs were indirectly measured by the loss of a ferrocene signal, as determined by DPV. The sensor exhibited selectivity for PCBs in the presence of other EDCs. The detection limit, 0.5 ppm, is well below the EPA’s 2001 enforceable MCL, and PCB concentrations at the MCL were detected in lake sediments, demonstrating the utility of the sensor for rapid screening of environmental samples. A more recent example of a beta-cyclodextrin-based sensor for planar PCBs demonstrated that other redox indicators such as methylene blue can be effectively used, although the detection limits were orders of magnitude higher than the previous sample. Similarly, an immunosen sor was designed to detect PCBs in complex matrices, again without differentiation between specific chemicals. Screen-printed electrodes were modified with PCB-specific antibodies pre-bound with enzyme-labeled PCB. Upon analyte binding, enzyme-PCB was competed off the electrode (Figure 1e). Importantly, this sensor is sensitive to a group of chemicals (PCBs) in complex solutions including food matrices, without requiring independent monitoring of each unique chemical. The reported linear range of detection was 0.01–50 ppm in standard solutions, with successful detection at the tolerance levels established by the European commission. These platforms represent a key improvement in the electrochemical detection of EDCs because they can detect families of compounds from complex solutions, rather than individual chemicals.

Receptor-based bioactivity sensor for xenoestrogens.—Many chemically dissimilar compounds act on a single hormone pathway. Thus, monitoring concentrations of single analytes can be less important than determining the total bioactivity of a mixture for a particular pathway. We recently reported an electrochemical biosensor for the class of chemically dissimilar molecules that interfere with native estrogen signaling (xenoestrogens) by binding to the human estrogen receptor beta. The biosensor was based on the aptamer of the xenoestrogen 17β-estradiol that was hybridized to N-doped TiO₂ nanotubes (NT). Single-stranded DNA modified with CdS quantum dots (DNA-CdS) was initially hybridized to the aptamer. Upon addition of 17β-estradiol, the DNA-CdS was displaced, resulting in a signal decrease. Through this aptasensor is sensitive, the ability to detect only a single compound can be limiting for environmental monitoring where bioactivity may be more relevant than targeted analyte detection. As an alternative strategy, several methods have been developed to detect PCBs as a class of chemicals without differentiating between specific PCBs. In one example, PCBs displaced a guest molecule in a host-guest complex. An electroactive ferrocene acted as the guest molecule to be displaced in a host-guest complex with beta-cyclodextrin that was attached to a modified pyrolytic graphite electrode. PCBs were indirectly measured by the loss of a ferrocene signal, as determined by DPV. The sensor exhibited selectivity for PCBs in the presence of other EDCs. The detection limit, 0.5 ppm, is well below the EPA’s 2001 enforceable MCL, and PCB concentrations at the MCL were detected in lake sediments, demonstrating the utility of the sensor for rapid screening of environmental samples. A more recent example of a beta-cyclodextrin-based sensor for planar PCBs demonstrated that other redox indicators such as methylene blue can be effectively used, although the detection limits were orders of magnitude higher than the previous sample. Similarly, an immunosen sor was designed to detect PCBs in complex matrices, again without differentiation between specific chemicals. Screen-printed electrodes were modified with PCB-specific antibodies pre-bound with enzyme-labeled PCB. Upon analyte binding, enzyme-PCB was competed off the electrode (Figure 1e). Importantly, this sensor is sensitive to a group of chemicals (PCBs) in complex solutions including food matrices, without requiring independent monitoring of each unique chemical. The reported linear range of detection was 0.01–50 ppm in standard solutions, with successful detection at the tolerance levels established by the European commission. These platforms represent a key improvement in the electrochemical detection of EDCs because they can detect families of compounds from complex solutions, rather than individual chemicals.
receptor ERs (Figure 1d). The platform is based on an electrochemical sandwich assay, with one half being ERs expressed on the surface of E. coli. The second half is a small, fibronectin-based monobody immobilized on an electrode that binds to ERs only in the presence of a xenoestrogen. This sensor successfully detected sub-pb levels of the native hormone, estradiol, as well as the activity of nM concentrations of combinations of xenoestrogens. Because the sensor reports on the total bioactivity of a solution, combinations of chemically dissimilar molecules were quantifiable over five orders of magnitude of concentration. Importantly, this feature also enabled the determination of the bioactivity of an unknown compound; significant estrogenic activity was detected from a microwaved BPA-free baby bottle, equivalent to 100 nM estradiol. As the sensor was developed on disposable electrodes, it is low-cost and portable. The E. coli-bound hormone receptor is most stable when the cells on which it is expressed are lyophilized. Those E. coli are stable when stored frozen for at least six months, which would enable storage and transport of these sensors for point-of-exposure monitoring. As regulatory agencies move away from simple quantification to studying the bioactivity of pollutants and analyzing environmental samples for unknown targets, assays based on bioactivity will become increasingly important.

Future Needs and Prospects

As the list of prevalent chemicals that act on native hormone pathways increases daily, the need for novel and creative detection strategies becomes ever more pressing. EDC detection comes with specific challenges not associated with other groups of small molecules, as they are classified based on their biological activity rather than their chemical similarity. Disparate structures that all target a single biological pathway pose challenges for conventional detection strategies, and further complications arise because EDC monitoring is so varied and application-dependent. Monitoring ranges from batch or continuous monitoring of environmental samples, in-home monitoring of consumer products, fundamental studies of the biological targets of EDCs, to, most challenging, evaluating whether EDCs are present without knowing the specific compounds present.

Moving forward, three key areas necessitate significant efforts in sensor development for EDCs. Electrochemical sensors are ideally suited to provide improvements in all of these areas, including: 1) environmental and in-home monitoring of specific compounds, 2) monitoring the bioactivity of polluted systems with potentially unknown contaminants, and 3) elucidation of the modes of action of emerging contaminants.

Improved sensors for point-of-exposure EDC detection are key, especially as contamination occurs in such a broad spectrum of locations, from public lands to private households. EDCs are increasingly found in household products, from bisphenols in kitchen plastics and the lining of aluminum cans to PCBs in household electronics. With the omnipresence of these chemicals comes a need for in-home testing. Thus, developing sensors for existing contaminants that are amenable to commercialization is an area primed for significant growth in the next few years.

Similarly, there are limited examples of strategies for effective, sensitive, inexpensive sensors for newly-emerging contaminants, including phthalates and PFAS. In the case of phthalates, their use in so many common products points to their pervasiveness as environmental pollutants. However, current strategies to monitor these compounds remain limited. Portable, inexpensive sensors will be invaluable for remediation efforts, especially as governments and businesses increase these efforts. Current detection strategies are insufficient for the broad implementation that is required to detect these compounds and elucidate their biological modes of action. As direct electrochemical detection of these classes of compounds is either impractical (phthalates) or impossible (PFAS), electrochemical strategies will require additional creativity, and the bioactivity of these compounds suggests that biomolecules can be successfully exploited in sensor development. With the rate at which industrial compounds are found to be endocrine disruptors, we expect new targets to emerge in the future that will similarly require unique sensors for environmental monitoring.

As it is increasingly apparent that we do not know all of the chemicals in our environment that may act as endocrine disruptors, sensors based on bioactivity rather than specific concentrations of chemicals become imperative. Though monitoring specific pollutants will always be important, complementary strategies that do not require knowledge of the components of a polluted system are equally important. Expanding on the electrochemical sandwich assay developed for estrogenic compounds will be an important thrust in the next few years. Developing equivalent systems for xeno-testosterone and chemicals that target the thyroid hormone receptor PPARγ will be the next steps in the development of a screening platform based on a bioactivity panel for human hormone receptors. Similarly, as many of these compounds have been found to also target the brain, it is vital to develop sensors that report on the neurological activity of EDCs. Because electrochemical platforms are modular and readily multiplexed, they are ideal for the development of such screens.

Finally, as researchers continue to develop new compounds that could interfere with native hormonal signaling, platforms to elucidate their mode of action will be essential. In the near future, such platforms will illuminate the biological targets of compounds that have only recently been identified as problematic, such as PFAS. We envision such electrochemical sensors being similar in nature to those described above for monitoring total bioactivity. However, as these sensors will likely be limited to laboratory settings, more complicated protein targets can be incorporated for screening. Long term, this technology will be essential as a pre-screen for industrial and pharmaceutical compounds prior to their broad implementation to ensure new compounds have no adverse effects on endocrine systems.

Overall, environmental monitoring of endocrine disruptors is a rapidly growing field. Electrochemical sensors offer low cost, versatility, portability, and modularity unavailable with other analytical methods. As creative methods to ensure biologically-relevant sensing improves, electrochemical sensors for EDCs will move to the forefront of detection strategies.

Conclusions

Endocrine disrupting compounds pose major threats to human health, and monitoring them with knowledge of their biological targets is key for efficient containment. Electrochemical sensors exist for many of these compounds, with both direct and biorecognition-based detection finding success. However, as the number of newly-identified endocrine disruptors is constantly increasing, there is a pressing need for sensors to: 1) directly detect specific chemical contaminants in point-of-exposure settings, 2) determine the total bioactivity of a combination of compounds based on their bio-interactions, negating the need for knowledge of the specific components, and 3) elucidate modes of action of newly-identified endocrine disruptors and evaluate the potential biological implications of new industrial chemicals. Better electrochemical sensors will be vital to the success of these efforts. Technological advances to address each need will significantly improve environmental monitoring and enable remediation.

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