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RESEARCH ARTICLE

# Integrated Monitoring of Endocrine-Disrupting Chemicals: Analytical Chemistry and Biological Assessment Approaches

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**Abstract:** Endocrine-Disrupting Chemicals (EDCs) in aquatic environments pose significant threats to ecosystems and human health. These compounds interfere with hormonal regulation at low concentrations and have non-monotonic dose-response relationships, posing challenges to conventional risk assessment. This review systematically compares current EDC monitoring methods, including instrumental chemical analysis and receptor-based biological detection systems, to evaluate sensitivity thresholds, sample throughput, and mechanistic selectivity of different analytical platforms. The detection limit of the target compound by liquid chromatography-tandem mass spectrometry is 0.05-5 ng/L, and the estrogenic activity detected by reporter gene assays (such as ER-CALUX) is 0.01-0.05 ng EEQ/L. This analysis demonstrates that neither of these methods can provide complete information for risk evaluation alone. In addition, a hierarchical assessment framework that combines chemical quantification with effect-based biological evaluation is presented. These technologies such as aptamer-functionalized biosensors, omics profiling, and adverse outcome pathway modeling provide promising directions. This integrated approach can conduct comprehensive EDC monitoring and solve the problem of known contaminants and uncharacterized bioactive substances in environmental samples.

**Keywords:** Endocrine Disruptors, Environmental Monitoring, Bioanalytical Methods, Effect-Based Assessment, Mass Spectrometry, Adverse Outcome Pathways

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## Introduction

The endocrine system coordinates important biological processes, including energy metabolism, bone formation, reproductive function, and behavioral regulation. However, this complex signal network is easy to interference from Endocrine-Disrupting Chemicals (EDCs) (substances that mimic, block, or interfere with the activity of natural hormones). Since early investigations in the 1990s established the link between environmental estrogen contamination and reproductive abnormalities in aquatic wildlife [1, 2], EDCs have received high attention. A decisive characteristic of EDCs is their ability to induce measurable biological changes at extremely low concentrations, with phenomena such as vitellogenin induction in male fish and decreased human quality observed at exposure levels of nanograms per liter [3]. In addition, many EDCs show

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non-monotonic dose-response patterns, where moderate concentrations of biological responses cannot be reliably predicted from higher dose observations, posing a challenge to established safety threshold determination [4].

Due to the extreme structural diversity of these compounds, EDC monitoring strategies face significant limitations. This heterogeneous group includes naturally occurring steroid hormones, artificially manufactured pharmaceutical estrogens, industrial plasticizers, brominated flame retardants, and agricultural chemicals [5]. This chemical heterogeneity means no single sample preparation protocol or detection instrument can efficiently address the complete range of EDCs in environmental assessment. Traditional targeted chemical analysis can provide concentration data, but not a deep understanding of biological activity. This is because compounds at the same mass concentration may exhibit several orders of magnitude receptor binding abilities. Moreover, targeted methods cannot address the complexity of mixtures exposed to actual environments, where the combined effects of multiple compounds may exceed predictions based on individual substance potencies [6, 7]. Although effect-based biological methods can detect total endocrine activity, they cannot determine the specific causative agents that cause the observed effects.

This review provides a systematic and comprehensive examination of monitoring methods suitable for EDC assessment, uniquely combining instrumental analytical methods and biological effect detection platforms within a unified evaluation framework. Unlike previous reviews that only focus on analytical chemistry or bioassay techniques, we provide quantitative comparison of sensitivity thresholds, sample processing capacity, and mechanistic selectivity of different analytical methods. We propose a hierarchical monitoring architecture directly applicable to regulatory monitoring programs and discuss technological innovations, including aptamer-functionalized sensing platforms, systems-level omics technologies, and conceptual models of adverse outcome pathway. Our contribution addresses the critical gap between chemical identification and biological relevance assessment, providing practical guidance for comprehensive EDC monitoring strategies.

This review has three main contributions:

(a) a systematic cross-platform comparison of sensitivity, throughput, and mechanistic selectivity between chemical and biological EDC detection methods

(b) a quantitative EBT-governed tiered monitoring framework directly applicable to regulatory programs; and

(c) identification of critical research gaps with prioritized recommendations for advancing integrated monitoring capabilities

## Materials and Methods

### Literature Search Strategy

This review was conducted based on a systematic literature search scheme. We searched the Web of Science, PubMed, and Scopus databases using keywords such as "endocrine disruptors," "bioassay," "mass spectrometry," and "environmental monitoring".

### Inclusion and Exclusion Criteria

Inclusion criteria include:

(1) Peer-reviewed articles published between 2019-2024

(2) Researches on quantitative detection limits or performance metrics

(3) Methodological papers describing validated analytical procedures. Exclusion criteria include conference abstracts, non-English publications, and studies without quantitative performance data. The timeframe from 2019 to2024 was selected to capture the latest technological breakthroughs in two key areas

(4) The rapid development of High-Resolution Mass Spectrometry (HRMS) instrumentation and non-target screening workflows that have greatly expanded the ability to identify unknown compounds

(5) Significant progress in bioassay standardization, including the development of internationally harmonized protocols (OECD Test Guidelines 455 and 458), the establishment of Effect-Based Trigger Values (EBTs), and

the increasing regulatory acceptance of effect-based methods in water quality frameworks (such as the EU Water Framework Directive)

### Data Extraction and Analysis

A total of 156 articles were initially identified, with 78 meeting inclusion criteria after screening. Data extraction focused on detection limits, dynamic ranges, sample throughput, and regulatory acceptance status.

## Results

### Mechanistic Basis and Environmental Distribution of EDCs

#### Molecular Targets and Modes of Action

The nuclear receptor family, including ligand-responsive transcription factors that control gene expression after hormonal stimulation, represents the most thoroughly studied category of EDC targets. Key members include estrogen receptors (ERα and ERβ), Androgen Receptor (AR), and thyroid hormone receptors. They can be inappropriately activated or blocked by EDCs, leading to abnormal cellular responses [1].

From a mechanistic perspective, EDCs may act as receptor activators, simulating the effects of natural hormones, or as a receptor blocker, preventing endogenous ligands from binding. In addition to these direct receptor interactions, EDCs can also disrupt hormonal balance through additional pathways: inhibiting enzymes involved in steroid synthesis (especially aromatase), displacing hormones from carrier proteins in blood, accelerating hormone breakdown in the liver, and disrupting feedback communication between the hypothalamus and pituitary gland [5].

Table 1 shows a systematic classification of major EDC categories according to their molecular targets and associated biological consequences.

Abbreviations: ER, estrogen receptor; AR, androgen receptor; TR, thyroid receptor; GR, glucocorticoid receptor; TPO, thyroid peroxidase; NIS, sodium-iodide symporter; E2, 17β-estradiol; EE2, 17α-ethinylestradiol; BPA, bisphenol A; NP, nonylphenol; YES, Yeast Estrogen Screen; YAS, Yeast Androgen Screen; CALUX, Chemical Activated LUciferase gene eXpression; ER-CALUX, Estrogen Responsive-CALUX; AR-CALUX, Androgen Responsive-CALUX.

The mechanistic diversity in Table 1 shows important patterns of monitoring design. The estrogen receptor-mediated disruption remains the most extensively studied pathway. Thyroid and glucocorticoid pathways, despite their increasing relevance to the environment, have received less attention. Compounds targeting the same receptor may exhibit different potency. For example, the estrogenic potency of 17α-ethinylestradiolis about 10,000-fold higher than that of bisphenol A. This change explains why only relying on chemical concentration data cannot predict biological effects, highlighting the necessity of integrating effect-based assessment methods.

**Table 1: Classification of endocrine-disrupting chemicals by mechanism and target**

| Disruption Mechanisms | Molecular Targets | Representative Compounds | Documented Effects | Key Bioassays |
|---|---|---|---|---|
| ER agonism | ERα, ERβ | E2, EE2, BPA, NP, Genistein | Vitellogenin induction, feminization, intersex | YES, ER-CALUX, E-Screen |
| ER antagonism | ERα, ERβ | Tamoxifen, Fulvestrant | Impaired estrogen-dependent processes | ER-CALUX (antagonist mode) |
| AR agonism | AR | Testosterone, Trenbolone | Masculinization, precocious maturation | YAS, AR-CALUX |
| AR antagonism | AR | Vinclozolin, Flutamide, p,p'-DDE | Demasculinization, cryptorchidism | AR-CALUX (antagonist mode) |
| Aromatase inhibition | CYP19A1 | Fadrozole, Tributyltin, Prochloraz | Masculinization, skewed sex ratios | H295R assay |
| Thyroid disruption | TR, TPO, NIS | Perchlorate, PCBs, PBDEs, Triclosan | Altered metamorphosis, neurodevelopmental deficits | T-Screen, TR-CALUX |
| GR modulation | GR | Dexamethasone, Budesonide | Immunosuppression, metabolic effects | GR-CALUX |
| Steroidogenesis perturbation | Multiple CYPs | Ketoconazole, Phthalates | Altered hormone profiles | H295R assay |

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## Environmental Distribution Patterns

EDCs enter aquatic systems through different routes, reflecting their different anthropogenic sources. Wastewater treatment facilities are important point sources as standard biological treatment processes can only partially removal many EDCs. Agricultural activities contribute the secretion of hormones by livestock and recorded endocrine activity of pesticides. The manufacturing process releases plasticizers, surface-active agented composition products and flame retardants into the receiving water [8, 9].

Table 2 shows the concentration ranges reported for priority EDCs indifferent environments.

**Table 2: Environmental occurrence of priority EDCs in matrices**

| Compound Class | Representative Analyte | River Water (ng/L) | WWTP Effluent (ng/L) | Sediment (ng/g dw) |
|---|---|---|---|---|
| Natural estrogens | Estrone | 0.5-47 | 3-120 | 0.2-15 |
| | 17β-Estradiol | 0.3-18 | 1-64 | 0.1-8 |
| Synthetic estrogens | 17α-Ethinylestradiol | <0.1-8.5 | 0.2-24 | <0.05-2 |
| Bisphenols | Bisphenol A | 5-1,200 | 25-6,800 | 2-680 |
| Alkylphenols | 4-Nonylphenol | 15-12,000 | 85-58,000 | 25-15,000 |
| Phthalates | DEHP | 80-65,000 | 250-180,000 | 150-72,000 |
| Flame retardants | BDE-47 | 0.01-25 | 0.05-85 | 0.5-450 |
| Pesticides | Atrazine | 2-6,500 | 15-1,800 | 1-180 |

Abbreviations: WWTP, wastewater treatment plant; dw, dry weight; DEHP, di(2-ethylhexyl) phthalate; BDE, brominated diphenyl ether.

The concentration ranges in Table 2 demonstrate significant variability. This change reflects the source proximity, physicochemical properties of compounds, and removal efficiency in wastewater treatment. Hydrophobic compounds (DEHP, BDE-47) preferentially accumulate in sediments, and polar substances remain in aqueous phases. These distribution patterns indicate that sediment sampling may be more suitable for hydrophobic EDCs. Water sampling remains crucial for polar compounds with direct relevance to aquatic exposure. These data are consistent with global monitoring results, confirming that these ranges represent watersheds of global urbanization.

## Instrumental Chemical Analysis

### Sample Preparation Approaches

Accurate EDC measurement requires attention to sample handling procedures. Solid-Phase Extraction (SPE) has become a main technique for processing water samples, with the advantages of reduced organic solvent consumption and suitability for a wide range of polarity analytes. Adsorption materials with balanced hydrophilic and lipophilic properties can retain multiple types of compounds [10].

For solid environmental matrices, pressurized solvent extraction provides efficient analyte recovery while minimizing the use of solvent. The Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) approach has been widely used to extract multiple EDC classes from complex matrices due to its simple implementation and favorable cost profile [11].

Table 3 provides a comparative overview of extraction techniques applicable to EDC analysis.

**Table 3: Extraction methodologies for EDC isolation**

| Technique | Applicable Matrices | Recovery Range | Solvent Use | Processing Time | Automation |
|---|---|---|---|---|---|
| SPE | Water, biological fluids | 65-125% | 5-25 mL | 30-90 min | High |
| LLE | Water, simple matrices | 55-110% | 50-300 mL | 30-60 min | Limited |
| SPME | Water, headspace | 45-95% | Solvent-free | 30-120 min | High |
| PLE | Sediments, soils, tissues | 70-115% | 15-60 mL | 15-45 min | High |
| QuEChERS | Soils, sediments, biota | 65-120% | 10-25 mL | 15-30 min | Moderate |

Abbreviations: SPE, solid-phase extraction; LLE, liquid-liquid extraction; SPME, solid-phase microextraction; PLE, pressurized liquid extraction.

Table 3 reveals a trade-off between recovery efficiency, solvent consumption, and automation potential. Solid-phase extraction achieves an optimal balance of aqueous samples, explaining its main regulatory adoption. QuEChERS provides practical advantages for resource-limited settings due to minimal equipment requirements and rapid processing. Compared to the methods ten years ago, current approaches have reduced solvent consumption by 80-90% while maintaining recovery efficiency. This progress reflects environmental sustainability and analytical optimization.

## Chromatographic Separation and Mass Spectrometric Detection

Liquid chromatography tandem mass spectrometry (LC-MS/MS) has emerged as the main platform for EDC quantification in environmental samples. This combination of chromatographic separation with highly selective mass detection has achieved excellent sensitivity, with conventional detection capabilities extending to sub-nanogram-per-liter concentrations [12].

Gas chromatography-mass spectrometry maintains correlation with volatile compound classes, especially legacy persistent pollutants. High-Resolution Mass Spectrometry (HRMS) instruments (including quadrupole time-of-flight and Orbitrap analyzers) greatly expand the ability to identify previously unrecognized EDCs through suspect and non-targeted screening workflows [13].

Table 4 compares the characteristics of instrumental platforms used for EDC determination.

Abbreviations: QqQ, triple quadrupole; QTOF, quadrupole time-of-flight; MRM, multiple reaction monitoring; ESI, electrospray ionization; EI, electron ionization; POPs, persistent organic pollutants.

**Table 4: Instrumental platforms for EDC analysis**

| Platforms | Ionization | Mass Resolution | Detection Limits | Identification Confidence | Optimal Applications |
|---|---|---|---|---|---|
| LC-QqQ (MRM) | ESI (+/−) | Unit mass | 0.05- ng/L | Level 1 (with standards) | Regulatory compliance, routine monitoring |
| LC-QTOF | ESI (+/−) | 15,000-40,000 | 0.5-0 ng/L | Level 2-3 | Suspect screening, metabolite ID |
| LC-Orbitrap | ESI (+/−) | 60,000-500,000 | 0.5-20 ng/L | Level 2-3 | Non-target screening |
| GC-QqQ (MRM) | EI | Unit mass | 0.1-0 ng/L | Level 1 (with standards) | POPs, volatile EDCs |
| GC-HRMS | EI | 10,000-120,000 | 0.5-5 ng/L | Level 2-3 | Unknown identification |

The platform comparison reveals a fundamental trade-off between sensitivity and identification. LC-QqQ achieves the lowest detection limits (0.05-5 ng/L) with Level 1 identification confidence, making it the best choice for regulatory compliance monitoring. However, despite a slightly higher detection limit, high-resolution platforms such as QTOF and Orbitrap are able to identify unknown compounds through precise mass measurements. The practical implication is that comprehensive monitoring programs should deploy complementary platforms. LC QqQ is the best choice for regulatory quantification, while LC-HRMS can conduct regular screening to identify newly emerging pollutants that need to be added to targeted methods.

An important analytical challenge in LC-MS/MS-based EDC analysis is the matrix effect. That is, the co-extracted sample components change the ionization efficiency of target analytes in the Electrospray Ionization (ESI) source, resulting in signal suppression or enhancement. In complex environmental matrices such as wastewater effluent and sediment extracts, matrix effects can cause quantification errors exceeding 50%, seriously affecting data accuracy. The use of stable isotope-labeled internal standards (such as $^{13}C$-labeled or deuterated analogs) represents the gold standard for matrix effect compensation. This is because these compounds co-elute with their natural analogues and experience the same ionization, providing accurate correction for extraction recovery and signal variability. For regulatory EDC monitoring programs requiring high quantitative accuracy, we strongly recommend adding isotopically labeled internal standards to all target analytes when commercially available.

High-Resolution Mass Spectrometry (HRMS) platforms are particularly suitable for discovering unknown EDC transformation products. Unlike triple Quadrupole (QqQ) instruments operating in Multiple Reaction Monitoring (MRM) mode, HRMS platforms (QTOF, Orbitrap) can simultaneously obtain full-scan accurate mass data for all ionizable compounds, while MRM mode is essentially limited to pre-defined target analyte lists. This feature supports three powerful analysis strategies:

(1) Suspected screening of the database for predicted transformation products can be conducted without reference standards

(2) Non-target screening identifies completely unknown compounds through accurate mass measurement and fragmentation interpretation

(3) A retrospective analysis of the archived data when new compounds of concern emerge. Given that many environmental degradation transformation products still retain or enhance endocrine activity, these capabilities are particularly valuable. For example, [14] demonstrated that HRMS-based suspect and non-target screening identified numerous micropollutants in stormwater runoff that would have been completely missed by traditional targeted methods

## Effect-Based Biological Assessment

### Rationale for Biological Detection Approaches

The effect-based approach has significant advantages in dealing with all substances that may interact with the biological target of interest, including transformation products and novel pollutants that do not exist in the target chemical method. These methods automatically consider the reaction conditions of all active mixed ingredients and provide direct information on their biological efficacy, rather than just reflecting the chemical components [15].

Regulatory frameworks have increasingly incorporated bioanalytical methods, and European institutions have set effect-based trigger values for key indicators. Switzerland has incorporated conventional biometric methods into its national water quality monitoring [7].

Different bioanalytical platforms have different monitoring purposes based on their characteristics. Simple in vitro reporter gene assays (such as yeast-based YES/YAS systems) are most suitable for rapid and high-throughput Tier-1 screening of large environmental samples. The advantages include low costs, robust growth, minimal biosafety requirements, and suitability for resource-limited laboratories. These assays are highly suitable for a wide range of monitoring projects, with the main goal of labeling samples with elevated endocrine activity for further investigation. Mammalian cell-based assays (such as ER-CALUX, AR-CALUX) are more appropriate when enhanced sensitivity and metabolic relevance are required. Due to their superior endogenous metabolic enzyme system, mammalian cell lines are particularly suitable for detecting pro-estrogens that require metabolic bioactivation. These assays are recommended for regulatory-grade assessments and for use where quantitative Effect-Based Trigger value (EBT) comparisons are required. Whole-organism models (such as fish embryo tests and amphibian metamorphosis assays) are still indispensable for mechanistic research and in vivo toxicity confirmation. They integrate the processes of Absorption, Distribution, Metabolism, And Excretion (ADME) that are lacking in cell-based systems, providing ecologically significant endpoints for risk assessment.

Biological methods and instrumental chemical analysis are highly complementary components in a comprehensive monitoring strategy, rather than competing alternatives. Bioassays are like an "active compass" that can comprehensively assess the overall potential risk of endocrine disruption in complex environmental mixtures. It includes unknown compounds, transformation products, and the effects of mixture interactions that cannot be predicted solely through chemical analysis. Chemical analysis is a “compound identifier”: when significant biological activity is detected by bioassays, targeted LC-MS/MS analysis is subsequently deployed to identify and quantify the specific chemical agents responsible for the observed effects. When significant biological activity is detected in a biological assay, targeted LC-MS/MS analysis is subsequently deployed to identify and quantify specific chemical reagents that contribute to the observed effects. This complementary workflow ensures that monitoring programs achieve both comprehensive hazard detection (through bioassays) and precise chemical identification (through LC-MS/MS), forming the conceptual foundation of the tiered assessment framework proposed in this review. As demonstrated by [16], this integrated approach has been successfully adopted by water quality managers and regulators in Europe and Australia.

## Reporter Gene Detection Systems

Reporter gene assays utilize genetically modified cells that express hormone receptors linked to easily measured reporter genes. When receptors are activated by EDCs in test samples, reporter gene expression produces quantifiable signals proportional to total endocrine activity.

Yeast-based platforms (including the Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS) assays) provide practical benefits such as robust growth characteristics and the absence of interfering endogenous receptors. Mammalian cell systems (such as the Estrogen Responsive Chemical Activated LUciferase gene eXpression (ER-CALUX) and Androgen Responsive - Chemical Activated LUciferase gene eXpression (AR-CALUX)) provide greater physiological relevance and enhanced detection sensitivity [17, 18].

Table 5 compares the performance attributes of reporter gene assay platforms.

**Table 5: Reporter gene assay systems for EDC detection**

| Assay | Host Cell | Target Receptor | Detection Threshold | Dynamic Range | Duration | Standardization |
|---|---|---|---|---|---|---|
| YES | S. cerevisiae | Human ERα | 0.1-0.3 ng EEQ/L | 3-4 orders | 48-72 h | ISO 19040-1 |
| YAS | S. cerevisiae | Human AR | 1-3 ng AEQ/L | 3 orders | 48-72 h | Limited |
| ER-CALUX | T47D (human) | Human ERα | 0.01-0.05 ng EEQ/L | 4-5 orders | 24 h | OECD TG 455 |
| AR-CALUX | U2OS (human) | Human AR | 0.03-0.1 ng AEQ/L | 4 orders | 24 h | OECD TG 458 |
| GR-CALUX | U2OS (human) | Human GR | 0.05-0.2 ng DEQ/L | 4 orders | 24 h | Limited |
| TR-CALUX | GH3 (rat) | Rat TRβ | 0.5-2 ng T3EQ/L | 3 orders | 24-48 h | Limited |

Abbreviations: EEQ, estradiol equivalents; AEQ, androgen equivalents; DEQ, dexamethasone equivalents; T3EQ, T3 equivalents; OECD TG, OECD Test Guideline.

Mammalian cell-based systems (ER-CALUX) achieve 3-10-fold lower detection thresholds than yeast-based alternatives (YES), reflecting the optimization of receptor expression. However, yeast assays have practical advantages of robust growth, lower biosafety requirements, and reduced costs. There is also a significant difference in the duration of assay: CALUX is completed within 24 hours, and yeast systems require 48-72 hours. Regulatory acceptance remains a key factor. ER-CALUX and AR-CALUX have been validated by OECD (TG 455, 458). In contrast, the lack of equal standardization in thyroid and glucocorticoid testing limits their current regulatory applicability.

An important biological difference between these two platform types is related to metabolic capacity. Mammalian cell lines (such as T47D cells used in ER-CALUX and U2OS cells used in AR-CALUX) have an endogenous Cytochrome P450 (CYP) metabolic enzyme system that enables biotransformation reactions for the bioactivation of xenobiotic compounds. This metabolic ability is critical for detecting pro-estrogens, which refers to compounds that have no biological activity in their maternal form but require metabolic activation to bind to estrogen receptors. For example, the pesticide methoxychlor requires CYP-mediated O-demethylation to form its estrogenically active metabolite HPTE. In contrast, yeast cells (Saccharomyces cerevisiae) used for YES and YAS assays lack most mammalian metabolic enzymes, which may underestimate the estrogenic potential of samples containing pro-estrogens. Consequently, for regulatory-grade assessments or suspected samples containing pro-estrogens, mammalian cell-based assays should be prioritized. Yeast-based assays are still valuable for initial broad screening considering cost and throughput as the main factors.

## Whole-Organism Testing Approaches

Living organism bioassays can capture comprehensive physiological responses, including absorption, tissue distribution, metabolic transformation, and excretion processes-factors that do not exist in cell-based systems. Fish development assays can detect the induction of vitellogenin and the change in gender. The amphibian metamorphosis experiments identify substances that may interfere with thyroid function by observing the changes in development time [19, 20].

## Effect-Based Trigger Values

Converting bioassay measurements into regulatory guidance requires establishing effect-based trigger values (EBTs)-activity thresholds indicating potential risk. For estrogenic activity in surface water, the recommended thresholds are typically in the range of 0.1-0.5 ng/L, expressed as estradiol equivalents [21]. Table 6 shows the proposed EBTs in multiple biological endpoints.

**Table 6: Effect-based trigger values for EDC monitoring**

| Endpoint | Bioassay | Matrix | Proposed EBT | Unit | Regulatory Context |
|---|---|---|---|---|---|
| Estrogenicity | ER-CALUX | Surface water | 0.1-0.5 | ng EEQ/L | EU WFD watch list |
| Estrogenicity | YES | Surface water | 0.2-1.0 | ng EEQ/L | Swiss monitoring |
| Estrogenicity | ER-CALUX | Drinking water | 0.1 | ng EEQ/L | Dutch guidance |
| Androgenicity | AR-CALUX | Surface water | 1-25 | ng DHT-EQ/L | Research proposal |
| Anti-androgenicity | AR-CALUX | Surface water | 5-50 | μg Flu-EQ/L | Research proposal |
| Glucocorticoid activity | GR-CALUX | Surface water | 10-100 | ng DEX-EQ/L | Research proposal |
| Thyroid agonism | TR-CALUX | Surface water | 10 | ng T3-EQ/L | Research proposal |

Abbreviations: EBT, effect-based trigger value; EEQ, estradiol equivalents; DHT-EQ, dihydrotestosterone equivalents; Flu-EQ, flutamide equivalents; DEX-EQ, dexamethasone equivalents; T3-EQ, triiodothyronine equivalents; WFD, Water Framework Directive.

The proposed EBTs for estrogenicity (0.1-0.5 ng EEQ/L) are derived from ecotoxicological data, which linked bioassay responses to adverse consequences for fish populations. The differences under different regulatory backgrounds reflect different protection objectives. The drinking water standard adopts more conservative thresholds than surface water monitoring. EBTs for non-estrogenic endpoints are still uncertain, representing a significant gap. The EBT framework represents a paradigm shift from compound-specific limits to comprehensive mixture evaluation, inherently accounting for additive effects from multiple compounds.

## Emerging Technologies

### Biosensor Platforms

Biosensor technologies combine biological recognition components with portable signal transduction systems to achieve on-site detection capabilities. Aptamer-based sensors have the advantages of chemical stability and regeneration potential. Using nanomaterials such as gold nanoparticles and graphene derivatives to improve signals allows us to detect substances at levels that matter for environmental monitoring [22, 23].

## Systems-Level Omics Technologies

Transcriptomic and metabolomic can comprehensively characterize the biological responses after EDC exposure. Specific gene expression characteristics of estrogen or androgen exposure have been established for model fish. Metabolomics captures downstream biochemical changes that reflect the functional consequences of EDC effects [24].

## Adverse Outcome Pathway Conceptual Framework

The Adverse Outcome Pathway (AOP) establishes a mechanistic link between the initiating events at the molecular level and the adverse consequences at the biological level related to regulatory decisions. Well-developed AOPs for EDCs include pathways linking estrogen receptor activation to reproductive impairment, as well as pathways linking aromatase inhibition to population-level sex ratio changes. This framework guides the selection of biological assays and supports extrapolation from cellular observations to predicted organism-level consequences [25, 26]. Ultimately, AOPs provide a key mechanism for translating high-throughput in vitro bioassay results into ecologically relevant in vivo risk assessments, laying a scientific and robust foundation for regulatory decision-making on EDC management in aquatic environments [16].

## Integrated Surveillance Strategies

### Tiered Assessment Architecture

Only chemical analysis or biological testing alone cannot provide complete information for EDC risk evaluation. An effective monitoring plan deploys these two methods in a complementary manner within a structured hierarchical framework [27].

Table 7 shows the components of a tiered assessment approach.

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**Table 7: Tiered framework for integrated EDC surveillance**

| Tier | Objective | Methods | Decision Criteria | Resource Intensity |
|---|---|---|---|---|
| 1: Screening | Identify samples with endocrine activity | Broad-spectrum bioassays (ER-CALUX, AR-CALUX) | Activity < EBT → No action; > EBT → Tier 2 | Low-Moderate |
| 2: Characterization | Quantify priority EDCs | Targeted LC-MS/MS; expanded bioassay panel | Chemicals explain activity → Risk assessment; Unexplained → Tier 3 | Moderate |
| 3: Identification | Identify unknown compounds | Effect-directed analysis; HRMS screening | Candidates identified → Tier 4 | High |
| 4: Confirmation | Verify identity and activity | Authentic standard confirmation | Confirmed → Update monitoring lists | Moderate-High |
| 5: Risk Integration | Evaluate risk; inform management | Exposure modeling; AOP integration | Risk assessment and recommendations | High |

Abbreviations: EBT, effect-based trigger value; HRMS, high-resolution mass spectrometry; AOP, adverse outcome pathway.

The tiered framework addresses the challenge of balancing comprehensive coverage with resource constraints. Tier 1 bioassay screening efficiently prioritizes the samples that require detailed investigation, with about70% of samples being resolved without further analysis. The decision criterion (Activity > EBT → Tier 2) provides objective and reproducible rules for routine monitoring. Tier 3 effect-directed analysis requires 2-4 weeks per sample and is appropriately reserved for samples of particular concern. Compared with previously proposed frameworks, our architecture explicitly incorporates adverse outcome pathway integration as a final synthesis step. This supplement enables the results to be translated into risk management recommendations for mechanical anchoring.

Specifically, the transition from Tier 1 to Tier 2 is controlled by a quantitative Effect-Based Trigger values (EBTs): only samples with measured bioactivity exceeding the predefined EBT thresholds (such as 0.1- 0.5 ng EEQ/L for estrogenicity in Table 6) can be subjected to Tier 2 for targeted LC-MS/MS quantification. This decision criterion controlled by EBT ensures objective and repeated priority, while avoiding unnecessary analysis resource expenditure on samples with minimal endocrine-related risks.

Figure 1 shows a flowchart of an integrated monitoring framework, illustrating the complete workflow from sample collection to data integration. The framework includes five sequential steps: Step 1: Environmental sample collection and pretreatment (SPE for water samples; QuEChERS or PLE for sediment/biota, with field blanks and procedural blanks for quality control); Step 2 : Parallel deployment of two analytical streams (Stream A: targeted LC-QqQ quantification followed by non-targeted HRMS screening; Stream B: in vitro reporter gene assays followed by in vivo whole-organism testing if necessary); Step 3 : Decision node controlled by EBTs (samples below EBTs are classified as low concern and returned to routine surveillance; samples exceeding EBTs will enter to Tier 2); Step 4: Bioactivity gap assessment (if targeted chemicals fully explain the observed bioactivity, compound-specific risk evaluation will be conducted; if significant bioactivity gap persists, HRMS will be upgraded to Tier 3 effect-directed analysis); and Step 5: Data integration and comprehensive ecological risk assessment, incorporating toxic unit calculations and AOP-anchored risk characterization [16].

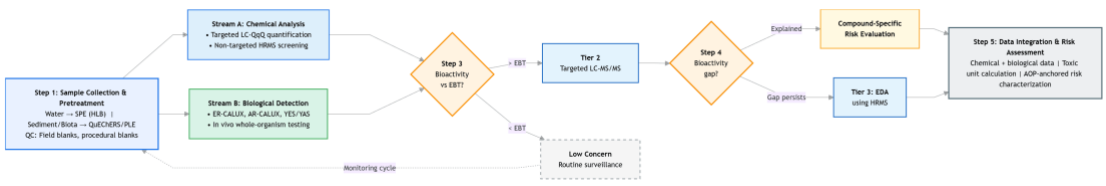


**Fig. 1: Integrated monitoring framework for endocrine-disrupting chemicals in aquatic environments**

Rectangles represent analytical processes; diamonds represent decision nodes; arrows indicate workflow direction. EBT, effect-based trigger value; EDA, effect-directed analysis; HRMS, high-resolution mass spectrometry; AOP, adverse outcome pathway.

### Effect-Directed Analysis

Effect oriented Analysis (EDA) uses iterative sample grading, biological activity testing of the obtained fractions, and high-resolution mass spectrometry identification of active ingredients. This method has successfully identified previously unrecognized EDCs, including transformation products and industrial chemicals not previously associated with endocrine activity [28].

## Discussion

### Outstanding Challenges

There are several factors that limit the progress of EDC surveillance capabilities. Understanding these challenges is essential for designing effective monitoring strategies.

Mixture interactions: Concentration addition models provide a reasonable prediction for EDCs to act through a shared receptor mechanism, but the interactions between compounds operating through different pathways are difficult to accurately predict. This limitation is due to the fact that current bioassays target a single receptor endpoint, and organisms undergo activation through multiple pathways simultaneously. Studies have shown that in complex environmental samples, mixture effects may deviate from additivity predictions by factors of 2-5.

This challenge is further compounded by the ‘cocktail effect’, where multiple EDCs with concentrations lower than their single unobserved effect concentrations (NOECs) can collectively trigger significant joint biological responses through increased concentration. Environmental monitoring studies have shown that the cumulative estrogenic activity of complex water samples often exceeds predictions based on individually quantified compounds. Unexplained ‘bioactivity gap’ are often attributable to the additive effects of unidentified mixture components. This phenomenon highlights the indispensable role of effect-based biological methods, which inherently integrate the combined effects of all bioactive compounds in a sample, whether they are individually identified or present above detection limits.

Transformation products: Parent EDCs undergo environmental and biological transformation to produce products that may retain or even enhance endocrine activity. For example, chlorination of bisphenol A during water treatment produces chlorinated derivatives with up to 10times estrogenic potency. Comprehensive monitoring must address these through non-targeted analytical approaches and biological activity screening.

Cellular to organism extrapolation: Cell-based bioassays do not directly translate into the impact on the entire organism. This limitation is due to the lack of consideration for Absorption, Distribution, Metabolism, and Excretion (ADME) processes in cell-based systems. The equivalent concentrations obtained from biological assays may overestimate or underestimate the in vivo effects by 1-2 orders of magnitude, depending on compound bioavailability. Physiologically-based pharmacokinetic modeling methods are being developed to address this gap.

Method standardization: More extensive regulatory implementation requires harmonized protocols, validated reference materials, and proficiency testing programs. The inter-laboratory variability for bioassay measurements ranges is 30-50% CV, and that for established chemical methods is <20% CV. Reducing this variability is crucial for regulatory acceptance.

### Priority Research Directions

Based on the comparison of monitoring approaches, we identify the following priority research directions:

(1) Mixture assessment advancement: Systematic evaluation of mixture interactions requires mechanistic modeling support. The integration of adverse outcome pathway networks provides a promising framework for predicting cross-pathway interactions

(2) Transformation product discovery: The combination of non-targeted high-resolution mass spectrometry with effect-directed analysis can identify bioactive transformation products. Establishing a spectral database of common EDC metabolites will accelerate identification workflows

(3) In vitro-to-in vivo extrapolation: The development of pharmacokinetic modeling based on physiology should focus on high priority compounds that exhibit significant differences in biological assays and biological level data

(4) Method standardization: The development of international guidelines for other biological endpoints (thyroid, glucocorticoids) should follow the successful OECD TG 455/458 model. The CV between target laboratories should be reduced to<30% within 5 years

(5) Predictive toxicology: The computational methods, including QSAR modeling and read-across methods, provide potential for active hazard identification before widespread environmental contamination occurs. The computational methods, including QSAR modeling and cross method approaches, provide potential for active hazard identification before widespread environmental pollution occurs

(6) Economic evaluation standardization: Future studies proposing a comprehensive monitoring framework should incorporate standardized cost-benefit analysis, systematically address capital costs, operational expense, sample throughput, and additional risk-relevant information obtained from integrated and single approaches methods. A unified economic reporting format will facilitate direct comparison between studies and support evidence-based decision-making by regulatory agencies with limited resources

## Limitations

This review has several limitations:

(1) Literature search is limited to English publications only. This may exclude relevant studies published in other languages, especially from regions with significant EDC contamination concerns such as China, Japan, and European countries with non-English scientific literature

(2) The search timeframe (2019-2024) is to capture recent methodological advances, but this approach may have excluded foundational studies and historically important validation data from earlier periods that remain relevant to current practice

(3) The heterogeneity of research reporting formats limits direct quantitative comparison of some performance parameters. In particular, the report on detection limits uses different conventions (method detection limit, quantification limit, and instrument detection limit), making cross-study comparisons more complex

(4) Cost-effectiveness analysis is constrained by inconsistent economic data reports in various studies. Equipment costs, consumable expenses, and labor requirements were rarely reported in standardized formats. This inconsistency limited our ability to provide comprehensive economic guidance for monitoring program design

(5) This review mainly focuses on aquatic environmental matrices (water, sediment, and wastewater discharge). Our conclusion may have limited applicability to other matrices such as air, soil, food, and biological tissues, which may be limited and need to be evaluated separately

(6) The rapid development of mass spectrometry and biometric platform technology means that some emerging methods may not be fully reflected. These novel methods may not have yet appeared in the peer-reviewed literature captured by our search strategy

# Conclusion

This review systematically evaluated the monitoring methods for endocrine-disrupting chemicals, revealing complementary strengths between chemical and biological methods. Our analysis has the following key findings:

(1) Detection capabilities: LC-MS/MS achieves detection limits of 0.05-5 ng/L for targeted EDCs, and ER-CALUX bioassays detect total estrogenic activity at 0.01-0.05 ng EEQ/L. Both of them provide enough sensitivity for detecting EDCs at environmentally relevant concentrations associated with adverse ecological effects

(2) Method applicability: Instrumental analysis provides the specificity and quantitative accuracy (CV <15%) required for regulatory compliance verification, and effect-based methods capture the biological relevance of complex exposures, including mixture effects and unknown bioactive substances. Neither of these methods is sufficient to be used alone

(3) Framework contribution: We propose a five-tier integrated assessment architecture that deploys broad-spectrum bioassays for preliminary screening (resolving about 70% of samples), targeted LC-MS/MS for priority compound quantification, and effect-directed analysis with HRMS for identifying unknown active substances

(4) Regulatory application: The proposed effect-based trigger values (0.1-0.5 ng EEQ/L for estrogenicity in surface water) provide actionable thresholds directly applicable to monitoring program decision-making, as demonstrated in Swiss national monitoring implementation

The priority areas for continued development include:

(a) improving EBTs for androgenic and thyroid endpoints

(b) expanding the bioassay protocols validated by the OECD

(c) improving mixture interaction prediction; and (d) developing field-deployable biosensor

Combining precise chemical quantification with effect driven biological assessment within the hierarchical framework is the most promising approach for achieving comprehensive EDC monitoring to protect ecosystems and human health. The proposed comprehensive framework provides an immediately implementable and scientifically robust comprehensive EDC monitoring strategy for environmental monitoring agencies, which involves known pollutants and unidentified bioactive substances.

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## Authors Contributions

Changjin Li and Zijun Dou: Investigation and writing original draft.
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Peng Cao: Conceptualization, methodology, writing original draft, writing review and editing, supervision, funding acquisition.
Yongbing Zhang: Writing original draft, writing review and editing, supervision.

## Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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