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Making waves: toward systems-level interpretation of hormonal and endogenous biomarkers in wastewater-based epidemiology

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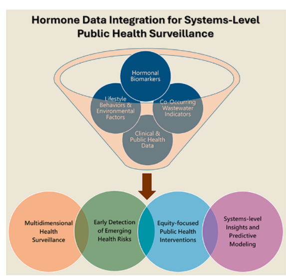
HIGHLIGHTS

- Hormone-based WBE expands wastewater surveillance beyond external exposure tracking toward interpretation of population-level physiological responses.
- Hormonal biomarkers in wastewater are highly context-dependent and require integration with clinical, environmental, behavioral, and co-occurring wastewater indicators for reliable interpretation.
- A systems-level and ethically governed framework is essential for advancing hormone-based WBE into a scientifically robust, context-aware, and actionable public health surveillance tool.

GRAPHICAL ABSTRACT



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ABSTRACT

Wastewater-based epidemiology (WBE) has proven invaluable for population health monitoring, most notably during the COVID-19 pandemic. Yet current WBE largely relies on exogenous markers such as drugs, pathogens, and their metabolites, limiting surveillance to what communities are exposed to. We argue for expanding WBE towards endogenous biomarkers, particularly hormones, which provide insights into physiological stress, metabolic function, and endocrine activity. Hormone-based WBE offers new opportunities to capture population-level biological responses to societal and environmental stressors, disasters, and chronic disease burdens at the community scale. This perspective outlines a systems-level framework for integrating hormonal signals in wastewater with clinical data, behavioral indicators, environmental factors, and digital markers to support more robust and context-aware public health surveillance. We highlight key technical considerations, interpretive challenges, and opportunities for translational pilot studies. By moving beyond exposure tracking toward more integrated interpretation of biological responses, hormone-informed WBE may contribute to more resilient, inclusive, and actionable public health infrastructure.

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1. Introduction

Wastewater-based epidemiology (WBE) has rapidly evolved into a powerful tool for population-level health surveillance (Juneja et al., 2023; Kumblathan et al., 2021; Zhu et al., 2025). It captures chemical and biological signals excreted in human waste, enabling a real-time, community-wide perspective on public health in a non-invasive and anonymized manner (Carneiro et al., 2025). WBE has been widely applied to monitor illicit drug use, assess pharmaceutical consumption, and, most prominently, track infectious disease outbreaks (Carmo dos Santos et al., 2024; Erickson et al., 2021; Huizer et al., 2021; Osena et al., 2025). During the COVID-19 pandemic, wastewater monitoring networks were rapidly scaled worldwide, demonstrating the ability of WBE to provide early warning signals, high-resolution temporal trends, and equitable population coverage beyond what clinical testing alone could achieve (Acosta et al., 2025; Chen et al., 2024; Pang et al., 2022). Despite these successes, the scope of WBE has remained largely focused on markers that reflect external exposures, primarily capturing what populations consume or encounter rather than how they physiologically respond.

In parallel, emerging public health challenges are increasingly shaped not only by exposure to external agents but also by how populations physiologically respond to stressors such as mental health burdens, chronic disease, and environmental pressures, responses that are not fully captured by traditional WBE markers. Some studies have recently explored the possibility of monitoring endogenous compounds, such as steroid hormones and metabolic indicators, as potential biomarkers in wastewater systems to reflect population-level physiological responses (Chen et al., 2014; Driver et al., 2022; Elliss et al., 2025; Gao et al., 2023; Shao et al., 2025; Thai et al., 2019). As interest in endogenous biomarkers has expanded, recent studies have also begun moving toward broader multi-biomarker and systems-oriented wastewater surveillance approaches that integrate endogenous indicators with wider health and environmental datasets, reflecting growing recognition that interpretation of population-level physiological signals requires greater contextualization and integration (Kasprzyk-Hordern et al., 2025). However, their application remains limited by multiple factors, including analyte instability, transformation and degradation during sewer transport, challenges in interpretation within complex wastewater matrices, as well as contributions from other sources such as pharmaceutical use, dietary inputs, and system-level processes that complicate source attribution and quantitative interpretation (Gao et al., 2023; Shao et al., 2025; Thai et al., 2019). In practice, prior efforts have largely remained focused on individual endogenous markers rather than broader public health applications. This highlights the need for approaches that move beyond single-analyte interpretation toward more integrative, context-aware frameworks.

To address this need, we outline a systems-level framework for wastewater surveillance of endogenous biomarkers, in which these signals are integrated with multiple complementary data streams to improve interpretation, reduce uncertainty, and enable more reliable population-level health insights. We use hormones as a key illustrative example of endogenous markers, given their role as central regulators of stress, metabolism, and endocrine function and their potential to provide informative signals of population-level physiological responses. Their continuous regulation of key biological processes enables them to reflect dynamic changes in health, behavior, and environmental adaptation. Hormones such as cortisol, estrogens, testosterone, insulin-related compounds, and thyroid hormones are excreted in urine and feces and therefore enter wastewater systems (Combalbert and Hernandez-Raquet, 2010; Grzegorzek et al., 2024).

Within this perspective, hormonal signals are considered in conjunction with co-occurring wastewater indicators, clinical data, environmental conditions, and behavioral metrics to provide a more robust and context-aware basis for interpretation. This approach moves beyond single-analyte analysis toward a multi-dimensional framework that enables more reliable insights into population-level health dynamics. By embedding hormonal data within this integrated system, challenges related to biomarker stability, signal variability, and multiple contributing pathways can be better contextualized and addressed. In this way, hormonal signals are not treated as standalone indicators but as components of a broader surveillance framework. We outline the analytical and interpretive considerations required for implementation, highlight opportunities for data integration, and identify key challenges that must be addressed to support practical applications.

2. From exposure to response: why hormones matter

Tracking exogenous markers alone in WBE does not capture the full picture of population health, as communities exposed to similar external stressors may exhibit markedly different physiological responses shaped by factors such as baseline health, resilience, nutrition, and chronic disease (Bagby et al., 2019). In this context, hormones, key regulators of stress, metabolism, and endocrine function, offer a means to capture aspects of these internal biological responses at the population scale (Axelrod and Reisine, 1984; Ranabir and Reetu, 2011; Tao and Cheng, 2023). When interpreted alongside conventional exposure markers, hormonal signals can extend WBE beyond documenting exposures to provide insight into population-level responses. However, hormonal signals in wastewater should not be interpreted as direct or one-to-one representations of physiological state. Instead, they reflect context-dependent signals that require careful interpretation within a broader analytical framework.

i. **Cortisol as a population stress barometer.** Cortisol is a well-established stress hormone that responds to psychological and physiological pressures (Knezevic et al., 2023). Wastewater measurements of cortisol may provide insight into collective stress dynamics following societal, economic, or environmental disruptions. However, previous studies have shown that steroid hormones may undergo transformation and degradation in wastewater systems, and cortisol levels can exhibit temporal variability (e.g., circadian patterns), which must be considered in sampling design and interpretation (Gao et al., 2023; Shao et al., 2025; Thai et al., 2019). When interpreted within a broader context, cortisol signals may still offer useful insights into population-level stress trends.

ii. **Thyroid hormones as indicators of metabolic and environmental stress.** Thyroxine (T_4) and triiodothyronine (T_3) regulate metabolism and respond to environmental and nutritional conditions (Babić Leko et al., 2021; Shahid et al., 2025; Yang et al., 2025). Wastewater measurements of these hormones may reflect population-level responses to factors such as temperature changes or pollutant exposure. However, as with other hormonal biomarkers, their interpretation requires consideration of stability, transformation, and potential variability within wastewater systems.

iii. **Insulin analogs as indicators of metabolic disease.** Insulin and its analogs are widely used in the management of diabetes and are linked to metabolic regulation (Sanlioglu et al., 2013). Wastewater detection of insulin-related compounds may provide information on community-level metabolic health patterns. However, insulin presents important interpretive challenges due to limited urinary excretion, potential contributions from treatment-related inputs, and signal variability within wastewater systems (Boogaerts et al., 2021; Hanefeld et al., 2014). As a result, insulin-related measurements are better interpreted as contextual indicators of metabolic health and treatment burden rather than direct representations of endogenous physiological activity.

3. Analytical and technical foundations

Realizing the potential of hormone-based wastewater epidemiology requires moving from conceptual vision to robust analytical and interpretive frameworks. While established analytical methods enable the detection of hormones at trace levels, their successful application in WBE depends not only on measurement sensitivity but also on biomarker stability, transformation, and interpretability within complex.

i. **Analytical Platforms.** Liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) remains the gold standard for hormone quantification in wastewater (Cavalier et al., 2024; Wang et al., 2024). Its high sensitivity enables the detection of diverse hormone classes, including glucocorticoids, sex hormones, thyroid hormones, and insulin-related compounds, often at concentrations below 10 ng/L. Combined with optimized solid-phase extraction (SPE), LC–MS/MS achieves high recovery for both free and conjugated forms (Antonelli et al., 2023). However, analytical sensitivity alone does not ensure reliable interpretation. Variability across laboratories in sample preservation, extraction protocols, and calibration approaches can limit comparability across studies. Moreover, distinguishing between different chemical forms, such as conjugated versus unconjugated hormones or natural versus synthetic analogs, may be necessary to better understand the origin and transformation of signals.
ii. **Sampling Dynamics.** Hormonal signals in wastewater are inherently dynamic, reflecting temporal variability in both human physiology and wastewater system conditions. For example, cortisol follows a circadian rhythm, with peak levels occurring in the morning and declining throughout the day, while acute stress events such as heatwaves at the community level or examination periods on university campuses may induce transient spikes (Andreadi et al., 2025). In addition, wastewater system factors such as flow variability, dilution, and sewer residence time can further influence observed concentrations. Grab sampling or infrequent sampling strategies may therefore fail to capture meaningful temporal patterns or may introduce bias. More representative approaches, such as 24-hour composite sampling, passive samplers, or high-frequency autosampling, are better suited to capturing temporal variability and providing more reliable estimates of population-level signals.
iii. **Biomarker Stability and Sewer Processes.** A fundamental requirement for hormone-based wastewater epidemiology is understanding how biomarkers behave between excretion and measurement. During sewer transport, hormones and related compounds are subject to physicochemical and biological processes, including microbial degradation, enzymatic transformation, temperature-dependent reactions, and interactions with suspended solids (Chen et al., 2014; Driver et al., 2022; Gao et al., 2023; Thai et al., 2019). These processes can alter both the chemical form and concentration of target analytes. For example, steroid hormones may undergo biodegradation or transformation, while conjugated forms can be deconjugated during sewer transit, modifying the measurable signal (Gao et al., 2023; Wang et al., 2024). The extent of these processes is influenced by system-specific factors such as hydraulic residence time, wastewater composition, microbial activity, and environmental conditions. As a result, the persistence and detectability of hormonal biomarkers are not solely intrinsic properties of the compounds but are also shaped by the characteristics of the wastewater system itself. Understanding these transformation pathways and system dynamics is therefore essential for evaluating biomarker behavior prior to measurement and for designing sampling strategies that more accurately capture population-level signals.
iv. **Normalization Approaches.** Interpreting hormone concentrations in wastewater requires normalization to account for dilution, population dynamics, and system variability. Flow-based normalization provides a baseline correction; however, in systems with transient or variable inputs, flow data alone may be insufficient. In addition, variability in biomarker origin and transformation can further complicate interpretation, particularly for compounds influenced by multiple contributing pathways. Human-specific biomarkers, such as creatinine, ammonium, or mitochondrial DNA, can serve as co-markers of population size and stability (Hutchison et al., 2022). A combined normalization approach incorporating both flow and human biomarkers can improve comparability across sites and overtime. Importantly, hormonal signals should be interpreted as context-dependent indicators rather than direct proxies of physiological state. Integrating hormone data with complementary information, such as co-occurring wastewater indicators, clinical data, environmental conditions, and behavioral metrics, provides the necessary context to reduce ambiguity and improve interpretive reliability.

4. Toward systems-level surveillance

Hormones in wastewater provide valuable physiological signals; however, when interpreted in isolation, they may be subject to ambiguity due to variability, transformation, and multiple contributing pathways. For example, a change in cortisol levels may reflect true population-level stress, temporal variability, or system-level influences (Thorn et al., 2009). This inherent uncertainty highlights the limitations of single-analyte interpretation and emphasizes the need for more context-aware approaches. To address these challenges, hormone-based WBE should be framed within an integrated, systems-level approach (Fig. 1). Rather than relying on individual biomarkers as direct indicators of physiological state, this framework emphasizes the interpretation of hormonal signals in conjunction with complementary data streams, transforming hormone-based WBE from a single-measurement paradigm into a multi-dimensional public health tool.

i. **Integration with Clinical and Public Health Data.** Hormonal signals in wastewater gain interpretive strength when evaluated alongside clinical and population-level data. For example, trends in cortisol may be examined in relation to hospital admissions for anxiety, mental health service utilization, or socioeconomic indicators. Similarly, insulin-related signals may be interpreted in the context of diabetes prevalence or treatment patterns. This integration is particularly important given the potential variability and indirect nature of hormonal signals in wastewater. Aligning wastewater measurements with independent clinical datasets helps validate observed trends and reduces uncertainty associated with interpretation.
ii. **Integration with Co-Occurring Wastewater Indicators.** Hormones can be analyzed alongside co-occurring chemical and microbial markers to provide a more comprehensive understanding of population health. For instance, cortisol trends may be evaluated in conjunction with antidepressant metabolites or substance-use indicators, while reproductive hormones may be interpreted alongside endocrine-disrupting compounds. Combining multiple wastewater indicators enables cross-validation of signals and helps distinguish physiological responses from potential confounding influences. This multi-marker approach strengthens interpretive confidence compared to relying on individual biomarkers alone.
iii. **Integration with Lifestyle and Behavioral Markers.** Behavioral patterns influence both hormone dynamics and wastewater composition. Indicators such as caffeine, alcohol metabolites, and dietary markers can provide context for interpreting hormonal

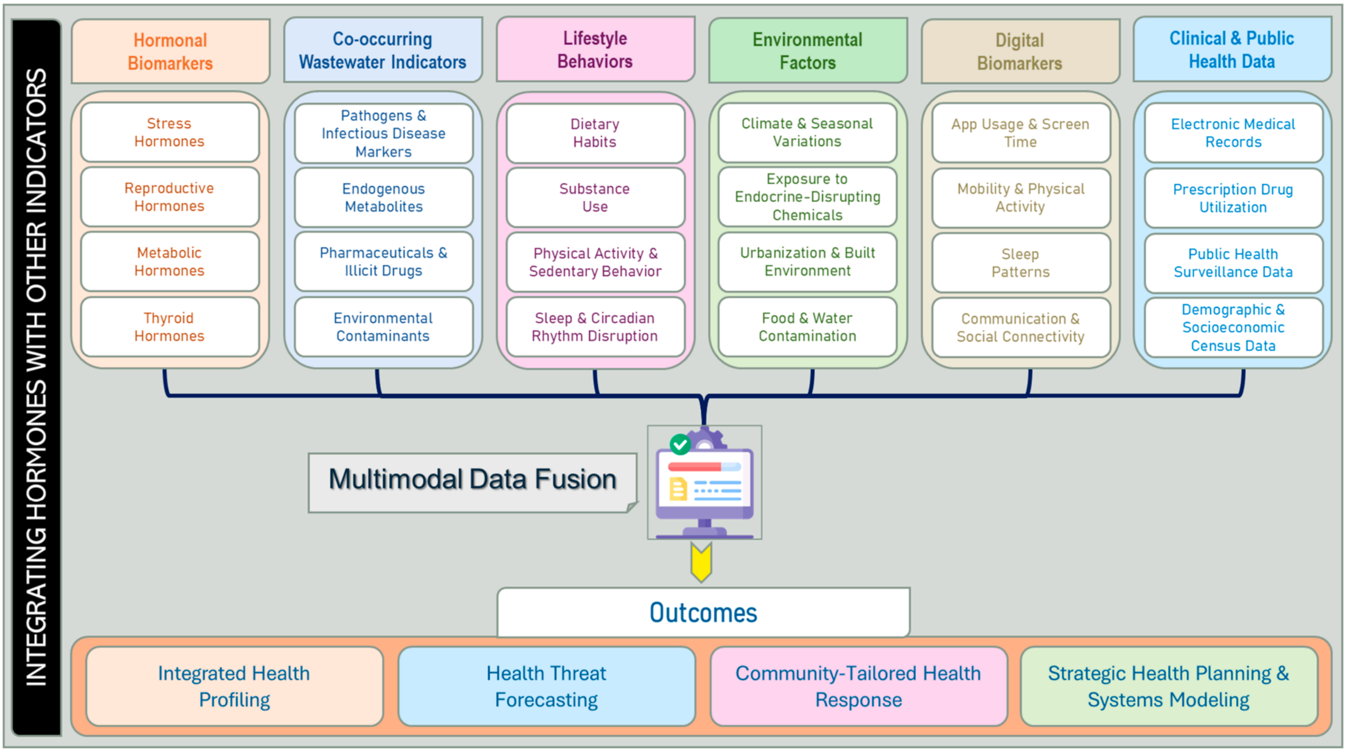

Fig. 1. Integrated hormone data framework for systems-level public health surveillance. Hormonal biomarkers, when combined with clinical data, co-occurring wastewater indicators, and lifestyle/environmental factors, support multi-dimensional health monitoring, early risk detection, equity-focused interventions, and predictive modeling.

signals (Sisti et al., 2015). For example, changes in cortisol levels observed alongside caffeine consumption patterns or sleep-related behavioral indicators may reflect shifts in daily routines rather than acute stress responses. Incorporating behavioral context helps disentangle overlapping influences and improves interpretation.

iv. **Integration with Environmental Factors.** Hormonal responses are influenced by environmental conditions such as temperature, air quality, and pollutant exposure (Çalıyurt, 2022; Rony and Alamgir, 2023; Street et al., 2024). These factors may affect both physiological responses and wastewater system dynamics. For example, elevated temperatures during heatwaves may increase physiological stress responses (e.g., cortisol levels) while also accelerating microbial activity and temperature-dependent degradation within sewer systems (Silva et al., 2019). Integrating environmental data with hormonal measurements therefore enables differentiation between true physiological responses to external stressors and apparent changes driven by system-level factors such as dilution, temperature, or transformation processes.

v. **Integration with Digital Markers.** Digital data streams, including mobility patterns, smartphone activity, and aggregated social indicators, provide additional context for interpreting population-level trends behavior (Mary Mammen et al., 2025; Trivedi et al., 2021; Zakaria et al., 2023, 2022). These data can offer a temporal and behavioral context that complements wastewater measurements. For example, changes in mobility or sleep-related activity may support interpretation of hormonal trends, helping to distinguish between behavioral shifts and physiological stress responses (Höhn et al., 2021).

Emerging studies are beginning to demonstrate aspects of such integrative approaches. Recent multi-biomarker wastewater surveillance efforts have combined endogenous and exogenous indicators to evaluate broader dimensions of community health and well-being (Elliss et al., 2025; Kasprzyk-Hordern et al., 2025). Similarly, studies evaluating glucocorticoids and other endogenous biomarkers in wastewater systems have highlighted both the potential and interpretive complexity of hormone-based WBE approaches (Driver et al., 2022; Shao et al., 2025). Although fully integrated operational systems remain limited, these studies illustrate the growing movement toward more comprehensive, systems-oriented, and context-aware WBE applications.

Taken together, these data streams form an integrated surveillance framework in which hormonal signals represent one component of a broader system. Within this context, hormones are not interpreted as standalone indicators but as part of a network of complementary signals that collectively inform population-level health assessment. This systems-level approach is particularly important given the known limitations of hormonal biomarkers, including variability, potential transformation, and multiple contributing pathways. By embedding hormone data within a multi-dimensional framework, these limitations can be addressed through cross-validation, contextualization, and integration with independent data sources. In this framework, hormone-based WBE evolves from a single-measurement concept into a context-aware, integrative tool capable of supporting more reliable, equitable, and actionable public health insights.

5. From insight to intervention: translational opportunities

The value of hormone-based wastewater epidemiology lies not only in detecting physiological signals but also in translating these signals into informed and timely public health insights (Table 1). However, translating these signals into actionable interventions requires careful interpretation within a broader analytical framework that accounts for system complexity and uncertainty. When integrated with complementary data streams, hormone-based WBE has the potential to support earlier detection of emerging health trends, improved resource targeting, and more adaptive public health strategies.

Table 1
Translational scenarios and ethical safeguards for hormone-based wastewater epidemiology (WBE). Representative applications showing how hormones in wastewater can inform timely, equitable public-health responses.

Application Domain	Target Hormone(s)	Public-Health Insight	Example Setting	Potential Intervention	Ethical / Governance Safeguards
Mental Health & Community Stress	Cortisol	Provides an early indicator of collective psychological stress, anxiety, or burnout across populations	Urban neighborhoods, schools, or workplace campuses	Deploy targeted mental-health outreach, counseling, and stress-mitigation programs	Ensure aggregate, anonymized reporting; obtain community consent; establish transparent communication channels
Metabolic Disease Surveillance	Insulin analogs, Thyroid hormones	Reveals shifts in diabetes or metabolic disease burden through wastewater signatures of treatment use or endocrine activity	Municipal wastewater networks in diverse socioeconomic regions	Support early public-health interventions, dietary initiatives, and preventive screenings	Maintain population-level data aggregation; protect health privacy; ensure cross-sector data governance
Reproductive Health & Equity	Estrogens, Testosterone	Tracks community-level reproductive hormone trends as indicators of contraceptive access, fertility patterns, or endocrine health	City or regional monitoring programs	Guide equitable distribution of reproductive health resources and policy evaluation	Avoid small-catchment targeting; implement demographic neutrality; involve community advisory oversight

Temporal Stress Trend Analysis. Cortisol, a primary stress-related hormone, may enable temporal and spatial analysisof population-level stress dynamics (Dziurkowska and Wesolowski, 2021). Wastewater-based monitoring of cortisol may help identify broader temporal patterns and relative changes associated with societal, environmental, or institutional stressors when interpreted over appropriate timescales and within a broader contextual framework. For example, in academic settings, longitudinal cortisol trends observed across exam periods, semester transitions, or post-crisis recovery windows may provide insight into changing population-level stress dynamics when evaluated alongside behavioral and institutional indicators. Similarly, at the community scale, temporal hormone patterns may help contextualize responses to environmental stressors, economic disruptions, or public health events. However, interpretation of cortisol signals requires careful consideration of temporal variability, including circadian rhythms, as well as system-related influences such as sampling design, population mixing, and biomarker transformation during sewer transport. As a result, hormone-based WBE is currently more suitable for evaluating broader temporal trends and relative changes over time. In addition, logistical and cost considerations may limit the feasibility of high-frequency hormone monitoring as a standalone surveillance strategy. Accordingly, the interpretive value of cortisol measurements is likely to be greatest when integrated with complementary temporal, behavioral, and public-health datasets within a broader systems-level framework.

i. **Chronic Disease Surveillance.** Hormone-related compounds associated with metabolic function, including insulin-related markers, may provide insights into community-level metabolic health patterns (Berenson et al., 2011). However, as discussed, insulin-related signals in wastewater present interpretive challenges due to limited excretion, variability, and potential contributions from treatment-related inputs. Accordingly, these signals are better interpreted as contextual indicators of disease burden, treatment patterns, or healthcare access rather than direct measures of endogenous physiological activity (Pearce, 2024). When combined with prescription data, demographic information, and complementary wastewater indicators, these measurements may contribute to a broader understanding of metabolic health trends at the population level.

ii. **Reproductive Health and Equity.** Reproductive hormones such as estrogens and testosterone may provide indirect insights into population-level reproductive health dynamics and access to healthcare services. However, interpretation of these signals requires careful consideration of multiple contributing pathways, including physiological excretion, pharmaceutical use, and environmental inputs. When analyzed within an integrated framework that incorporates demographic, healthcare access, and environmental data, these hormonal patterns may help identify disparities in reproductive health services and inform more equitable public health interventions. At the same time, applications involving reproductive hormones require careful consideration of privacy, governance, and potential political or legal misuse of wastewater-derived population data.

The transition from data to intervention in hormone-based WBE requires careful consideration of uncertainty, context, and system-level influences. Rather than serving as direct triggers for intervention, hormonal signals are best positioned as early indicators or complementary evidence that can inform decision-making processes when interpreted alongside other data sources. In this way, hormone-based WBE supports a shift from reactive to more anticipatory and adaptive public health strategies, while maintaining appropriate caution in interpretation.

6. Challenges and ethical imperatives

While hormone-based wastewater epidemiology (WBE) offers a promising extension of population-level surveillance, its successful application depends on addressing several technical, interpretive, ethical, and strategic challenges. These challenges are not unique to hormonal biomarkers but are particularly important given the variability, transformation, and context-dependence of these signals in wastewater systems:

i. **Detection, Stability, and Standardization.** Despite advances in analytical techniques, translating hormone measurements into reliable population-level insights remains challenging. A key limitation is that measured concentrations may be influenced by processes occurring during sewer transport, including transformation, degradation, and partitioning, which can obscure the relationship between observed signals and their underlying sources (Chen et al., 2014; Driver et al., 2022; Gao et al., 2023; Shao et al., 2025; Thai et al., 2019). These uncertainties complicate interpretation and highlight that biomarker suitability cannot be assessed based on analytical detectability alone. Instead, factors such as stability, persistence, and system-dependent variability must be considered when determining whether a biomarker can provide meaningful and interpretable information. Furthermore, variability in sampling protocols, preservation methods, extraction procedures, and analytical calibration across laboratories may limit comparability between studies.

ii. **Interpretive Challenges: Context, Variability, and Multiple Contributions.** Hormonal signals are biologically rich but inherently complex, varying with age, sex, circadian rhythms, health status, and behavioral factors (Meléndez-Fernández et al., 2023; Mong et al., 2011; Neves et al., 2022). Variability in excretion dynamics, fluctuations in population behavior, and contributions from multiple pathways can all influence measured concentrations at the population scale. In particular, hormonal signals in wastewater may reflect contributions from multiple pathways, including physiological excretion as well as pharmaceutical use, dietary inputs, and system-level processes (Almazrouei et al., 2023; González et al., 2020, 2020). In addition, wastewater system processes, including dilution, transport, mixing, and transformation, further complicate signal attribution. As a result, hormonal measurements often represent aggregated and context-dependent signals rather than direct reflections of specific physiological states, limiting the feasibility of establishing one-to-one relationships between measured concentrations and underlying biological conditions (Elliss et al., 2025; Gao et al., 2023; Shao et al., 2025).

iii. **Ethical Challenges: Privacy, Equity, and Responsible Use.** Hormonal signals may reflect sensitive aspects of population health, including stress, reproductive function, and metabolic status. Misinterpretation or misuse of such data could lead to stigmatization or unintended consequences, particularly for vulnerable communities. These concerns are amplified when monitoring small catchment areas (e.g., dormitories or workplaces), where inferred patterns may be perceived as targeting specific groups. These concerns may be particularly important in the context of reproductive hormones and hormone-related compounds, where wastewater surveillance could raise broader societal, political, and legal concerns related to reproductive privacy and potential misuse of population-level data. Recent public debate in the United States has highlighted concerns that wastewater surveillance infrastructure could potentially be used to monitor reproductive or sexual-health-related biomarkers in ways that may contribute to demographic targeting, stigmatization, or politically motivated surveillance (D'Aoust, 2025). Although hormone-based WBE may offer valuable public-health insights, such applications require careful governance, transparency, ethical oversight, and community engagement to ensure responsible implementation and protection against misuse.

iv. **Strategic Challenges: Scaling, Validation, and Interdisciplinary Collaboration.** Advancing hormone-based WBE from conceptual frameworks to operational systems requires coordinated efforts across multiple disciplines, including analytical chemistry, environmental engineering, epidemiology, public health, and data science. In particular, there is a need for systematic validation studies that evaluate biomarker stability, transformation, and interpretability across diverse wastewater systems and population contexts. High-frequency or near-real-time monitoring of hormonal signals may also face practical limitations related to analytical cost, sampling logistics, and interpretive uncertainty. Current limitations in data availability, standardization, and cross-sector collaboration may constrain large-scale implementation.

Rather than viewing these challenges as barriers, they highlight the importance of adopting integrative and context-aware approaches to hormone-based WBE. The systems-level framework proposed in this work is specifically designed to address these limitations by embedding hormonal signals within a broader network of complementary data streams. Through cross-validation, contextualization, and integration with independent datasets, uncertainties associated with individual biomarkers can be reduced. In this context, hormonal signals are best understood as contributing components within a multi-dimensional surveillance system, supporting pattern recognition and trend analysis rather than serving as standalone indicators. By embracing this framework, hormone-based WBE can evolve in a way that is both scientifically rigorous and practically meaningful, enabling more reliable and equitable public health insights.

7. Calls to action & future directions

To advance hormone-based wastewater epidemiology, we propose several priority directions that build upon both prior work and the challenges identified in this study.

i. **Establish Longitudinal Baseline Datasets.** Developing baseline datasets across diverse settings, including urban, rural, campus, and climate-impacted communities, is essential for interpreting hormonal signals in wastewater. Given the variability and uncertainty associated with individual biomarkers, longitudinal data are particularly important for distinguishing meaningful trends from background variability and system-level influences. These datasets will help define expected ranges, temporal patterns, and thresholds that may inform future public health applications.

ii. **Conduct Targeted Validation and Pilot Studies.** Pilot studies should evaluate hormone-based WBE in real-world contexts, focusing on specific applications such as stress dynamics, metabolic health trends, or environmental response. Importantly, these studies should include systematic evaluation of biomarker stability, transformation pathways, and interpretability under different wastewater system conditions. Such validation efforts are critical for identifying which hormones are most suitable for WBE applications and for defining the limitations and appropriate use cases of each biomarker.

iii. **Develop Standardized Analytical and Interpretive Frameworks.** Advancing hormone-based WBE requires standardized protocols for detection, normalization, and reporting. In addition to analytical standardization, there is a need for consistent approaches to interpretation that account for variability, multiple contributing pathways, and system-level influences. Establishing shared frameworks and reference datasets will improve comparability across studies and support broader adoption.

iv. **Strengthen Cross-Sector Integration and Data Linking.** The value of hormone-based WBE depends on its integration with complementary data streams, including clinical, environmental, and behavioral datasets. Strengthening data integration capabilities will enhance interpretive reliability by providing independent lines of evidence and contextual support for observed patterns. Interdisciplinary collaboration across environmental engineering, public health, data science, and policy will be essential for advancing this integrative approach.

v. **Advance Ethical Governance and Responsible Implementation.** Ethical considerations must remain central to the development and application of hormone-based WBE. Given the sensitivity and potential complexity of hormonal data, governance frameworks should emphasize transparency, community engagement, data privacy, and equitable use. Ensuring that hormone-based monitoring supports public health without unintended harm will be critical for building trust and long-term adoption.

vi. **Toward a Context-Aware Vision for Hormone-Based WBE.** Taken together, these priorities reflect a shift from viewing hormonal biomarkers as standalone indicators to understanding them as components of a broader, integrated surveillance system. The future of hormone-based WBE lies not in the direct interpretation of individual signals, but in the ability to contextualize these signals within a multi-dimensional framework that accounts for variability, uncertainty, and system-level influences. Over time, with continued validation, standardization, and integration, hormone-based WBE may evolve into a valuable complement to existing public health surveillance systems. Importantly, this evolution should be guided by a cautious and evidence-based approach, where applications are developed incrementally and supported by robust validation rather than assumed a priori. In this context, hormone-based WBE represents not a replacement for existing approaches, but an opportunity to expand the scope of wastewater surveillance toward capturing aspects of population-level physiological response.

8. Conclusions

- Hormone-based wastewater epidemiology (WBE) extends current approaches beyond tracking external exposures toward capturing aspects of population-level physiological response.
- Hormonal signals in wastewater provide context-dependent information related to stress, metabolic function, and endocrine activity; however, they are most informative when interpreted within an integrated framework rather than as standalone indicators.
- Key Priorities:

  Evaluation of biomarker stability, transformation, and system dynamics, alongside standardized detection and normalization methods.

  Integration of hormonal data with clinical, environmental, behavioral, and wastewater-derived indicators to support robust interpretation.

  Development of longitudinal datasets and targeted pilot studies to assess suitability and define appropriate use cases.

  Ensuring transparency, privacy protection, and equitable application of hormone-based monitoring.

CRediT authorship contribution statement

Ahmed S. Abouhend: Writing – original draft, Visualization, Resources, Investigation, Conceptualization. **Walid A. Hanafy:** Writing – review & editing, Visualization. **Christopher Watt:** Writing – review & editing. **Raphael C. Nnachi:** Resources. **Prashant Shenoy:** Writing – review & editing, Conceptualization. **Caitlyn S. Butler:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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