ABSTRACT

HIRONS, NATALIE LIANNE. Estimating Chronic Exposure to Steroid Hormones in Water. (Under the direction of Damian Shea.)

Ecological risk assessment (ERA) is a useful process contributing to safeguarding the earth’s ecosystems. Exposure assessment is a vital component of ERAs, as chemical exposure information is used to make decisions involving ecological risk. So, the continued advancement of sampling methodology is essential to better understanding chemical exposure. While traditional sampling methodologies, including grab sampling, only capture a snapshot of exposure, the development of passive sampling devices (PSDs) has allowed for estimations of chronic contaminant exposure. For many emerging contaminants, including many endocrine disruptors, determining chronic exposure is pertinent to assessing toxicological risk, and unlike current available sampling methodologies that tend to target specific classes of compounds, a more universal PSD (uPSD) is necessary to capture both hydrophobic contaminants and more hydrophilic contaminants, such as steroid hormones.

In this work, two novel PSDs, a cartridge uPSD and a fiber uPSD, were evaluated as tools to improve our understanding of chronic exposure to steroid hormones, namely estrogens and androgens, in surface waters. A number of studies have demonstrated that chronic exogenous hormone exposure in the low nanogram-per-liter range can result in irreversible alterations in development and reproduction of individual organisms, which could threaten the reproductive success of certain aquatic populations. Consequently, as hormone use in agriculture for the efficient growth and production of meat has increased and as the science community has become increasingly aware of potential hormone
contamination of surface waters via wastewater effluent, increased hormone monitoring efforts have revealed the presence of hormones in surface waters. The goal of this work was to develop novel surface-water monitoring tools to quantitatively assess chronic exposure to free estrogens and androgens.

Both the cartridge uPSD and fiber uPSD contained the same polymeric sorbent, Oasis HLB®; however the cartridge uPSD consisted of a porous, stainless steel casing, while the fiber uPSD consisted of a polyethersulfone membrane. Both devices were calibrated in the laboratory using an uptake experiment, in order to determine sampling rates for all hormones, and an elimination experiment, to determine the effect of flow rate on elimination and to evaluate the suitability of using performance reference compounds (PRCs) during in situ applications.

Over the 30-day uptake experiment, all hormones remained in the linear uptake phase for both devices, demonstrating that both devices have the capacity to function as time-integrative devices. Sampling rates for the cartridge uPSD ranged from 0.09 – 0.11 L/d, and sampling rates for the fiber uPSD ranged from 0.04 – 0.09 L/d. Neither device demonstrated clear correlations of sampling rates to log $K_{ow}$ values; however calibrations of both devices to contaminants with expanded ranges of log $K_{ow}$ values is necessary to confirm these results.

In the 30-day elimination experiment, neither device demonstrated any effect of flow rate on elimination rate; however, for two hormones, estrone and androstenedione, a correlation was observed. These results suggest that the use of PRCs may not be necessary to correct for differences in flow rates between laboratory calibration and field deployment;
however, PRCs may be necessary to monitor changes in uptake due to biofouling or temperature in situ.

The final component to validating the use of both devices included field deployments in surface waters receiving inputs from wastewater treatment plants and concentrated animal feeding operations. Mean estimated water concentrations from cartridge uPSD and fiber uPSD residues were in general agreement with mean grab sample mean concentrations of surface waters.

Ultimately, these results validate that both the cartridge uPSD and fiber uPSD offer potential as tools to provide quantitative estimates of chronic steroid hormone exposure in surface waters.
Estimating Chronic Exposure to Steroid Hormones in Water

by
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A thesis submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Master of Science

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DEDICATION

I dedicate this work to my family, who as a whole, has provided me with more than I could ask for.
BIOGRAPHY

With a family nickname like “Miss Chievous,” her innate curiosity was obvious by the age of two. Though somewhat tempered since her kitten-swimming-lessons-in-the-toilet days, that same innate curiosity has been a driving force in her life, and paired with father and grandfather engineers, it is not surprising that her educational path led to the field of science.

Upon completion of the International Baccalaureate Programme at Marietta High School, Marietta, Georgia, Natalie pursued a degree in Environmental Health Science at the University of Georgia in the College of Public Health. The range of exposure in coursework piqued her interest in a multidisciplinary approach to science, aiming to understand how human populations affect their environment, in turn how those environmental changes affect ecosystems and human health, and ultimately what, if any, action is necessary to mediate those alterations. In her third year, she took a course that gave a name to her previously faceless interest, Environmental Toxicology. Under the mentorship of Dr. Mary Alice Smith, Natalie decided to pursue a graduate career in toxicology.

The summer of 2006 marked a culminating point in her life, with the completion of her B.S.E.H., marriage, and a move seven hours away from her childhood home to commence graduate studies in the Department of Environmental and Molecular Toxicology at North Carolina State University in Raleigh, North Carolina. She hopes, upon completion of her Master’s degree, to pursue a career applying scientific knowledge to real-world challenges facing our environment.
ACKNOWLEDGEMENTS

I would like to begin by extending my gratitude to Dr. Mary Alice Smith for planting the seed of toxicology in my learning experience. Her class inspired the direction of my graduate studies.

This work would not be accomplished without the support and wisdom of my advisor, Damian Shea, and committee members, Chris Hofelt and Pat McClellan-Green, thank you. I would also like to thank Greg Cope for keeping the door open to my questions, my technical support team and lab members, Norman Glassbrook, Peter Lazaro, Kelly O’Neal, Xiang Kong, and Xin Rui-Xia, lending both technical expertise and open ears to weekly troubles and joys, and another thank you to my fellow “tox” buddies for their support.

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INTRODUCTION

1. Environmental Exposure

Sources

The scientific community has become increasingly aware that many compounds released into the environment have the potential to block or mimic the actions of sex steroids (1, 2). So, the possibility of agonistic or antagonistic activity is potentially dangerous because sex steroids, particularly androgens and estrogens, irreversibly program aspects of sexual differentiation and reproduction early in development (3). Ultimately, exposure to exogenous steroid hormones, even at low levels, during these susceptible windows can result in detrimental and irreversible alterations in development and potentially, reproductive success of the population (4, 5).

Aquatic inputs of hormones chiefly derive from agricultural sources, either due to runoff from waste-amended fields or waste lagoon spills, or municipal waste sources, in effluent from wastewater treatment plants (WWTP) or sewage treatment plants (STP) (6-10).

Over the past 20 years, large-scale concentrated animal feeding operations (CAFOs) have flourished for the space- and resource-efficient production of meat. CAFOs are characterized by the high-density maintenance of livestock and the use of pharmaceuticals and automation to maximize productivity. Accordingly, CAFOs produce large amounts of organic wastes, particularly manure. In 2002, the US EPA implemented rules, as mandated by the Clean Water Act, to ensure that CAFOs appropriately manage manure in order to...
protect the Nation’s water quality. CAFOs are a major source of release of bioactive steroidal compounds (6, 8, 11).

Estrone, 17α-estradiol, and 17β-estradiol are the most abundant forms of parent estrogens released into the environment by CAFOs (2, 8, 12). Estrogens are largely eliminated by swine and poultry in urine (70-99%) (13, 14), indicating that these compounds are released as polar, glucuronic acid or sulfate, conjugates (8, 15, 16). Polar conjugates are significantly more mobile in aqueous environments than their parent compounds and may facilitate the transport of estrogens from CAFOs to surface waters. Once in receiving waters, conjugated estrogens can undergo biotic or abiotic hydrolysis yielding a highly estrogenic parent compound (10, 17). Unlike swine and poultry, cattle typically excrete estrogen in feces, largely as unconjugated, or free, estrogens which are less mobile in aquatic environments (13).

Among the androgens, 17α-testosterone, 17β-testosterone, and androstenedione are most commonly associated with CAFOs (12). Like estrogens, androgens are eliminated from swine predominantly as polar conjugates which would enhance their environmental mobility and transport to surface waters (14).

Unlike CAFOs, surface water contamination of natural and synthetic hormones by WWTPs is not a recent phenomenon. However, only in the past two decades when feminization of male fish has been observed downstream of WWTP outfalls have monitoring efforts been made to determine the extent of hormone loading by WWTPs. While removal of steroid hormones from influent is dependant upon treatment technology
employed, resulting in varying removal efficiencies among treatment facilities, one study estimated that ~ 20% of human-derived estrogens survive standard sewage treatment (18).

The most abundant forms of estrogens released by WWTPs include the parent forms of natural estrogens, estrone (E1) and 17β-estradiol (E2), and the synthetic oral contraceptive, ethynyl estradiol (EE2) (19, 20). These natural and synthetic estrogens enter WWTPs as polar, primarily glucuronic acid, conjugates, which results in enhanced mobility in the aquatic environment (21).

Similar to CAFO-derived androgens, the most common androgens associated with WWTP effluent are 17α-testosterone, 17β-testosterone, and androstenedione, although significantly less work has been done on environmental androgens (22, 23). Like estrogens, androgens enter WWTPs primarily as glucuronic acid conjugates, to a lesser extent sulfate conjugates, and are released as free androgens, chiefly due to transformation by bacteria concurrent in wastewater influent (21, 24). However, studies have demonstrated major removal of androgens from effluent in conventional wastewater treatment processes (22-24).

Regardless of their source (CAFO or WWTP), all androgens and estrogens have the potential to undergo hydroxylation prior to urinary or fecal elimination (25-27). Patterns of hydroxylation can be complex and likely vary between sexes and among age groups (28, 29). Hydroxylated steroid derivatives have significantly greater aqueous mobility than the parent steroids. The hydroxyl groups also provide additional sites for conjugation to sulfate or glucuronic acid. However, bacteria deriving from fecal inputs, namely Escherichia coli, convert hormone conjugates to their unconjugated, or free, form (8, 18, 21). Free hormones have a greater propensity to sorb to solid particles than their conjugate forms, and hence,
free hormones, particularly testosterones, are largely removed via sludge in wastewater treatment (24). Androgens and estrogens have moderately high partitioning coefficients (log \textit{K}_{ow} values \sim 3-4), and therefore can be sorbed to sediments or suspended organic material (Table 1). In soil, sludge, or sediment, there is the potential for biological uptake, degradation and transformation to less mobile or more mobile forms (11). So, while polar conjugates have increased aqueous mobility, bacterial deconjugation could result in free steroids released to the aquatic environment. In summary, estrogens and androgens contaminate the environment from CAFOs and WWTPs largely as parent compounds, hydroxylated derivatives, or polar conjugates, with environmental polar compounds being the most mobile and the parent compound being the least mobile.

**Environmental Concentrations**

Reported values of estrogens and androgens in surface waters downstream of CAFOs and WWTPs widely vary (Table 2). However, concentrations in WWTP effluent can be translated to rough environmentally-relevant averages. Reported WWTP effluent mean concentrations for estrogens range from non-detects – 1.8 \(\mu\text{g/L}\), with maximum concentrations as high as 3.7 \(\mu\text{g/L}\), while downstream average concentrations range from non-detects –1.1 \(\mu\text{g/L}\), with maximum concentrations as high as 2.7 \(\mu\text{g/L}\) and many average concentrations in the nanogram-per-liter range. Published concentrations of androgens in WWTP effluent and in downstream surface waters are significantly less frequent than for estrogens. Average androgen concentrations both in WWTP effluent and in downstream surface waters are reported in the low nanogram-per-liter range (0.1 – 2.8 ng/L). Similarly, reported values of estrogens related to agricultural operations are more widely published.
than for androgens. Average lagoon concentrations of estrogens range from 9.5 – 34.6 μg/L, with maximum concentrations of 150 μg/L measured. Average concentrations of estrogens in surface waters near CAFOs range from non-detects to 2.4 ng/L, with maximum concentrations reported at 9.3 ng/L, and nearby groundwater averages ranging from 0.8 – 2.2 ng/L with maximums reported at 3.5 ng/L. Both groundwater and surface water concentrations of androgens near CAFOs exhibit average and maximum values in the low nanogram-per-liter range (0.6 – 5.0 ng/L). Surface waters receiving mixed-use impacts have average estrogen concentrations ranging from 19 – 160 ng/L with maximum concentrations reported at 831 ng/L and average androgen concentrations at 116 ng/L with reported maximums as high as 214 ng/L.

2. **CHRONIC TOXICITY & EFFECTS**

**Biochemical to Individual Level**

Contamination of surface waters with natural and synthetic steroid hormones chiefly arises from agricultural uses for efficient growth promotion in animals and from humans naturally and for contraception and various hormone replacement therapies. In addition to understanding environmental exposure to steroid hormones, understanding the levels and time points at which aquatic organisms are impacted is essential to protecting critical populations and ultimately, ecosystem integrity.

Organisms susceptible to impacts by steroid hormones primarily include vertebrates, although some invertebrates, such as select mollusks, are also susceptible. This vulnerability is due to the presence or absence of an estrogen or androgen receptor, as exogenous hormones act through the same pathways as endogenous hormones (8, 30). Hence, any
organism expressing the estrogen or androgen receptor is susceptible to impacts from exogenous estrogens or androgens. The general mechanism of action for estrogens and androgens involves entry of the hormone into the nucleus, first crossing the cell membrane into the cytosol, binding to its associated receptor at the ligand-binding domain and crossing the nuclear membrane. This ligand-receptor complex then binds to DNA estrogen or androgen response elements via the receptor’s DNA-binding domain and with the recruitment of other transcription-associated proteins, controls gene expression, either upregulating or downregulating associated genes (31).

As stated above, the most common parent estrogens and androgens released into the environment include estrone, 17α-estradiol, and 17β-estradiol and 17α-testosterone (epitestosterone), 17β-testosterone, and androstenedione, respectively (2, 8, 12, 19, 20). However, all estrogens do not equally activate the estrogen receptor with the same potency, and likewise, not all androgens activate the androgen receptor with equal potency. Hence, hormones are measured in toxicity equivalents (TEQs) based on their relative activation of their associated receptors to the most potent natural hormone. For instance, 17β-estradiol is the most potent natural estrogen, having a TEQ of 1.0, and all other natural estrogens have a TEQ \( \leq 1.0 \) due to lower potencies (8). However, synthetic hormones, such as ethynyl estradiol (EE2) or 17β-trenbolone, have the potential to activate endogenous hormone receptors with equal or greater potency than the most biologically-active natural hormone (22, 30, 32). Similarly, androgens and estrogens excreted as metabolites or polar conjugates generally have lower biological activity than their parent forms, with polar conjugates having the least potency and parent hormones being the most potent (25-27).
At the biochemical level, numerous studies have demonstrated altered levels of circulating androgens or estrogens upon exposure to exogenous hormones (5, 32-35). In a short-term in vivo study exposing juvenile male turbot (Psetta maxima) to 3.5 ng/L of EE2, altered sex steroid profiles were observed in males with androgen to estrogen hormone ratios in the testes and plasma reaching values observed in females (35). Another study exposing male zebra fish to EE2 demonstrated decreased levels of circulating androgens (33). Conversely, female fathead minnows (Pimephales promelas) exposed to 17α- and 17β-trenbolone, synthetic androgens used in primarily in beef cattle growth production, demonstrated altered plasma hormone profiles and decreased production of vitellogenin (VTG), a female egg yolk precursor protein (5, 32, 34). VTG synthesis is an estrogen receptor–mediated response, which occurs naturally in female fish following endogenous estrogen exposure through blood plasma (36). So, in studies where male fish have been exposed to the strong estrogen agonist, EE2, VTG induction in has been observed (30, 37). Additionally, alterations in secondary sex characteristics have been observed upon exposure to exogenous hormones (32, 34). For instance, female fathead minnows exposed to 17α- and 17β-trenbolone develop the typically-male secondary sex characteristics of dorsal (nuptial) tubercles (32, 34), and while these changes in secondary sex characteristics do not directly impact reproduction capabilities, there is potential for alterations in sexual or mating behavior as a result.

Linking molecular and biochemical hormonal responses to mechanism of action is relatively straightforward, while connecting molecular and biochemical responses to population-level effects proves more challenging. Some studies focused at individual-level
effects, including endpoints such as survival, growth, and reproduction, relate more closely to population-level effects (5). One study, however, attempted to demonstrate a predictive link between biochemical observations and population-level effects (5). In this study, fecundity was predicted by observed VTG production based on the established link between VTG production in female fish and egg production. Other studies, focused on individual-level effects, have demonstrated alterations in development and reductions in reproductive success upon exposure to exogenous hormones (33, 38-40). Particularly, reduced fecundity in female fathead minnows upon exposure to < 3 ng/L 17α- and 17β-trenbolone has been observed (32, 34). Numerous studies have focused on the effects of the synthetic estradiol, EE2, in male fish (33, 38-40). A no-observed-effects-concentration (NOEC) based on growth, survival, and reproductive endpoints in the male fathead minnow was determined to be 1.0 ng/L (39). Another study demonstrated altered reproductive hierarchies through the disruption of sexual selection in group-spawning zebrafish when exposed to environmentally relevant concentrations of EE2 (33). Two lifecycle studies demonstrated demasculinization through gonadal feminization of males and a decrease egg fertilization success following exposure to < 1 ng/L and 3 ng/L of EE2, respectively (38, 40).

Although many studies have demonstrated individual-level effects, few studies have demonstrated potential population-level effects of hormone exposure. In one in-vivo study, exposing zebrafish (Danio rerio) to environmentally-relevant levels of EE2 (5 ng/L) in a lifecycle assessment resulted in significantly reduced fecundity in the F1 generation and complete population failure upon lifelong exposure (41). A recent 7-year, whole-lake field study was conducted maintaining a constant 5-6 ng/L exposure of EE2 to determine if low-
level, chronic exposure would adversely impact the sustainability of wild fish populations (4). The results of this study demonstrated that chronic exposure of the fathead minnow to low EE2 concentrations led to feminization of males (VTG production, intersex), altered reproduction in females (oogenesis disruption), and a near extinction of the fathead minnow from the lake. Hence, exposure to low, environmentally-relevant concentrations of exogenous hormones can impact sustainability of wild fish populations.

3. PASSIVE SAMPLING DEVICES

With the understanding that EDCs, like steroid hormones, have the potential to cause irreversible alterations in organism development and reproduction when exposure is chronic at low nanogram-per-liter levels, the ability to detect and quantify low-level, chronic exposure is very important to protecting the integrity of ecosystems impacted by EDCs.

Traditional contaminant monitoring methods include grab sampling, which involves the collection of a water sample, transport to laboratory facilities, extraction, and analysis to give an estimated aqueous concentration, and biological sampling, which involves the collection of native or transplanted organisms, transport to laboratory facilities, sacrifice and extraction, and analysis. Grab sampling can be a limiting method of environmental sampling, as grab samples only represent exposure at a single moment in time. To gain an estimate of chronic exposure, numerous samples must be collected sequentially over the exposure time period, which may be very costly and impractical. Additionally, grab sampling may require large sample volumes (42) in order to detect low or sub nanogram-per-liter concentrations, which again may present problems of practicality, both in collection and extraction in the laboratory. Finally, grab samples measure the total concentration of a
chemical within the sample, which may not be representative of the bioavailable fraction of
the chemical in that sample.

Although biological samples potentially allow for a measure of bioavailable
exposure to chemicals, they present their own limitations as well. Sampling of native
organisms leaves a large uncertainty in exposure history and may be restrictive due to
endangerment of certain species. Regardless of using native or transplanted organisms,
biological samples may miss exposure to non-bioaccumulative compounds or may not
account for chemical metabolism or depuration in the organism, ultimately leading to
uncertainty in how well measured exposure correlates to actual exposure (42). Other
potential points of uncertainty include intra-species differences in organism metabolic rate,
health, and other behaviors, including extent of locomotion, feeding, and mating or
reproduction.

A tool to overcome the limitations of grab sampling and biological sampling is the
passive sampling device (PSD), which is a device that passively accumulates and
concentrates contaminants over time to give a time-weighted-average (TWA) estimate of
chronic exposure. Contaminants passively diffuse into the PSD until a steady-state
condition, or equilibrium is reached. Hence, uptake curves for PSDs are logarithmic,
containing an initial linear uptake phase, a curvilinear portion, and a final equilibrium phase
(43). When PSDs are deployed during the linear uptake phase, a quantitative estimation of
chronic exposure integrated over time can be calculated. First, PSDs are calibrated in
laboratory uptake experiments to yield integrative sampling rates, which are then used to
calculate chronic exposure estimates in field samples. Laboratory sampling rates are a
measure of the sampling rate of the device, measuring the volume of water quantitatively sampled per unit time (L/d), and are determined using the following equation,

\[ R_s = \frac{N_t}{C_w t} \]  

(1)

where \( R_s \) is the sampling rate (L/d), \( N_t \) is the amount (ng) of analyte accumulated in the sampler at the end of exposure, time in days, \( C_w \) is the average concentration of freely-dissolved analyte in water (ng/L), and \( t \) is the duration of the exposure measured in days (44).

In field samples, estimates of chronic exposure (\( C_w \)) can be determined by rearranging equation 1 as follows,

\[ C_w = \frac{N_t}{R_s t} \]  

(2)

where \( N_t \) is the amount (ng) of analyte in the PSD at the end of the deployment period, \( t \) in days, \( R_s \) is the laboratory-derived sampling rate (L/d), and \( t \) is the duration of the deployment in days (44).

However, field-deployed samplers are subject to varying environmental conditions, such as temperature, flow rate, and biofouling, which can affect the uptake rate of analytes into the device. For instance, uptake rates for more hydrophobic compounds (\( \log K_{ow} \geq 4-5 \)) can be controlled by flow rate, as flow rate controls the thickness of the aqueous boundary layer (ABL). Higher flow rates decrease ABL thickness, which result in faster uptake for hydrophobic compounds (45). So, environmentally-produced alterations in uptake can lead to misrepresentations in estimates of exposure, if not taken into account. Fortunately, changes in uptake rate due to environmental conditions can be corrected for through the use of performance reference compounds (PRCs), which are analytically non-interfering.
compounds that share similar chemical properties to analytes of interest (46). PRCs are added to samplers prior to deployment and elimination over the duration of deployment is monitored. So, with the understanding of isotropic exchange kinetics, changes in elimination rate of PRCs are proportional to changes in uptake rate of targeted analytes (46-48).

Release kinetics of compounds from PSDs can be modeled assuming first-order release kinetics using the following equation (47),

$$N_t = N_0 \times e^{-k_e t}$$  (3)

where $N_t$ is the amount (ng) of PRC remaining in the sampler at time $t$ in days, $N_0$ is the amount (ng) of PRC initially loaded into the sampler, $k_e$ is the elimination or exchange rate constant (d$^{-1}$), and $t$ is the time in days.

Hence, field $k_e$ values for PRCs can be compared to laboratory-derived $k_e$ values for PRCs through an environmental adjustment factor (EAF), which is calculated using equation 4 (46),

$$EAF \equiv k_{ePRC-f} / k_{ePRC-cal}$$  (4)

where $k_{ePRC-f}$ represents the PRC elimination rate constant in the field (d$^{-1}$) and $k_{ePRC-cal}$ represents the PRC elimination rate constant determined in a laboratory calibration experiment (d$^{-1}$). Ultimately, changes in uptake rate are corrected for by applying the EAF to resultant PSD-estimated water concentrations.

Despite these advances in passive sampling technology over the past 20 years, further research is necessary. Early passive sampling technologies included dialysis membrane devices (49) and have since evolved to devices, such as the carbon-filled dosimeter (50), the permeation sampler (51), and more recently, the semi-permeable
membrane device (SPMD) (52), that aim to quantify chemical exposure to micropollutants with greater accuracy (42). Importantly, many of the newer PSDs, including solid-phase micro extraction (SPME) devices (53) and polyethylene devices (54), are designed to sample compounds sharing similar chemical properties, namely hydrophobic organic compounds like PCBs, PAHs, and other organophosphates and organochlorines (42). However, few samplers have been developed to target sampling of polar compounds, those samplers including the organic version of the Chemcatcher (55) and the polar organic chemical integrative sampler (POCIS) (56). Despite these technological advances, a recent review of PSD technology stressed the need for the development of samplers targeting the class of polar organic compounds (42). Moreover, sampling of compounds varying across chemical classes currently requires the use of multiple sampling technologies, which is costly, requires additional time-consuming analysis, and may ultimately exclude certain classes of compounds from monitoring efforts (57). As more polar compounds, like synthetic endocrine disrupting compounds (EDCs) and steroid hormones, become a part of our slew of environmental contaminants, a more holistic approach to environmental sampling will be required, and as an extension, the development of a PSD with the capacity to sample compounds with a wide range of physicochemical properties would provide a tremendous advantage to future environmental assessment and monitoring efforts. In this work, two novel universal passive sampling devices (uPSDs) previously developed in our laboratory were calibrated to quantify select estrogens and androgens in surface waters. These devices are potential solutions to further understanding chronic exposure to hormones in surface waters deriving from CAFOs and WWTPs and determining if hormone loading from
CAFOs and WWTPs occur at levels sufficient to cause adverse ecological effects. Ultimately, these devices can be used in expanded environment monitoring efforts, encompassing a wide suite of compounds in addition to steroid hormones.
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Table 1. Selected Physicochemical Properties of Steroidal Hormones.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>MW(^a) (g mol(^{-1}))</th>
<th>(S_w) (^b) (mg L(^{-1}))</th>
<th>(\log K_{ow}) (^c)</th>
<th>(\log K_{oc}^d) (L kg(^{-1}) C)</th>
<th>Typical (K_f^e) (L kg(^{-1}))</th>
<th>Half-Lives (d) in Soil and Water</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Estrogens:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrone (E1)</td>
<td>270.4</td>
<td>0.8-13(^{g, k})</td>
<td>2.5-3.4(^{g, k})</td>
<td>3.0-4.2(^{k})</td>
<td>2.2-48.1(^{k})</td>
<td>0.1-10.9</td>
</tr>
<tr>
<td>17(\alpha) Estradiol (E2)</td>
<td>272.4</td>
<td>3.2-13.3(^i)</td>
<td>3.4-4.0(^{i})</td>
<td>NA(^f)</td>
<td>NA(^f)</td>
<td>NA(^f)</td>
</tr>
<tr>
<td>17(\beta) Estradiol (E2)</td>
<td>272.4</td>
<td>3.9-13.3(^{k, l})</td>
<td>3.1-4.0(^{k, l})</td>
<td>2.76-5.28(^{k, l})</td>
<td>2.3-83.2(^{k})</td>
<td>0.2-9.7</td>
</tr>
<tr>
<td>Estradiol (E3)</td>
<td>288.4</td>
<td>3.2-30.2(^{k, l})</td>
<td>2.5-2.8(^{k, l})</td>
<td>4.64-5.32(^{l})</td>
<td>NA(^f)</td>
<td>NA(^f)</td>
</tr>
<tr>
<td>Ethynyl estradiol (EE2)</td>
<td>296.4</td>
<td>4.8-19.1(^{k, l})</td>
<td>3.7-4.7(^{k, l})</td>
<td>2.9-5.22(^{k})</td>
<td>2.1-23.4(^{k})</td>
<td>3.1-9.6</td>
</tr>
<tr>
<td><strong>Androgens:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Androstenedione (A)</td>
<td>286.4</td>
<td>37-41(^{h})</td>
<td>NA(^f)</td>
<td>3.69-3.77(^{h})</td>
<td>19.3-142(^{h})</td>
<td>NA(^f)</td>
</tr>
<tr>
<td>Epitestosterone (aT)</td>
<td>286.4</td>
<td>NA(^f)</td>
<td>NA(^f)</td>
<td>NA(^f)</td>
<td>NA(^f)</td>
<td>NA(^f)</td>
</tr>
<tr>
<td>17(\beta) Testosterone (T)</td>
<td>286.4</td>
<td>18-25(^{k})</td>
<td>3.0-3.6(^{k, w})</td>
<td>3.2-3.5(^{k, w})</td>
<td>4.6-42.7(^{k})</td>
<td>0.3-7.3</td>
</tr>
</tbody>
</table>

\(^a\) Molecular Weight; \(^b\) Water Solubility; \(^c\) Octanol-Water Partition Coefficient; \(^d\) Organic Carbon-Water Partition Coefficient; \(^e\) Solid-Water Distribution Coefficient; \(^f\) Not Available; \(^g\) Value from Temes et al. 1999 [36]; \(^h\) Value from Lee et al. 2003 [22]; \(^i\) Value from Hanelman et al. 2003 [13]; \(^j\) Value from Lai et al. 2002 [58]; \(^k\) Value from Lai et al. 2000 [59]; \(^l\) Value from Yamamoto et al. 2003 [60]; \(^m\) Value from Johnson et al. 2006 [2]; \(^n\) Value from Carbella et al. 2008 [61]; \(^o\) Value from Das et al. 2004 [62]; \(^p\) Value from Jurgens et al. 2002 [63]; \(^q\) Value from Ying et al. 2002 [64]; \(^r\) Value from Casey et al. 2004 [65].
Table 2. Selected Examples of Steroid Hormone Median<sup>a</sup> or Mean<sup>b</sup> (Maximum) Concentrations (ng/L).

<table>
<thead>
<tr>
<th>Hormone</th>
<th>WWTP Source</th>
<th>Agricultural Source</th>
<th>Mixed-Use Source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effluent</td>
<td>Nearby Surface Water</td>
<td>Lagoon</td>
</tr>
<tr>
<td>Estrone (E1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.4&lt;sup&gt;a&lt;/sup&gt;, 17.3&lt;sup&gt;b&lt;/sup&gt; (76.0)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.d.&lt;sup&gt;k.h&lt;/sup&gt; (1,600)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15,667&lt;sup&gt;b&lt;/sup&gt; (74,700)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2&lt;sup&gt;b&lt;/sup&gt; (3.5)&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>28.7&lt;sup&gt;b&lt;/sup&gt; (75.0)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>n.d.&lt;sup&gt;k.h.i&lt;/sup&gt;</td>
<td>5,299&lt;sup&gt;a&lt;/sup&gt; (5400)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34,633&lt;sup&gt;b&lt;/sup&gt; (150,000)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.0&lt;sup&gt;c&lt;/sup&gt;, 9.0&lt;sup&gt;b&lt;/sup&gt; (48.0, 70.0)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.3&lt;sup&gt;b&lt;/sup&gt; (3.4)&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17.0&lt;sup&gt;b&lt;/sup&gt; (96.0)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.4&lt;sup&gt;b&lt;/sup&gt;, 0.7&lt;sup&gt;b&lt;/sup&gt; (4.1)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>665&lt;sup&gt;b&lt;/sup&gt; (680)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;b&lt;/sup&gt; (1.6)&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.8&lt;sup&gt;b&lt;/sup&gt; (2.0)&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5&lt;sup&gt;b&lt;/sup&gt; (47.0)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.3&lt;sup&gt;d&lt;/sup&gt; (82.1)&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5&lt;sup&gt;b&lt;/sup&gt;, 3.4&lt;sup&gt;d&lt;/sup&gt; (18.0)&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17α Estradiol (αE2)</td>
<td>n.d.&lt;sup&gt;k.h&lt;/sup&gt; (5)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>665&lt;sup&gt;b&lt;/sup&gt; (680)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;b&lt;/sup&gt; (1.6)&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5&lt;sup&gt;c&lt;/sup&gt;, 1.0&lt;sup&gt;b&lt;/sup&gt; (4.5)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>n.d.&lt;sup&gt;k.h.i&lt;/sup&gt;</td>
<td></td>
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</tr>
<tr>
<td>11.0&lt;sup&gt;b&lt;/sup&gt; (48.0)&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,786&lt;sup&gt;b&lt;/sup&gt; (3,650)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.138&lt;sup&gt;b&lt;/sup&gt; (2.670)&lt;sup&gt;j&lt;/sup&gt;</td>
<td>1,127&lt;sup&gt;b&lt;/sup&gt; (-)&lt;sup&gt;u&lt;/sup&gt;</td>
<td>0.8&lt;sup&gt;b&lt;/sup&gt; (1.3)&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.8&lt;sup&gt;b&lt;/sup&gt; (7.4)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>n.d.&lt;sup&gt;k.h&lt;/sup&gt; (n.d.)&lt;sup&gt;j&lt;/sup&gt;</td>
<td>1,225&lt;sup&gt;b&lt;/sup&gt; (-)&lt;sup&gt;u&lt;/sup&gt;</td>
<td>n.d.&lt;sup&gt;k.h&lt;/sup&gt; (-)&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.8&lt;sup&gt;b&lt;/sup&gt; (-)&lt;sup&gt;j&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

<sup>a</sup>Median concentration, <sup>b</sup>Mean concentration, <sup>c</sup>Maximum concentration.
Table 2. Continued

<table>
<thead>
<tr>
<th></th>
<th>n.d.ª, 6.0ª (3.0, 64.0)ª</th>
<th>n.d.ª, (-)ª</th>
<th>n.d.ª, (5.5)ª</th>
<th>0.3ª, 0.6ª (3.8)ª</th>
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<tbody>
<tr>
<td>Estriol (E3)</td>
<td>1.9ª (14.7)ª</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.7ª (1.3)ª</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.9ª (12.0)ª</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0ª (3.5)ª</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.4ª, 0.9ª (5.2)ª</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethynyl estradiol (EE2)</td>
<td>1.1ª (18.0)ª</td>
<td>4,283.3ª (10,900)ª</td>
<td>&lt;9.5ª (2.3)ª</td>
<td>19ª (31)ª</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2,569ª (3,000)ª</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.2ª (7.0)ª</td>
<td>403ª (520)ª</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>401.5ª (759.0)ª</td>
<td>n.d.ª (n.d.)ª</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0ª, 9.0ª (15.0, 42.0)ª</td>
<td>&lt;0.0ª (2.3)ª</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.7ª (2.4)ª</td>
<td>n.d.ª (4.3)ª</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0ª (3.4)ª</td>
<td>0.4ª, 0.8ª (5.1)ª</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n.d.ª (7.5)ª</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.4ª (1.7)ª</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.7ª, 1.4ª (8.9)ª</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ANDROGENS:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Androstenedione (A)</td>
<td>1.8ª (2.5)ª</td>
<td></td>
<td>2.1ª (2.6)ª</td>
<td>1.6ª (2.1)ª</td>
</tr>
</tbody>
</table>

29
Table 2. Continued

<table>
<thead>
<tr>
<th>Epitestosterone (μT)</th>
<th>17β Testosterone</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2.8 (2.1)</td>
<td>5.0 (6.0)</td>
<td>0.6 (0.4)</td>
<td>116 (214)</td>
<td></td>
</tr>
</tbody>
</table>

1 Median concentration; a Mean concentration; b Desbrow et al. 1999 [10]; c Lee et al. 2004 [28]. d Medians and maximums in 2 different studies by Termes et al. 1999 [10]; e Servos et al. 2005 [60]; f Wang et al. 2005 [67]; g Belfroid et al. 1999 [68]; h Baronti et al. 1999 [69]; i Kuch and Ballschmiter 2001 [70]; j not detected; k Vuillet et al. 2008 [71]; l Fine et al. 2003 [72]; m Furuchi et al. 2006 [6]; n Raman et al. 2004 [73]; o passive sampling device (POCIS) used by Matthiessen et al. 2006 [2]; p Kolpin et al. 2002 [74]; q Snyder et al. 1999 [75]; r Huang et al. 2001 [76]; s Kolodziej et al. 2004 [9]
Chapter 1. Calibration and Field Verification of a Cartridge-Based Passive Sampling Device for Estimating Chronic Exposure to Steroid Hormones in Water

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Environmental Science and Technology
Abstract. A novel passive sampling device (PSD) was developed in our laboratory to take a more holistic approach to aquatic exposure. The cartridge-style universal passive sampling device (uPSD) was designed with the purpose of sampling compounds varying in physicochemical properties, including more polar compounds. In this work, the uPSD was calibrated under controlled laboratory conditions over a 30-day period to determine sampling rates of eight common steroid hormones, five estrogens and three androgens. All eight hormones remained in the linear uptake phase over the 30-day exposure, resulting sampling rates ranged from 0.09 – 0.11 L/d. In an elimination experiment, uPSDs were subject to different flow rates, mimicking different stream flow velocities, and elimination was monitored over 30 days. Flow-rate does not appear to have an effect on mass transfer kinetics. Finally, uPSDs were validated in a 30-day field experiment sampling downstream of a wastewater treatment plant where uPSD-derived estimates closely mirrored mean concentrations (ng/L range) using repeated grab sampling.
Introduction

As an emerging class of contaminants, endocrine active chemicals (EACs) have the potential to adversely affect highly-valued and critical populations in aquatic ecosystems (1, 2, 3). Adverse effects typically have been observed when exposure is chronic and at very low concentrations (4, 5). So, the ability to quantify low-level, chronic exposure to EACs in surface waters is essential to assessing ecosystem health and further, in characterizing and managing risks associated with EACs. EACs of particular importance, natural and synthetic hormones, are found in both human and animal wastes and have the potential to contaminate surface waters (6-8). Because endogenous androgens and estrogens program aspects of sex differentiation and reproduction during development, exogenous steroid exposure during those susceptible windows can result in detrimental and irreversible alterations in development and by extension, reproductive success of the population (2, 9-11).

So, detection and quantification of emerging contaminants are of great importance to environmental and public health protection. Surface water monitoring of natural and synthetic steroid hormones can prove difficult because toxicologically-significant concentrations are often low, at the part-per-trillion (ppt) level (2, 12). While detecting low, toxicologically-significant concentrations is possible by traditional methods (grab sampling), this sampling only gives an exposure estimate of a single point in time. To obtain an estimate of chronic exposure, grab samples must be taken frequently. Developments in sampling technology, namely passive sampling devices (PSDs), have allowed for estimations of chronic exposure for a broad suite of chemicals. PSDs concentrate contaminants over time, hence integrating exposure over time. Prior to use in field exposure
estimation, PSDs are calibrated under controlled conditions in the laboratory to determine
effective sampling rates for each compound, and effective sampling rates are determined
during an initial period of linear uptake using the following equation,

$$ R_s = \frac{N_t}{C_w t} $$

where $R_s$ is the sampling rate (L/d), $N_t$ is the amount (ng) of analyte accumulated in the
sampler at the end of exposure, time in days, $C_w$ is the average concentration of freely-
dissolved analyte in water (ng/L), and the duration of the exposure, $t$, where time is
measured in days (13). Once laboratory-derived sampling rates have been determined, eq 1
can be rearranged to solve for $C_w$, the freely-dissolved field exposure estimate (ng/L),

$$ C_w = \frac{N_t}{R_s t} $$

where $N_t$ is the amount (ng) of analyte in the PSD at the end of the deployment period, $t$, in
days, $R_s$ is the laboratory-derived sampling rate (L/d), and $t$ is the duration of the
deployment in days (13).

So, estimating exposure in the field using eq 2 requires laboratory-derived sampling
rates, determined under a controlled flow-through system. Differences in field conditions
compared to calibration conditions, such as stream flow velocity, temperature, or biofouling,
can affect the uptake rate of chemical into the PSD (14). Assuming isotropic exchange
kinetics, changes in uptake rate are directly proportional to changes in elimination, and so
the use of performance reference compounds (PRCs) have been used to translate
environmental changes in elimination to changes in uptake (15, 16). PRCs are analytically
non-interfering compounds that share similar chemical properties to compounds of interest
and are added to samplers prior to deployment in the field (14). Elimination over the
duration of the deployment can be modeled assuming first-order release kinetics using the following equation (15),

\[ N_t = N_0 \times e^{-k_e t} \]  

(3)

where \( N_t \) is the amount (ng) of PRC remaining in the sampler at time \( t \) in days, \( N_0 \) is the amount (ng) of PRC initially loaded into the sampler, \( k_e \) is the elimination or exchange rate constant (d\(^{-1}\)), and \( t \) is the time in days. By comparing laboratory PRC \( k_e \) values to field PRC \( k_e \) values, an environmental adjustment factor (EAF) can be calculated and applied to resultant PSD-estimated water concentrations (14),

\[ EAF \equiv \frac{k_e^{PRC-f}}{k_e^{PRC-cal}} \]  

(4)

where \( k_e^{PRC-f} \) represents the PRC elimination rate constant in the field (d\(^{-1}\)) and \( k_e^{PRC-cal} \) represents the PRC elimination rate constant determined from the laboratory calibration experiment (d\(^{-1}\)).

Most PSDs, such as the semipermeable membrane device (SPMD) (17) and polyethylene samplers (18), are designed to sample compounds sharing similar chemical properties, namely hydrophobic organic compounds. Few samplers have been developed to estimate aquatic exposure to polar compounds; those samplers include the organic version of the Chemcatcher (19) and the polar organic chemical integrative sampler (POCIS) (20). However, the development of a more universal PSD (uPSD) to monitor compounds differing in physicochemical properties concurrently would be a tremendous advantage in assessing risks associated with numerous classes of compounds, from pesticides to steroid hormones. Further, the development of uPSDs to integrate steroid hormone exposure over time would provide a tool for better understanding chronic aquatic exposure of hormones in future.
environmental assessments. The purpose of this work is to present the first laboratory-
derived sampling rates for eight select androgens and estrogens in a cartridge-style uPSD
developed in our laboratory, determine if flow-rate affects elimination kinetics from the
uPSD, and to provide a first field verification of cartridge uPSD sampling rates for those
steroid hormones.

**Experimental Section**

**Materials.** Acetone (ACE), dichloromethane (DCM), and hexane were purchased from J.T.
Baker Inc. (Phillipsburg, NJ). Acetonitrile (ACN) and ethyl acetate were purchased from
Burdick and Jackson™ (Muskegon, MI). Methanol was purchased from Fisher Scientific
(Fairlawn, NJ). All hormone standards were brought up in methanol or acetonitrile.
Androstenedione (A) was purchased from Steraloids Inc. (Newport, RI). Epitestosterone,
17β-testosterone, estrone (E1), 17α-estradiol (αE2), 17β-estradiol (E2), estriol (E3), ethynyl
estradiol (EE2), and diethylstilbestrol (DES), used as a surrogate internal standard, were
purchased from Sigma Aldrich Co. (St. Louis, MO). Recovery internal standard, benzo-a-
pyrene-d_{12}, was purchased from Cambridge Isotope Laboratory (Andover, MA). 17β-
estradiol-2,4,16,16-d_{4} was used as a performance reference compound (PRC) (C/D/N
Isotopes Inc., Quebec, Canada). The derivatizing reagents, trimethylsilyldimethylamine with
10% hexamethyldisilazane (TMSDMA + 10% HMDS), were purchased from Fisher
Scientific Inc. (Pittsburg, PA) and Sigma Aldrich Co. (St. Louis, MO), respectively, and the
derivatizing solvent, acetonitrile with 100mM n-methylmorpholine and 50mM
trifluoroacetic acid (ACN w/ 100mM nMM and 50mM TFA), components were purchased
from Sigma Aldrich Co. (St. Louis, MO). Oasis® HLB SPE columns were purchased from Waters Corporation (Milford, MA).

Cartridge uPSDs were constructed using stainless steel inlet filters manufactured by Upchurch Scientific (Oak Harbor, WA) and purchased through Fisher Scientific Inc. (Pittsburg, PA), having a 10μm pore diameter, external diameter of 1.25cm, length of 2.8cm, and interior surface area of approximately 6.19 cm². Inlet filters were packed with 200 ± 1 mg Oasis® HLB sorbent (Waters Corporation, Milford, MA) and enclosed with an impermeable Teflon frit. Cartridge uPSDs were washed prior to use in 6 1-hour repeats using DCM, alternating with ACE. Cartridge uPSDs used on laboratory and field experiments were fortified with 500ng PRC per device immediately prior to deployment.

All glassware was silanized using Sylon CT (Sigma Aldrich Co., St. Louis, MO) before use. Aluminum foil was baked (300 °C) overnight before use, and all glassware was solvent-rinsed using ACE, DCM, and HEX and then baked (300 °C) overnight prior to use.

**Uptake Experiment.** An uptake experiment was conducted, exposing cartridge uPSDs to selected androgens and estrogens. The experiment utilized a flow-through system that pumped a mixture of selected androgens and estrogens at 6 mL/min to a 4-L amber glass bottle with a 100 ng/L nominal concentration of each hormone. Cartridge uPSDs were suspended in the 4-L amber bottle, and mixing was induced by shaking the exposure bottle at a constant speed of 150rpm on a shaker table for the duration of the exposure. Cartridge uPSDs were removed in triplicate on days 0, 1, 5, 10, 15, 20, 27, and 30 and either processed immediately or stored at -20 °C and analyzed within 48 hours after sampling. One-liter water samples were collected three times per week to ensure constant exposure. Water
samples not immediately processed were stored at 4°C and extracted within 48 hours after sampling.

Elimination Experiment. A flow-through elimination system was created to simulate 3 stream flow velocities within a single 19 L aquarium (~24 °C). Three channels were created within the aquarium, and varying flow velocities were achieved by altering channel dimensions such that channel 1 had the highest flow velocity of approximately 30 cm/s, channel 2 flow velocity was approximately 15 cm/s, and channel 3 had the slowest flow velocity of approximately 7 cm/s. Flow was maintained using pumps, one to recycle water within the system, another to supply new de-ionized water to maintain a constant water concentration of approximately zero. Cartridge uPSDs, loaded with 500ng of PRC and hormones, were suspended in the center of each channel for the duration of the 30-day experiment and were sampled in triplicate on days 0, 1, 5, 10, 15, 20, 27, and 30. Samples not immediately processed after sampling were stored at -20 °C and analyzed within 48 hours after storage.

Field Set-Up and Sample Collection. Cartridge uPSDs were deployed at two sites: 1) in the dilution zone of a municipal wastewater treatment plant outfall, and 2) in surface waters near a concentrated animal feeding operation (CAFO).

Cartridge uPSDs were deployed in triplicate downstream of a municipal wastewater treatment facility. At the end of the 30-day deployment, samplers were taken back to the laboratory, wiped clean, and immediately processed. Seven water samples (each consisting of a composite of six samples taken over a 24 hour period) were taken over the course of the
30-day period, on days 0, 4, 7, 12, 18, 24, and 30 and were filtered through a 0.7 μm glass-fiber filter (Whatman) prior to the processing below.

Cartridge uPSDs were deployed in triplicate in surface waters near a CAFO with grab samples of water collected every other day over the 30-day deployment period. At the end of deployment, uPSDs were transported to the laboratory, wiped clean, and immediately processed, as described below. The 15 water samples were filtered prior to extraction and extracted using the methods below.

**Sample Processing.** Prior to extraction, excess water was removed from cartridge uPSDs by centrifugation, placing samplers in a 15-mL plastic centrifuge tube and centrifuging at 3000 rpm for 15 min at 23 °C. 500 ng of the surrogate internal standard was run with each sample. Cartridge uPSDs were shaker-table extracted twice using 15 mL ethyl acetate for 1 hour at 180 rpm for each extraction. Eluent was transferred to a 50-mL glass vial and evaporated to dryness under a gentle stream of nitrogen at 50 °C. The sample was reconstituted in three 0.5-mL ethyl acetate rinses and filtered through a 0.45 μm PTFE Whatman® UNIPREP syringeless filter into a deactivated 2-mL glass autosampler vial and again evaporated to dryness under nitrogen. 200 μL of derivatization solvent, as well as 100 μL of derivatization reagent, were added to each autosampler vial. The vials were then sealed with PTFE-lined rubber crimp caps, and samples were then incubated at 60 °C for 1.25 hr. Samples were then cooled to room temperature, 500 ng internal standard was added, and brought up to ~ 0.5 mL in ACN prior to analysis by GC-MS.

Prior to extraction, water samples were spiked with 500 ng of surrogate internal standard. Water samples were then extracted using Oasis® HLB solid phase extraction
cartridges (6 mL, 500 mg; Waters Corporation, Milford, MA). Cartridges were conditioned with 5 mL ethyl acetate, washed with 5 mL MeOH, and equilibrated with 5 mL de-ionized water, and samples were then pressure-extracted at ~10 mL/min. Steroids were eluted with 5 mL ethyl acetate. Eluent was evaporated to dryness under a gentle stream of nitrogen at 50 °C. The sample was reconstituted in three 0.5-mL ethyl acetate rinses and filtered through a 0.45 μm PTFE Whatman® UNIPREP syringeless filter into a deactivated 2-mL glass autosampler vial and again evaporated to dryness under nitrogen. Samples were then derivatized, using the method described above for cartridge uPSD samples, brought up to ~0.5 mL in ACN, and analyzed by GC-MS.

**Instrumental Analysis.** All hormone quantification was performed by GC-MS, using an Agilent 6890 GC equipped with a Restek 30 m × 0.25 mm Rtx-5 (film thickness 0.25 μm) MS with Integra-Guard column, connected to an Agilent 5973 MSD. Extracts were injected in pulsed splitless mode and flow was kept constant at 0.9 mL/min for the duration of the run. The temperature program for hormone analysis was as follows: initial temperature 150 °C for 1 min with a ramp of 10 °C/min to 230 °C with no hold time and a second ramp of 25 °C/min to 310 °C and a final hold time of 8 min; transfer line temperature 290 °C, MS source temperature 300 °C. Selected ion monitoring (SIM) was used for analysis. Response factors were generated using a 7-point calibration curve (0.001 μg/mL – 1.0 μg/mL). Method detection limits for cartridge uPSDs ranged from <1 - <10 ng/device and were <1 ng/L for water samples. Information about hormone quantification and qualifier ions and retention times can be located in supporting information (Table SI 1).
Quality Control. Data quality was assessed using procedural blanks, cartridge uPSD blanks, matrix spikes, and replicate analyses, as well as surrogate internal standards. Method blanks were clean with no qualitative evidence of contamination. Surrogate and matrix spike recoveries were between 55 - 129%, and results were not corrected for these recoveries.

Results and Discussion

Uptake Curves. The eight androgens and estrogens (log $K_{ow}$s ranging from 2.5 – 4.0) investigated in this study are listed in Table 1. Uptake curves for four representative steroid hormones, two estrogens and two androgens, are shown in Figure 1; uptake curves for all hormones in this study are given in supporting information (see below). All hormones remained in the linear uptake phase throughout the 30-day period with variability among triplicates greatest at day 30 ($R^2 \geq 0.86$ for all 8 curves). This validates the appropriateness of using a linear model to derive sampling rates (eq 1) and also illustrates that linear uptake over a 4-week period can be assumed in field sampling.

Traditional PSDs, like SPMDs (17), demonstrate linear uptake over a 30-day period only for compounds with log $K_{ow} \geq 4.5$ (13, 15). For compounds with log $K_{ow} < 4.5$, deviations from linearity begin anytime from day 2 in polydimethylsiloxane devices (PDMS) (21) to days 7 to 15 in the SPMD (13). The use of traditional PSDs to estimate exposure to compounds, like hormones, with log $K_{ow} < 4.5$ poses a potential problem in assuming linear uptake over a typical sampling period (~ 4 weeks) (22), and thus, using a linear model (eq 1 and 2) to estimate $C_w$ would be inappropriate. While shortening the sampling period is possible to avoid deviations from linear uptake, the advantages of using
PSDs over traditional sampling methods starts to diminish when shortening sampling deployment.

Another factor that could influence the deployment duration of cartridge uPSDs is a nonzero intercept. In order to maintain the use of the linear model (eqs 1 and 2), deployment periods must be long enough to diminish the initial uptake contribution leading to nonzero intercepts. Intercepts observed in this study ranged from 6.4 – 15.6 ng (Figure 1). Although other studies have demonstrated that deployment periods of a few days are sufficient to negate the contribution of initial uptake (13, 15), Booij et al. (23) observed greater contributions of initial uptake that necessitated longer deployment windows, as high as 53 days. This study also established that a maximum intercept contribution equal to 10% of the total amount accumulated in the PSD was sufficient to estimate minimum exposure time required to negate nonzero intercepts in field deployment (23). By this criterion, minimum field exposures between 9 – 22 days for cartridge uPSDs were calculated using linear regressions generated from uptake curves in this study. So, typical field deployments of 3 – 4 weeks are suitable for providing measurable exposure to the androgens and estrogens from this study while negating contributions of initial uptake into the cartridge uPSD.

Differences in uptake were observed, with uptake slightly slower for the three estrogens, 17α-estradiol, 17β-estradiol, and estriol, compared to all three androgens and the two estrogens, estrone and ethynyl estradiol (SI figure 1). Log $K_{ow}$ does not appear to be an accurate predictor of uptake; this is not surprising due to the uPSD’s chemically-inactive stainless steel membrane which should not demonstrate any partitioning preference due to polarity. Slower uptake may be attributed to differences in functional groups among the
analytes, as both androgens and estrogens contain the same basic structure but differ in functional groups. The three hormones demonstrating slightly slower uptake contain two or more (sterically-unhindered) hydroxyl groups, unlike the other 5 hormones that contain zero or one hydroxyl groups. Ethynyl estradiol is an exception, as this compound has two hydroxyl groups; however, one of the hydroxyl groups may be sterically hindered due to the presence of a methyl functional group bonding at the same carbon. Ultimately, hydroxyl groups represent ionizable functional groups whose presence or absence may govern uptake or diffusion of steroid hormones into the sorbent matrix of the cartridge uPSD.

**Sampling Rates.** Effective sampling rates ($R_s$) for target androgens and estrogens were calculated using eq 1 at each time point beyond day one. If any analyte did not remain in the linear uptake phase, $R_s$’s would only be calculated within the region of linear uptake. Mean $R_s$’s are reported in Table 1 with corresponding standard deviations. Cartridge uPSD sampling rates were determined under controlled laboratory conditions, including a stipulation of using eq 1 requiring relatively constant aqueous concentrations of hormones ($C_w$), which was verified by collecting and analyzing samples at least three times every week. Average water concentrations ranged from 47 – 64 ng/L. $R_s$’s remained constant throughout the exposure (Table 1) and mean $R_s$’s were constant across analytes in this study, ranging from 0.09 – 0.11 L/d.

The only other PSD with published use for monitor steroid hormones in surface waters is the POCIS (20), which has been used in qualitative (ng/POCIS) or semi-quantitative (YES-screened extracts) until recently (1, 20). Arditoglou et al. (24) recently published laboratory-determined POCIS $R_s$’s for selected estrogens and progestogens. When
comparing $R_s$’s generated in this study to those generated by Arditsoglou et al. (24) for select estrogens, the apparent volume of water quantitatively extracted from the cartridge uPSD is lower than for the POCIS, with uPSD $R_s$’s ranging between 0.09 and 0.11 L/d and POCIS $R_s$’s between ~0.11 and 0.22 L/d (Table 1). However, a couple factors to consider include differences in surface area between the two PSDs and how $R_s$’s relate to log $K_{ow}$ values. POCIS surface area (~18 cm$^2$) (20) is considerably larger than the cartridge uPSD surface area (~6.19 cm$^2$); so, normalizing $R_s$’s to surface area would produce higher $R_s$’s per device for the cartridge uPSD. Additionally, when comparing $R_s$’s across log $K_{ow}$’s for estrogens, POCIS $R_s$’s appear to vary more across log $K_{ow}$’s. For instance, there is less than a 1.3-fold difference in uPSD $R_s$’s, compared to a ~2-fold difference in POCIS $R_s$’s, for 5 select estrogens (log $K_{ow}$ values ranging from 2.5 – 4.7, a 150-fold difference). Further research establishing uPSD $R_s$’s for compounds with wider ranging log $K_{ow}$’s is necessary to be able to definitively compare log $K_{ow}$-dependence of uPSD and POCIS $R_s$’s. However, the relatively constant $R_s$’s observed in this study suggests that uPSD uptake rates may be independent (or less dependent) of $MW$ or log $K_{ow}$ values, and hence, a more universal $R_s$ may be used in the future for analytes without laboratory-calibrated $R_s$’s.

**Elimination Rate Constants.** Because environmental conditions have been shown to impact uptake rate in field deployments (14), an elimination calibration experiment was performed to determine the effect of one environmental variable, flow rate, on depuration. Assuming isotropic exchange kinetics, any observed change in elimination due to flow rate will proportionately impact uptake (15, 16).
In the elimination calibration experiment, cartridge uPSDs were initially loaded with a known amount of each steroid hormone and then subject to three different flow conditions (~30, 15, and 7 cm/s). Release kinetics to determine elimination rate, or exchange rate ($k_e$), constants were modeled using eq 3, which follows a first-order exponential elimination model and has been used previously to model elimination in other PSDs (13, 14, 25).

Elimination curves of one representative androgen and estrogen are presented in Figure 2. High variability observed among time points indicates potential problems with initial loading in this experiment. $k_e$ values determined using eq 3 ranged from 0.013 – 0.022 d$^{-1}$ in channel 1, 0.021 – 0.034 d$^{-1}$ in channel 2, and 0.006 – 0.020 d$^{-1}$ in channel 3 (Table 2), signifying no effect of flow rate on $k_e$. However, coefficients of correlation ($R^2$ values) are very low (Table 2), indicating elimination does not follow first-order kinetics; this observation is not surprising due to the biphasic nature of the uPSD sorbent, Oasis HLB®.

Additionally, there was no apparent correlation between log $k_e$ and log $K_{ow}$, which supports observations in the uptake experiment that mass transfer may be independent of physicochemical properties of analytes as long as water can get into the uPSD (little or no biofouling). Importantly though, these compounds are not expected to be under aqueous boundary layer control because log $K_{ow}$’s < 4.5. So regardless of stream flow velocity, the aqueous boundary layer would not be the rate-limiting step; rather mass transfer of these compounds would be under membrane control (19).

**Performance Reference Compounds.** Performance reference compounds have been utilized to allow the use of laboratory-derived $R_s$’s to more accurately estimate $C_w$ in-situ (eq 2) while accounting for differences between laboratory and field conditions, such as flow-
rate, biofouling, and temperature (14, 16, 26, 27). PRCs are chemically non-interfering compounds not normally found in nature that are loaded into the PSD prior to field deployment. PRC elimination is typically modeled assuming first-order exponential decay, eq 3, to determine a PRC $k_e$. Differences in laboratory and field PRC elimination are accounted for using eq 4 by calculating environmental adjustment factor (EAF), and ultimately, the EAF is applied to equation 3 to provide for a more accurate estimate of in-situ $C_w$.

As observed in the elimination experiment, the use of a $k_e$ determined by eq 3 is not appropriate because elimination from uPSDs does not appear to follow a first-order exponential model. So, in order to account for differences in laboratory and field conditions, ratios of percent PRC are used in field calculations,

$$EAF = \frac{[(N_{0,\text{field}} - N_{t,\text{field}})]}{[(N_{0,\text{cal}} - N_{t,\text{cal}})]}$$

(5)

where $N_{0,\text{field}}$ is the amount (ng) initially loaded in the uPSD prior to field deployment, $N_{t,\text{field}}$ is the amount (ng) of PRC remaining in the uPSD at the end of the deployment, $N_{0,\text{cal}}$ is the amount (ng) of PRC initially measured in the uPSD in laboratory calibration, and $N_{t,\text{cal}}$ is the amount (ng) of PRC measured in the uPSD at the end of the laboratory calibration period.

**Field Verification.** The primary purpose of developing the uPSD in this work is to estimate average concentrations of androgens and estrogens in water. At the end of field deployment, uPSDs are analyzed for contaminants, and contaminant residues are used to estimate average water concentrations using eq 2. Applying eq 5 allows for PRC-corrected average water concentrations to account for differences between laboratory and field conditions. Using $R_s$
data in Table 1, we can estimate concentrations of steroid hormones in surface waters receiving inputs from a wastewater treatment plant and downstream of an agricultural area.

Concentrations of hormones dissolved in water were estimated using eq 2, the mean sampling rates (Table 1) and hormone residues in the field-deployed uPSDs. Concentrations were estimated first using only laboratory-derived $R_s$’s and then concentrations were estimated by applying a PRC-correction in samples collected in wastewater effluent (eq 5) (Table 3). These estimated concentrations were then compared to measured hormone concentrations from grab samples of wastewater effluent in Figure 3. The estimated:observed comparisons in Figure 3 were based on field measurements using the mean of seven grab samples over a 30-day period and the mean of triplicate cartridge uPSD residues deployed for 30 days. Approximations using mean uPSD residues were calculated both with and without a PRC-correction. In both approximations (with and without PRC correction), most hormones are overestimated, generally under a factor of two (Figure 3). Surprisingly, the mean uPSD estimates (without any PRC correction) were in good agreement with observed mean concentrations with ratios very close to one. With the exception of estriol (ratio of 1.52), all ratios for mean uPSD estimates without PRC correction, were between 0.75 and 1.25, indicating < 25% deviation from estimated to observed values. All PRC-corrected estimates were overpredictions with ratios ranging from 1.37 – 2.43 (Figure 3), indicating that PRC-corrected estimates demonstrated a larger deviation from observed values than estimates without a PRC correction.

Interestingly, in the case of epitestosterone ($\alpha$T), an estimated:observed ratio was not able to be calculated because $\alpha$T was not detected in any of the water samples. This does not
mean that αT was not present in the sampled water, rather that concentrations could have been below method detection limits for the volume of water extracted. Cartridge uPSD residues placed mean estimates between 0.12 – 0.18 ng/L, which although low, the estimates indicate that αT contributes to the overall androgenicity of the effluent (Table 3). So, the case of αT shows that while repetitive or exhaustive grab sampling may underestimate or completely miss exposure, the use of uPSDs allows very low chronic exposures to be captured and quantified.

Concentrations of hormones in surface waters near a CAFO were estimated using eq 2, the mean sampling rates (Table 1) and hormone residues in the field-deployed uPSDs. These estimated concentrations were then compared to measured hormone concentrations from surface water grab samples in Figure 4. The grab sample values were based on field measurements using the mean of 15 grab samples over a 30-day period, and uPSD-estimated values were calculated from the mean of triplicate cartridge uPSD residues deployed over the 30-day period. For all estrogens, uPSD-derived estimations were generally a factor of 2-3 below the grab sample means. Grab sample means ranged from 2.4 – 26.6 ng/L, and cartridge uPSD estimated means ranged from 1.59 – 18.6 ng/L (Figure 4). The systemic negative bias of hormone exposure across all estrogens by cartridge uPSD residues could be due to complexation of hormones by dissolved organic carbon (DOC). It is likely that the uPSD only accumulates hormones (or any other chemical) that are freely dissolved in water; chemicals complexed by DOC would not exchange with the uPSD polymer phase upon contact. In contrast, grab samples were filtered but then exhaustively extracted with organic solvent yielding a total concentration that would include freely dissolved and DOC-
complexed hormones. An additional explanation could be competition for uPSD sorption sites by other organic compounds in water. This would reduce the amount of hormone sorbed to the PSD. The standard conditions employed in the calibration of the uPSD used de-ionized water with no DOC or competing organic compounds.

So, cartridge uPSDs have proven useful in providing quantitative estimates of steroid hormone exposure. uPSDs offer many advantages over traditional sampling methodologies, including reducing cost by decreasing required sample size, saving time in both the laboratory and field by taking and processing fewer samples, representing the bioavailable fraction of exposure, and capturing and quantifying transient or trace exposure that could otherwise be missed by grab sampling. Additionally, elimination experiment results (Figure 2) and field validation (Figure 3) have demonstrated that PRC-correction may not be necessary to gain an estimate of chronic exposure to hormones using cartridge uPSDs.

**Supporting Information**

Additional information on all uptake curves and all elimination curves is provided. The material is available free of charge on the internet at http://pubs.acs.org.
References


Table 1. Summary of Sampling Rates and Select Physicochemical Properties of Steroid Hormones.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Symbol</th>
<th>MW&lt;sup&gt;a&lt;/sup&gt;</th>
<th>S&lt;sub&gt;w&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Log K&lt;sub&gt;ow&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Sampling Rates, R&lt;sub&gt;s&lt;/sub&gt; ± S.D.&lt;sup&gt;d&lt;/sup&gt;</th>
<th>POCTIS Sampling Rates, R&lt;sub&gt;p&lt;/sub&gt; ± S.D.&lt;sup&gt;e&lt;/sup&gt;</th>
<th>POCTIS Sampling Rates, R&lt;sub&gt;p&lt;/sub&gt; ± S.D.&lt;sup&gt;f&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Estrogens:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>nPSD</td>
<td>Pest-POCIS&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Pharm-POCIS&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Estrone</td>
<td>E1</td>
<td>270.4</td>
<td>0.8-13&lt;sup&gt;j,k&lt;/sup&gt;</td>
<td>2.5-3.4&lt;sup&gt;j,k&lt;/sup&gt;</td>
<td>0.11 ± 0.05</td>
<td>0.1292 ± 0.0121</td>
<td>0.1199 ± 0.0177</td>
</tr>
<tr>
<td>17α Estradiol</td>
<td>αE2</td>
<td>272.4</td>
<td>3.2-13&lt;sup&gt;j,l&lt;/sup&gt;</td>
<td>3.4-4.0&lt;sup&gt;j,k&lt;/sup&gt;</td>
<td>0.10 ± 0.05</td>
<td>0.1451 ± 0.0141</td>
<td>0.1216 ± 0.0031</td>
</tr>
<tr>
<td>17β Estradiol</td>
<td>E2</td>
<td>272.4</td>
<td>3.0&lt;sup&gt;j,k&lt;/sup&gt;</td>
<td>3.1-4.0&lt;sup&gt;j,k&lt;/sup&gt;</td>
<td>0.10 ± 0.05</td>
<td>0.1144 ± 0.0150</td>
<td>0.1145 ± 0.0139</td>
</tr>
<tr>
<td>Estriol</td>
<td>E3</td>
<td>288.4</td>
<td>30.2&lt;sup&gt;j,k&lt;/sup&gt;</td>
<td>2.5-2.8&lt;sup&gt;j,k&lt;/sup&gt;</td>
<td>0.09 ± 0.05</td>
<td>0.1305 ± 0.0098</td>
<td>0.1571 ± 0.0041</td>
</tr>
<tr>
<td>Ethynyl estradiol</td>
<td>EE2</td>
<td>296.4</td>
<td>19.1&lt;sup&gt;j,k&lt;/sup&gt;</td>
<td>3.7-4.7&lt;sup&gt;j,k&lt;/sup&gt;</td>
<td>0.11 ± 0.05</td>
<td>0.2137 ± 0.0456</td>
<td>0.2217 ± 0.0525</td>
</tr>
<tr>
<td><strong>Androgens:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>nPSD</td>
<td>Pest-POCIS&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Pharm-POCIS&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>A</td>
<td>286.4</td>
<td>37-41&lt;sup&gt;j&lt;/sup&gt;</td>
<td>NA&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.11 ± 0.05</td>
<td></td>
<td></td>
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<tr>
<td>Epitestosterone</td>
<td>αT</td>
<td>288.4</td>
<td>NA&lt;sup&gt;g&lt;/sup&gt;</td>
<td>NA&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.11 ± 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17β Testosterone</td>
<td>T</td>
<td>288.4</td>
<td>18-25&lt;sup&gt;j&lt;/sup&gt;</td>
<td>3.0-3.6&lt;sup&gt;j&lt;/sup&gt;</td>
<td>0.11 ± 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Molecular Weight (g mol<sup>-1</sup>); <sup>b</sup> Water Solubility (mg L<sup>-1</sup>); <sup>c</sup> Octanol-Water Partition Coefficient; <sup>d</sup> Standard deviation, R<sub>s</sub> in L d<sup>-1</sup>; <sup>e</sup> Not Available; <sup>f</sup> Value from Ternes et al. 1999 [28]; <sup>g</sup> Value from Lee et al. 2003 [29]; <sup>h</sup> Value from Hanselman et al. 2003 [30]; <sup>i</sup> Value from Lai et al. 2002 [31]; <sup>j</sup> Value from Lai et al. 2000 [32]; <sup>k</sup> Value from Yamamoto et al. 2003 [33]; <sup>l</sup> Value from Johnson et al. 2006 [7]; <sup>m</sup> Arditisoglou et al. 2008 [24].
Table 2. Summary of Elimination Rates ($k_e$, d^{-1}) of Steroid Hormones under Different Flow Velocities: Channel 1 (~ 30 cm s^{-1}), Channel 2 (~ 15 cm s^{-1}), and Channel 3 (~ 7 cm s^{-1}).

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Channel 1</th>
<th>Channel 2</th>
<th>Channel 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_e$</td>
<td>$R^2$</td>
<td>$k_e$</td>
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<tr>
<td>Androstenedione</td>
<td>0.013</td>
<td>0.13</td>
<td>0.022</td>
</tr>
<tr>
<td>Epitestosterone</td>
<td>0.013</td>
<td>0.11</td>
<td>0.021</td>
</tr>
<tr>
<td>17β-Testosterone</td>
<td>0.013</td>
<td>0.10</td>
<td>0.021</td>
</tr>
<tr>
<td>$d_5$-Estradiol (PRC)</td>
<td>0.019</td>
<td>0.16</td>
<td>0.030</td>
</tr>
<tr>
<td>17α-Estradiol</td>
<td>0.019</td>
<td>0.16</td>
<td>0.030</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>0.020</td>
<td>0.17</td>
<td>0.031</td>
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<td>Estriol</td>
<td>0.022</td>
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<td>0.034</td>
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<td>Estrone</td>
<td>0.021</td>
<td>0.22</td>
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<tr>
<td>Ethynyl estradiol</td>
<td>0.017</td>
<td>0.16</td>
<td>0.022</td>
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</table>
Table 3. Mean Water Concentrations Measured from 7 Grab Samples, Estimated from Triplicate Cartridge uPSD Residues, and Estimated Using a PRC Correction from Triplicate Cartridge uPSD Residues.

<table>
<thead>
<tr>
<th>HORMONE</th>
<th>Mean Measured [Water]$^a$</th>
<th>Mean [uPSD]$^a$</th>
<th>PRC-Corrected Mean [uPSD]$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epitestosterone</td>
<td>0.00 ± 0.00</td>
<td>0.12 ± 0.01</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>17α-Estradiol</td>
<td>0.89 ± 0.51</td>
<td>1.06 ± 0.13</td>
<td>1.67 ± 0.08</td>
</tr>
<tr>
<td>Estriol</td>
<td>1.69 ± 0.93</td>
<td>2.57 ± 0.09</td>
<td>4.09 ± 0.36</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>2.27 ± 1.65</td>
<td>2.61 ± 0.41</td>
<td>4.16 ± 0.70</td>
</tr>
<tr>
<td>Ethynyl estradiol</td>
<td>2.50 ± 1.05</td>
<td>2.16 ± 0.26</td>
<td>3.43 ± 0.35</td>
</tr>
<tr>
<td>Estrone</td>
<td>2.77 ± 1.41</td>
<td>3.25 ± 0.36</td>
<td>5.19 ± 0.75</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>3.63 ± 1.17</td>
<td>3.79 ± 0.57</td>
<td>6.06 ± 1.28</td>
</tr>
<tr>
<td>β-Testosterone</td>
<td>4.11 ± 2.01</td>
<td>4.26 ± 0.54</td>
<td>6.85 ± 1.50</td>
</tr>
</tbody>
</table>

$^a$ concentrations ± s.d. (standard deviation) in ng L$^{-1}$
FIGURE LEGENDS

Figure 1. Uptake curves for four representative steroid hormones, two estrogens and two androgens: 17β-estradiol, ethynyl estradiol, epitestosterone, and 17β-testosterone. (●)s represent means, and vertical lines represent standard deviations (n = 3) of cartridge uPSD samples. Based on linear regression, $R^2 \geq 0.86$ for all 8 uptake curves.

Figure 2. Elimination curves of two representative steroid hormones, one estrogen and one androgen: 17β-estradiol and epitestosterone, subject to three different flow velocities: 30 cm/s in channel 1 (●), 15 cm/s in channel 2 (○), and 7.5 cm/s in channel 3 (▼). Vertical lines represent standard deviations of triplicate cartridge uPSD samples.

Figure 3. Ratio of freely dissolved steroid hormone concentrations derived from cartridge uPSD residues (predicted) to that measured in filtered water collected downstream from municipal wastewater treatment plant with (○) and without corrections using PRCs (●), with vertical bars representing standard deviations based on triplicate values.

Figure 4. Mean surface water estrogen concentrations (ng/L) measured from grab samples near a concentrated animal feeding operation, n = 15, (●) and estimated from cartridge uPSD residues, n = 3, (○), with vertical bars representing standard deviations.
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SUPPORTING INFORMATION

for

Calibration and Field Verification of a Cartridge-Based Passive Sampling Device for Estimating Chronic Exposure to Steroid Hormones in Water

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Supporting Information provides uptake curves representing all 8 androgens and estrogens in this study, as well as elimination curves under different flow velocities for all 8 hormones in this study.
Table SI 1. Summary of chromatographic and mass spectrometric parameters used to identify and quantify hormones during analysis. (Retention time in minutes; quantification and qualification ions based on $m/z$ charge. RIS, recovery internal standard; SIS, surrogate internal standard; PRC, performance reference compound.)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time</th>
<th>Quantification Ion</th>
<th>Qualification Ion 1</th>
<th>Qualification Ion 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>benzo-a-pyrene-$d_{12}$</td>
<td>RIS</td>
<td>14.49</td>
<td>264.2</td>
<td>260.1</td>
</tr>
<tr>
<td>diethylstilbestrol</td>
<td>SIS</td>
<td>11.733</td>
<td>412.2</td>
<td>413.2</td>
</tr>
<tr>
<td>17β-estradiol-2,4,16,16-$d_{4}$</td>
<td>PRC</td>
<td>13.219</td>
<td>420.3</td>
<td>287.2</td>
</tr>
<tr>
<td><strong>Estrogens:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17α-estradiol</td>
<td></td>
<td>13.025</td>
<td>416.3</td>
<td>285.2</td>
</tr>
<tr>
<td>17β-estradiol</td>
<td></td>
<td>13.249</td>
<td>416.3</td>
<td>285.2</td>
</tr>
<tr>
<td>estriol</td>
<td></td>
<td>14.254</td>
<td>504.3</td>
<td>345.2</td>
</tr>
<tr>
<td>estrone</td>
<td></td>
<td>13.109</td>
<td>342.2</td>
<td>257.1</td>
</tr>
<tr>
<td>ethynyl estradiol</td>
<td></td>
<td>13.81</td>
<td>425.2</td>
<td>440.3</td>
</tr>
<tr>
<td><strong>Androgens:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>androstenedione</td>
<td></td>
<td>13.245</td>
<td>286.2</td>
<td>244.2</td>
</tr>
<tr>
<td>17α-testosterone</td>
<td></td>
<td>13.083</td>
<td>360.2</td>
<td>270.2</td>
</tr>
<tr>
<td>17β-testosterone</td>
<td></td>
<td>13.411</td>
<td>360.2</td>
<td>270.2</td>
</tr>
</tbody>
</table>
SI FIGURE LEGENDS

**Figure SI 1.** Uptake curves for 8 steroid hormones, 5 estrogens and 3 androgens: estrone, 17α-estradiol, 17β-estradiol, estriol, ethynyl estradiol, androstenedione, epitestosterone, and 17β-testosterone. (●) represent means, and vertical lines represent standard deviations (n = 3) of cartridge uPSD samples. Based on linear regression, $R^2 \geq 0.86$ for all 8 uptake curves.

**Figure SI 2.** Elimination curves of 8 steroid hormones, 6 estrogens and 3 androgens: estrone, 17α-estradiol, 17β-estradiol, estriol, ethynyl estradiol, 17β-estradiol-2,4,16,16-$d_4$ (PRC), androstenedione, epitestosterone, and 17β-testosterone, subject to three different flow velocities: 30 cm/s in channel 1 (●), 15 cm/s in channel 2 (○), and 7.5 cm/s in channel 3 (▼).
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Chapter 2. Development and Field Validation of a Fiber-Based Passive Sampling Device for Measuring Free Estrogens and Androgens in Surface Waters

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Abstract. Aqueous sampling rates for eight steroid hormones were determined for a novel passive sampling device (PSD), the fiber universal passive sampling device (uPSD). While the calibration in this work targeted estrogens and androgens, the fiber uPSD was designed with the potential for sampling a wide suite of contaminants differing in physicochemical properties. Over the 30-day uptake experiment, all hormones remained in the linear uptake phase with sampling rates ranging from 0.04 – 0.09 L/d. A 30-day elimination experiment was conducted to determine the effect of flow rate on elimination; no correlation was observed for six of the eight hormones studied, indicating that the use of performance reference compounds (PRCs) to relate differences in field to calibration conditions may not be necessary for differences in flow rate. Field deployments of fiber uPSDs in surface waters downstream of municipal wastewater discharge had time-weighted average (TWA) concentrations estimated using laboratory-derived sampling rates that were consistent with routine grab sampling. Ultimately, this work indicates that fiber uPSDs can be used as a quantitative tool for measuring steroid hormones in surface waters.
INTRODUCTION

Natural and synthetic hormones and a number of pharmaceuticals and personal care products (PPCPs) and other compounds comprise a group of emerging surface water contaminants known jointly as endocrine active compounds (EACs). EACs are discharged into surface waters via wastewater treatment plant effluent, runoff from waste-amended fields, or agricultural waste lagoon spills (1-4). In particular, natural and synthetic hormones are used in agriculture for efficient growth production in animals or given to humans for contraception and various hormone therapies. EACs have the potential to adversely impact various aquatic populations by acting as steroid receptor agonists or antagonists (5). Sex steroids irreversibly program aspects of sex differentiation and reproduction early in development through their receptor activity (6). So, exogenous exposure to EACs could result in adverse and irreversible alterations in development in individuals, which potentially could impact the reproductive success of the population (7, 8).

Surface water monitoring of pollutants is of great importance to protecting environmental and public health. Further, developing sampling technologies to encompass emerging contaminants, such as synthetic and natural hormones, in addition to more historic pollutants, like PAHs or PCBs, is important in gaining a full understanding of environmental exposure.

While grab sampling is used as a current sampling methodology for natural and synthetic hormones, this technique is limiting. For contaminants like steroid hormones where chronic exposure is the primary concern, grab sampling fails to offer a measure of chronic exposure unless intensive sampling is conducted over a duration of time, potentially
posing problems in feasibility. In addition, where the fraction of exposure freely-dissolved, or biologically available (bioavailable), is most important in relating measured exposure to actual exposure, grab sampling does not give an estimate of the bioavailable exposure. However, sampling technology has evolved with the development of numerous passive sampling devices (PSDs), including the most-studied and well-known semipermeable membrane device (SPMD) (9). PSDs passively extract contaminants from surrounding water, concentrating them to yield a time-integrated exposure estimate of the bioavailable fraction. Most PSDs are designed to target sampling of specific classes of compounds, specifically more hydrophobic organic contaminants (10); those samplers developed to monitor more polar compounds include the polar organic chemical integrative sampler (POCIS) (11) for surface waters and the organic version of the Chemcatcher (12) for groundwater. However, in order to determine environmental exposure to a full suite of contaminants varying in physicochemical properties, multiple sampling technologies or strategies must be employed, which can be costly, require additional time-consuming analysis, and may ultimately exclude certain compounds from monitoring efforts (13). If polar compounds, like EACs, become incorporated into routine environmental monitoring, a more holistic approach to environmental sampling will be required. Further, the development of a more universal PSD (uPSD) with the ability to sample compounds with a wide range of physicochemical properties would be an important tool for future environmental assessment and monitoring efforts. The objectives of the current study were to determine sampling rates of eight androgens and estrogens commonly found in surface waters in a novel cartridge-style uPSD and to use these sampling rates to determine steroid
hormone concentration estimates in surface waters impacted by WWTPs and agricultural operations.

**EXPERIMENTAL SECTION**

*Chemicals and Materials*

Epitestosterone, 17β-testosterone (T), estrone (E1), 17α-estradiol (αE2), 17β-estradiol (E2), estriol (E3), ethynyl estradiol (EE2), and diethylstilbestrol (DES), used as a surrogate internal standard, were purchased from Sigma Aldrich Co. (St. Louis, MO), and androstenedione (A) was purchased from Steraloids Inc. (Newport, RI). All hormone standards were prepared in acetonitrile or methanol. 17β-estradiol-2,4,16,16-\textit{d}_4, used as a performance reference compound (PRC), was purchased from C/D/N Isotopes Inc. (Quebec, Canada), and benzo-a-pyrene-\textit{d}_{12}, used as a recovery internal standard, was purchased from Cambridge Isotope Laboratories (Andover, MA). Acetone (ACE), dichloromethane (DCM), and hexane were purchased from J.T. Baker Inc. (Phillipsburg, NJ). Acetonitrile (ACN) and ethyl acetate were purchased from Burdick and Jackson™ (Muskegon, MI), and methanol was purchased from Fisher Scientific (Fairlawn, NJ). Trimethylsilyldimethylamine with 10% hexamethyldisilazane (TMSDMA + 10% HMDS) was used as the derivatizing reagent. TMSDMA was purchased from Fisher Scientific Inc. (Pittsburg, PA), and HMDS was purchased from Sigma Aldrich Co. (St. Louis, MO). The derivatizing solvent used was acetonitrile with 100mM n-methylmorpholine and 50mM trifluoroacetic acid (ACN w/ 100mM nMM and 50mM TFA), with components purchased from Sigma Aldrich Co. (St.
Louis, MO). Oasis® HLB SPE columns were purchased from Waters Corporation (Milford, MA).

Glassware was silanized using Sylon CT (Sigma Aldrich Co., St. Louis, MO) before use, and all glassware was solvent-rinsed using ACE, DCM, and HEX and then baked (300 °C) overnight prior to use. Aluminum foil was baked (300 °C) overnight before use.

Fiber–uPSD Construction and Preparation

Fiber uPSDs were constructed using tubular polyethersulfone membranes purchased through Spectrum Laboratories, Inc. (Rancho Dominguez, CA), having a 0.2 μm pore diameter, internal diameter of 1.0 mm, and once prepared had length of ~ 18.4 cm and surface area of approximately ~5.8 cm². Membranes were packed with Oasis® HLB sorbent (Waters Corporation, Milford, MA), cut in half, the ends were tied closed with cotton string (~ 31.6 cm length), and the fiber uPSDs were weighed (90 – 95 mg). Fiber uPSDs were washed prior to use in 3 1-hour repeats using MeOH and dried overnight in a fume hood. Fiber uPSDs were fortified with 500 ng PRC per device immediately prior to deployment.

Uptake Experiment

Fiber uPSDs were exposed to selected androgens and estrogens in an uptake calibration experiment. A flow through system was constructed in which mixture of selected androgens and estrogens were pumped at 6 mL/min to a 4-L amber glass bottle with a 100 ng/L nominal concentration of each hormone in de-ionized water. Fiber uPSDs were suspended in the 4-L amber bottle, and mixing was induced by shaking the exposure bottle at 150rpm on a shaker table for the duration of the 30-day exposure. Fiber uPSDs were sampled in triplicate on days 0, 1, 5, 10, 15, 20, 25, and 30 and either processed immediately
or stored at -20 °C and analyzed within 48 hours after sampling. One-liter water samples were collected three times a week to ensure constant water concentrations of all hormones. Those water samples not immediately processed were stored at 4°C and extracted within 48 hours after sampling.

Elimination Experiment

An elimination experiment was conducted, determining the effects of flow rate on elimination of hormones in fiber uPSDs. A flow-through system was created within a 19-L aquarium by dividing the aquarium into three channels and maintaining constant flow through a pump system. Three different flow velocities, simulating stream flow, were achieved by altering channel dimensions such that channel 1 had the highest flow velocity of approximately 30 cm/s, channel 2 flow velocity was approximately 15 cm/s, and channel 3 had the slowest flow velocity of approximately 7 cm/s. Fiber uPSDs were loaded with 500 ng of PRC prior to use and then suspended across the center of each channel for the duration of the 30-day experiment. They were sampled in triplicate on days 0, 1, 5, 10, 15, 20, 25, and 30. Those samples not immediately processed after sampling were stored at -20 °C and analyzed within 48 hours after storage.

Field Set-Up and Sample Collection

Fiber uPSDs were deployed at two sites: 1) downstream of a municipal wastewater treatment plant, and 2) in surface waters near a concentrated animal feeding operation (CAFO).

Fiber uPSDs were deployed in triplicate downstream of a municipal wastewater treatment facility. At the end of the 30-day deployment, samplers were taken back to the
laboratory, wiped clean, and immediately processed as described below. Seven water samples (each consisting of a composite of six samples taken over a 24 hour period) were taken over the course of the 30-day period, on days 0, 4, 7, 12, 18, 24, and 30 and were filtered through a 0.7 μm glass-fiber filter (Whatman) prior to the processing below.

Fiber uPSDs were deployed in triplicate in surface waters near a CAFO with water grab samples collected every other day over the 30-day deployment period. At the end of deployment, uPSDs were transported to the laboratory, wiped clean, and immediately processed, as described below. The 15 water samples were filtered prior to extraction, methods are listed below.

Sample Processing

Prior to extraction, fiber uPSDs were gently wiped with a light-duty tissue wiper (VWR International, West Chester, PA) to remove any excess water on the fiber. 500 ng of DES, the surrogate internal standard, was run with each sample. Fiber uPSDs were extracted in 2-mL glass serological pipets connected to a SPE manifold via adapters using 1.5 mL ethyl acetate in 2 1-hr static extractions. Eluent was collected in 5-mL glass vials and evaporated to dryness under a gentle stream of nitrogen (50 °C). The sample was reconstituted in ethyl acetate with 3 0.5-mL rinses, transferred to a 0.45 μm PTFE Whatman® UNIPREP syringeless filter reservoir, and filtered into a 2-mL deactivated autosampler vial. Again, the sample was evaporated to dryness under a gentle stream of nitrogen. Samples were then derivatized by adding 200 μL of derivatization solvent, as well as 100 μL of derivatization reagent, sealing with PTFE-lined rubber crimp caps, and
incubating at 60 °C for 1.25 hr. After derivatization, samples were cooled to room temperature, 500 ng internal standard was added, and samples were analyzed by GC-MS.

Water samples were spiked with 500 ng of surrogate internal standard prior to extraction and were extracted using Oasis® HLB solid phase extraction cartridges (6 mL, 500 mg; Waters Corporation, Milford, MA). Cartridges were conditioned with 5 mL ethyl acetate, washed with 5 mL MeOH, and equilibrated with 5 mL de-ionized water, and samples were then loaded and pressure-extracted at ~ 10 mL/min. Hormones were eluted with 5 mL ethyl acetate, and eluent was collected in a 10-mL glass vial and evaporated to dryness under a gentle stream of nitrogen (50 °C). Hormones were reconstituted in ethyl acetate in 3 0.5-mL rinses, transferred to a 0.45 μm PTFE Whatman® UNIPREP syringeless filter reservoir, and then filtered into a deactivated 2-mL glass autosampler vial. Again, samples were evaporated to dryness under a gentle stream of nitrogen and then derivatized, using the method described above for cartridge uPSD samples. Samples were spiked with 500 ng of recovery internal standard and brought to ~ 0.5 mL with ACN prior to analysis by GC-MS.

Instrument Analysis and Quality Control

Hormone quantification was performed using an Agilent 6890 GC equipped with a Restek 30 m × 0.25 mm Rtx-5 MS, 0.25 μm film thickness, with Integra-Guard column, connected to an Agilent 5973 MSD. Samples were injected in pulsed splitless mode with flow maintained at 0.9 mL/min throughout the run. The temperature program for hormone analysis was as follows: initial temperature of 150 °C for 1 min with a ramp of 10 °C/min to 230 °C and an immediate second ramp of 25 °C/min to 310 °C with a final hold time of 8
min; transfer line temperature of 290 °C, MS source temperature of 300 °C. Selected ion monitoring (SIM) was used for analysis. Response factors were generated using a 7-point calibration curve (0.001 μg/mL – 1.0 μg/mL). Method detection limits for fiber uPSDs ranged from <1 - <10 ng/device and were <1 ng/L for water samples.

Data quality was assessed using procedural blanks, fiber uPSD blanks, matrix spikes, and replicate analyses, as well as surrogate internal standards. Method blanks were clean with no qualitative evidence of contamination. Surrogate and matrix spike recoveries were between 58 - 122%, and results were not corrected for these recoveries.

RESULTS AND DISCUSSION

Calibration Experiment

*Passive sampling theory.* Similar to other PSDs, fiber uPSDs must be calibrated under laboratory-controlled conditions prior to use in the field (11, 14). Calibration experiments, generally involving an uptake experiment, generate uptake curves for analytes of interest and integrative sampling rates, ultimately used to calculate time-weighted-average (TWA) exposure estimates in field samples. Sampling rates for fiber uPSDs are calculated using eq 1,

$$R_s = \frac{N_t}{C_w t} \quad (1)$$

where $R_s$ is the sampling rate (L/d), $N_t$ is the amount (ng) of analyte accumulated in the sampler over the duration of exposure, $C_w$ is the average concentration of freely-dissolved analyte in water (ng/L), and $t$ is the duration of the exposure where time is measured in days (15).
Estimates of chronic exposure ($C_w$) can be determined from field sample fiber uPSD residues by rearranging equation 1 such that,

$$C_w = \frac{N_t}{R_s t}$$  \hspace{1cm} (2)

where $N_t$ is the amount (ng) of analyte in the uPSD at the end of the deployment period, $t$, in days, $R_s$ is the laboratory-derived sampling rate (L/d), and $t$ is the duration of the deployment in days ($15$).

Similarly, release kinetics can be used to calibrate PSDs or to monitor release of performance reference compounds (PRCs) used in the field. Elimination has traditionally been modeled using a first-order exponential decay equation,

$$N_t = N_0 \times e^{-k_e t}$$  \hspace{1cm} (3)

where $N_t$ is the amount (ng) of PRC remaining in the sampler at the end of the sampling period, $N_0$ is the amount (ng) of PRC initially loaded into the sampler, $k_e$ is the elimination or exchange rate constant (d$^{-1}$), and $t$ is the duration of the sampling period in days ($16$).

**Uptake curves.** Three distinct phases have been observed in uptake curves for PSDs, an initial linear phase, central curvilinear portion, and final equilibrium phase ($17$). Hence, samplers can be calibrated for use as an equilibrium sampler if curves approach the asymptotic equilibrium or an integrative sampler if curves remain in the linear portion of the uptake curve. Deviations from linearity over the course of a laboratory calibration or field deployment have been observed with other PSDs ($15$, $16$, $18$). Those samplers that demonstrate deviations from linearity such that equilibrium is reached on the uptake curve have been used as equilibrium samplers in the field ($19$). However, samplers with curves not yet at equilibrium or those in the curvilinear portion of the uptake curve cannot be used...
unless field deployment is shortened to ensure that compounds remain in linear uptake throughout the duration of deployment (18); importantly, advantages of PSDs, including time and cost, over traditional sampling methods (i.e. grab sampling) diminishes as sampling period is shortened. For some PSDs, deviations from linearity only occur for a group of compounds within their sampling suite; for instance, traditional PSDs like SPMDs (9) only show linear uptake over a 30-day period for analytes having log $K_{ow} \geq 4.5$, while those analytes with log $K_{ow} < 4.5$ have shown deviations from linearity as early as day 2 in polydimethylsiloxane devices (PDMS) (20) and 7 days in the SPMD (15). So, for steroid hormones or other more polar compounds, the use of traditional PSDs to determine a TWA exposure estimate is not realistic using a linear model.

The androgens and estrogens targeted in this study, along with select physicochemical properties, are listed in Table 1. The uptake kinetic curves of three representative hormones are shown in Fig. 1 (uptake curves for all eight hormones not shown). The fiber uPSD exhibited integrative uptake for all eight steroid hormones throughout the 30-day exposure with correlation coefficients of linear regression greater than 0.94 for all eight curves. Because all hormones remained in the linear uptake phase throughout the laboratory calibration, the use of a linear model (eq 1) to derive $R_s$‘s is appropriate and further, the use of eq 2 to estimate $C_w$ from field-deployed fiber uPSDs is appropriate for field deployments up to 30 days.

In addition to deviations from linearity, linear regressions of uptake curves with nonzero intercepts could necessitate shortening sampling periods. In this work, intercepts were determined by linear regression and ranged from -7.6 – 1.4 ng (Figure 1). Hence,
deployment periods must be sufficiently long to diminish the contribution of variability in initial uptake. Other studies have demonstrated deployment periods as short as a few days were sufficient to discount the contribution of initial uptake \((15, 21)\); however, longer deployment windows, as high as 53 days, have been required when contributions of initial uptake were greater \((16)\). Booij et al. \((16)\) established a criterion for field deployment that the maximum intercept contribution equal to 10% of the total amount accumulated in the disk was sufficient to determine minimum exposure time required in order to counteract nonzero intercepts. Using this criterion, minimum field deployments generated by linear regression of fiber uPSD uptake curves ranging between 5–13 days are sufficient. So, field deployments of 2 weeks are suitable to provide measurable exposure to select androgens and estrogens while discounting contributions of initial uptake into the fiber uPSD.

Differences in uptake among the hormones were observed, with uptake being faster for androgens than for estrogens and estriol having the highest uptake among the estrogens (Figure 1). Differences in uptake across analytes have been observed in other PSDs with similar membrane material (polyethersulfone) \((18)\), with uptake correlated to log \(K_{ow}\). Our results indicate that uptake may also be correlated with log \(K_{ow}\); in addition, presence or absence of ionizable functional groups may control transport across the polyethersulfone membrane or diffusion within the sorbent matrix.

**Sampling rates.** Effective sampling rates were calculated using eq 1 at each point beyond day one for all androgens and estrogens studied in this work. Mean \(R_s\)’s are reported in Table 1 with corresponding standard deviations. Fiber uPSD \(R_s\)’s were determined under controlled laboratory conditions, including constant flow and aqueous concentrations of
hormones ($C_w$). Constant $C_w$, a stipulation of using eq 1, was verified by collecting water samples at least three times every week, analyzing samples within 48 hrs of collection. Average $C_w$’s for all hormones ranged from 38 – 64 ng/L. Sampling rates ranged from 0.04 – 0.09 L/d and $R_s$’s remained constant over the duration of the experiment, suggesting that uptake is not dependent on the length of exposure period (Table 1). E3 exhibited the highest average $R_s$ (0.09 L/d) and EE2 exhibited the lowest average $R_s$ (0.04 L/d); consequently, the log $K_{ow}$ of E3 is the lowest of all steroid hormones (≈ 2.7), and the log $K_{ow}$ of EE2 is the highest of all steroid hormones (≈ 4.2) (Table 1). However, plotting $R_s$ versus log $K_{ow}$ values for individual steroid hormones reveals that a clear correlation between log $K_{ow}$ and $R_s$ does not exist (Figure 2). This observation is consistent with other polyethersulfone membrane devices (18, 22). However, a few studies have observed that correlations between $R_s$ and log $K_{ow}$ can be observed within certain classes of compounds, for instance neutral or basic pharmaceuticals or compounds sharing similar base structures within a therapeutic class (18, 23). Based on the results in Figure 2, no correlation between $R_s$ and log $K_{ow}$ is discernable; however, future calibration experiments with an expanded suite of compounds would be necessary to conclusively determine if a trend exists.

The POCIS (Alvarez 2004), another polyethersulfone membrane device, was designed to monitor more polar compounds (log $K_{ow} < 4.5$) and is the only device with published use monitoring steroid hormones in surface waters (22, 24). The POCIS has been used in a qualitative and semi-quantitative capacity for hormone monitoring until recently (11, 24); $R_s$’s for six select estrogens were published by Arditoglou et al. (22). In comparing $R_s$’s generated by Arditoglou et al. (22) to those generated in this study, the
sampling efficiency of the POCIS is greater than that of the fiber uPSD (Table 1), ranging between a factor of 1.5 and 5.5 greater. However, surface area of the POCIS (~ 18 cm²) is considerably larger than fiber uPSD (~ 5.8 cm²). So, $R_s$’s of the fiber uPSD and POCIS cannot be compared directly; normalization of $R_s$’s by uptake surface area allows for a more accessible comparison. Surface-area (SA) normalization exhibits fiber uPSD and POCIS $R_s$ values within a factor of two, with fiber uPSD SA-normalized $R_s$’s ranging from 0.008 – 0.016 L/d*cm² and POCIS SA-normalized $R_s$’s ranging from 0.006 – 0.012 L/d*cm² for the five overlapping estrogens in Arditoglou et al. (22) and in this study.

**Elimination**

Environmental conditions, such has flow-rate, temperature, and biofouling, can affect the uptake rate of chemicals into PSDs, most studies focusing on the SPMD (16, 21, 25, 26). So, differences in these conditions between controlled-laboratory exposures and field exposures could result in the use of inaccurate $R_s$’s to calculate $C_w$ or could greatly restrict the use of the laboratory-derived $R_s$’s to deployments with environmental conditions matching those controlled in the laboratory. Booij et al. (16) demonstrated the use of performance reference compounds (PRCs) in field deployments to correct for those differences in conditions between the laboratory and the environment. PRCs are analytically non-interfering compounds that share similar physicochemical properties to the analytes of interest but are not normally found in nature and are fortified or loaded into the PSD prior to field deployment. Assuming isotropic exchange kinetics, the rate of PRC loss or elimination is proportional to the rate of uptake; theory and empirical evidence for nonpolar organic compounds have demonstrated that uptake and elimination of compounds are controlled by
the same molecular processes (27, 28). Elimination from PSDs has been modeled assuming first-order release kinetics by eq 3 (16, 29), and PRC loss in the laboratory and the field have been compared by calculating an environmental adjustment factor (EAF) and then applying the EAF to PSD-estimated \( C_w \) from eq 2 (14),

\[
EAF \equiv \frac{k_{ePRC-f}}{k_{ePRC-cal}}
\]

where \( k_{ePRC-f} \) is the PRC elimination rate constant derived from eq 3 in the field (d\(^{-1}\)) and \( k_{ePRC-cal} \) is the PRC elimination rate constant derived from eq 3 determined under controlled conditions in the laboratory (d\(^{-1}\)). So, by comparing in situ PRC loss to PRC loss in the laboratory (under the same conditions used to generate \( R_s \) values), differences between field conditions and laboratory conditions that affect the usability of laboratory-derived \( R_s \)'s can be accounted for.

**Elimination rate constants and PRCs.** In this work, an elimination experiment was conducted to determine if one environmental condition, flow-rate, had an effect on rate of elimination. Fiber uPSDs were initially loaded with ~ 500ng of each hormone and PRC and were then subject to three different flow conditions over a 30-day period. Release kinetics were modeled to determine \( k_e \) values (eq 3), and elimination curves of one representative androgen and estrogen are presented in Figure 3. \( k_e \)'s derived from eq 3 ranged from 0.002 – 0.025 d\(^{-1}\) in the highest flow channel (channel 1), from 0.001 – 0.018 d\(^{-1}\) in the channel with intermediate flow (channel 2), and from 0.001 – 0.17 d\(^{-1}\) in the lowest flow channel (channel 3) (Table 2). Correlation coefficients (\( R^2 \) values) ranged from 0.01 to 0.85 across all three channels, indicating first-order exponential regression may not be appropriate for modeling hormone release from fiber uPSDs. This lack of fit is not necessarily surprising for two
reasons: firstly, surficial loss from the polyethersulfone membrane may be contributing to initial loss; secondly, the biphasic nature of the uPSD sorbent, Oasis HLB®, may exhibit two different $k_e$ values based on different binding affinities and different elimination rates of the two polymer binding sites. However, by statistical analysis stream flow velocities of ~ 30, 15, and 7 cm/s did not have an effect on elimination for the PRC and six of the eight hormones studied. Correlations between flow rate and elimination were observed for estrone and androstenedione; however, no relationship between these two compounds can be postulated to explain why these compounds demonstrated elimination correlated to flow rate and the other six compounds did not. Further research is necessary to fully elucidate this relationship. Importantly however, the range of flow rates in this study should be large enough to discern flow-rate dependence based on other published works (18, 28, 30). Hence, the use of PRCs to correct for differences in flow velocity may not be necessary for fiber uPSDs; however, PRCs may be necessary to account for changes in uptake due to biofouling if used in future deployments. Additionally, in order to determine if the aqueous boundary layer is rate-limiting or if mass transfer is under membrane control, elimination experiments would need to be conducted for compounds with log $K_{ow}$’s ≥ 4.5 (30).

In this work, PRC elimination was monitored during field deployments. Because determination of $k_e$ by eq 3 does not appear appropriate, EAFs were determined by taking a ratio of percent PRC loss in the field to percent PRC loss during laboratory calibration,

$$EAF \equiv \left[ \frac{(N_{0,\text{field}} - N_{t,\text{field}})}{(N_{0,\text{field}})} \right] / \left[ \frac{(N_{0,\text{cal}} - N_{t,\text{cal}})}{(N_{0,\text{cal}})} \right]$$

(5)

where $N_{0,\text{field}}$ is the amount (ng) initially loaded in the uPSD prior to field deployment, $N_{t,\text{field}}$ is the amount (ng) of PRC remaining in the uPSD at the end of the deployment period, $N_{0,\text{cal}}$
is the amount (ng) of PRC initially measured in the uPSD prior to laboratory calibration, and $N_{t,\text{cal}}$ is the amount (ng) of PRC measured in the uPSD at the end of the laboratory calibration period.

**Field Validation**

By calibrating the fiber uPSD in laboratory experiments, the fiber uPSD can then be used to estimate chronic exposures to estrogens and androgens in surface waters. Following field deployments, fiber uPSDs are analyzed for contaminants, and contaminant residues are then used to estimate average water concentrations using eq 2. Using laboratory-derived $R_s$’s (Table 1), chronic estimates of hormone exposure in surface waters impacted by municipal effluent and a concentrated animal feeding operation (CAFO) can be calculated from hormones residues measured in field-deployed fiber uPSDs (Table 3). Estimated concentrations from fiber uPSD residues can be compared to mean concentrations of measured grab samples.

At the municipal wastewater effluent site, samples were collected in the dilution zone of the effluent outfall. Estimated effluent concentrations were derived from the mean of triplicate fiber uPSD samples, and measured concentrations were derived from the mean of seven grab samples collected over a 30-day sampling period. Mean fiber uPSD estimates were very similar to observed mean concentrations (Figure 4); however, mean fiber uPSD estimates were generally lower than mean observed concentrations with the exception of ethynyl estradiol, which was overpredicted by a factor of two using the mean fiber uPSD estimate compared to the mean observed concentration (Table 3). Additionally, in the case of epitestosterone, grab sampling did not detect any epitestosterone contamination in seven
sampling events over the 30-day sampling period; however, mean fiber uPSD estimates demonstrated average chronic epitestosterone estimates around 0.10 ng/L (Table 3). Although this concentration is low, chronic contamination at this level would contribute to overall androgenicity of surface waters receiving inputs from municipal effluents. So, this example verifies that repetitive grab sampling may not capture or may underestimate chronic exposure and that the use of PSDs, like fiber uPSDs, would capture chronic exposures to low-level contaminants like hormones.

At the CAFO site, samples were collected in surface waters near the agricultural operation. Concentrations of hormones in surface waters near a CAFO were estimated using eq 2, the mean sampling rates (Table 1) and hormone residues in the field-deployed fiber uPSDs. These estimated concentrations were then compared to measured hormone concentrations from surface water grab samples in Figure 5. Grab sample values were based on field measurements using the mean of 15 grab samples over a 30-day period, and uPSD-estimated values were calculated from the mean of triplicate fiber uPSD residues deployed over the 30-day period. For all estrogens, uPSD-derived estimations were about a factor of three lower than grab sample means. Grab sample means ranged from 2.4 – 26.6 ng/L, and cartridge uPSD estimated means ranged from 1.6 – 13.1 ng/L (Figure 5). The observed systemic negative bias of estrogen exposure by fiber uPSD residues could possibly be explained by the role of dissolved organic carbon (DOC). The concentration estimated from uPSD residues theoretically represents the freely-dissolved fraction of exposure; however, grab sample concentrations could additionally include the contribution of hormones bound
to DOC. Hence, grab samples may represent both the freely-dissolved concentration and the DOC-bound hormone concentration.

**CONCLUSION**

Laboratory calibration experiments were conducted over a period of 30 days, measuring sampling rates for eight common steroid hormones, three androgens and five estrogens, ascertaining the relationship between stream flow rate and rate of elimination of hormones from the fiber uPSD, and establishing the usability of PRCs to correct for differences in laboratory and environmental conditions. Sampling rates for each analyte were constant over the 30-day study, indicating independence of sampling rate from hormone concentration. Sampling rates have no clear correlation to log $K_{ow}$ values, however calibration of fiber uPSDs with an expanded suite of compounds differing in log $K_{ow}$ values would allow for more conclusions to be drawn. Elimination from fiber uPSDs does not appear to follow a first-order exponential elimination model. In addition, elimination rate was not correlated to flow rate for six of the eight hormones studied, indicating that the use of PRCs to correct for differences in flow rate between laboratory calibration and field conditions may not be necessary. PRCs may be useful in correcting for differences in uptake due to temperature or biofouling in situ. Sampling rates applied to field-deployed fiber uPSDs generated reasonable estimates of TWA concentrations of steroid hormones in surface waters.
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    Holistic Passive Integrative Sampling Approach for Assessing the Presence and
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Table 1. Summary of Physicochemical Properties and Sampling Rates (L/d) for Steroid Hormones.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>CAS No.</th>
<th>MW</th>
<th>Log $K_{ow}$</th>
<th>Mean $R_1 ± SD$</th>
<th>Mean $R_2 ± SD$</th>
<th>Mean $R_3 ± SD$</th>
<th>fiber uPSD</th>
<th>Pest POCIS$^c$</th>
<th>Pharm POCIS$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Androgens:</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Androstenedione (A)</td>
<td>63-05-8</td>
<td>286.4</td>
<td>NA$^b$</td>
<td>0.08 ± 0.01</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Epitestosterone (αT)</td>
<td>481-30-1</td>
<td>288.4</td>
<td>NA$^b$</td>
<td>0.07 ± 0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>17β Testosterone (T)</td>
<td>58-22-0</td>
<td>288.4</td>
<td>3.0 - 3.6$^c$</td>
<td>0.08 ± 0.01</td>
<td></td>
<td></td>
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<tr>
<td><strong>Estrogens:</strong></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Estrone (E1)</td>
<td>53-16-7</td>
<td>270.4</td>
<td>2.5 - 3.4$^e$</td>
<td>0.05 ± 0.01</td>
<td>0.1292 ± 0.0121</td>
<td>0.1199 ± 0.0177</td>
<td></td>
<td></td>
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<tr>
<td>17α Estradiol (αE2)</td>
<td>57-91-0</td>
<td>272.4</td>
<td>3.4 - 4.0$^f$</td>
<td>0.05 ± 0.01</td>
<td>0.1451 ± 0.0141</td>
<td>0.1216 ± 0.0031</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>17β Estradiol (E2)</td>
<td>50-28-2</td>
<td>272.4</td>
<td>3.1 - 4.0$^e$</td>
<td>0.05 ± 0.01</td>
<td>0.1444 ± 0.0150</td>
<td>0.1145 ± 0.0139</td>
<td></td>
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<td></td>
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<tr>
<td>Estriol (E3)</td>
<td>50-27-1</td>
<td>288.4</td>
<td>2.5 - 2.8$^c$</td>
<td>0.09 ± 0.01</td>
<td>0.1305 ± 0.0098</td>
<td>0.1571 ± 0.0041</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ethynyl estradiol (EE2)</td>
<td>57-63-6</td>
<td>296.4</td>
<td>3.7 - 4.7$^c$</td>
<td>0.04 ± 0.01</td>
<td>0.2137 ± 0.0456</td>
<td>0.2217 ± 0.0525</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ CAS = Chemical Abstract Service, MW = Molecular Weight (g/mol), $K_{ow}$ = Octanol-Water Partition Coefficient, $R_1 ± SD$ = Sampling Rate ± Standard Deviation (L/d).
$^b$ Not Available
$^c$ Value obtained from Lee et al. 2003 [2]
$^d$ Value obtained from Johnson et al. 2006 [2]
$^e$ Value obtained from Ternes et al. 1999 [2]
$^f$ Value obtained from Lai et al. 2000 [2]
$^g$ Value obtained from Lai et al. 2002 [2,4]
$^h$ Value obtained from Yamamoto et al. 2003 [2,5]
$^i$ Values obtained from Arditisoglou et al. 2008 [2,9]

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Table 2. Elimination Rates \( (k_e, \text{1/d}) \) of Hormones Subject to Different Flow Conditions: Channel 1 \((\sim 30 \text{ cm/s})\), Channel 2 \((\sim 15 \text{ cm/s})\), and Channel 3 \((\sim 7 \text{ cm/s})\).

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Channel 1</th>
<th>Channel 2</th>
<th>Channel 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( k_e )</td>
<td>( R^2 )</td>
<td>( k_e )</td>
</tr>
<tr>
<td>Estrone (E1)</td>
<td>0.019</td>
<td>0.80</td>
<td>0.014</td>
</tr>
<tr>
<td>17α-Estradiol (αE2)</td>
<td>0.018</td>
<td>0.80</td>
<td>0.010</td>
</tr>
<tr>
<td>17β-Estradiol (E2)</td>
<td>0.017</td>
<td>0.79</td>
<td>0.010</td>
</tr>
<tr>
<td>Estriol (E3)</td>
<td>0.019</td>
<td>0.85</td>
<td>0.018</td>
</tr>
<tr>
<td>Ethynyl estradiol (EE2)</td>
<td>0.025</td>
<td>0.75</td>
<td>0.016</td>
</tr>
<tr>
<td>( d_4 )-Estradiol (PRC)</td>
<td>0.019</td>
<td>0.74</td>
<td>0.011</td>
</tr>
<tr>
<td>Androstenedione (A)</td>
<td>0.002</td>
<td>0.09</td>
<td>0.001</td>
</tr>
<tr>
<td>Epitestosterone (αT)</td>
<td>0.007</td>
<td>0.50</td>
<td>0.004</td>
</tr>
<tr>
<td>17β-Testosterone (T)</td>
<td>0.006</td>
<td>0.32</td>
<td>0.005</td>
</tr>
</tbody>
</table>
Table 3. Mean Water Concentrations Measured from 7 Grab Samples and Estimated from Triplicate Fiber uPSD Residues (mean ± standard deviation).

<table>
<thead>
<tr>
<th>HORMONE</th>
<th>Mean Measured [Water]$^a$</th>
<th>Mean [uPSD]$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epitestosterone</td>
<td>0.00 ± 0.00</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>17α-Estradiol</td>
<td>0.89 ± 0.51</td>
<td>0.65 ± 0.06</td>
</tr>
<tr>
<td>Estriol</td>
<td>1.69 ± 0.93</td>
<td>0.84 ± 0.22</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>2.27 ± 1.65</td>
<td>1.49 ± 0.06</td>
</tr>
<tr>
<td>Ethynyl estradiol</td>
<td>2.50 ± 1.05</td>
<td>4.11 ± 0.22</td>
</tr>
<tr>
<td>Estrone</td>
<td>2.77 ± 1.41</td>
<td>1.69 ± 0.14</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>3.63 ± 1.17</td>
<td>2.18 ± 0.17</td>
</tr>
<tr>
<td>β-Testosterone</td>
<td>4.11 ± 2.01</td>
<td>3.22 ± 0.45</td>
</tr>
</tbody>
</table>

$^a$ concentrations ± s.d. (standard deviation) in ng L$^{-1}$
FIGURE LEGENDS

**Figure 1.** Uptake curves for three representative steroid hormones: 17α-estradiol, estriol, and androstenedione. The symbol, (●), represents mean values, and vertical lines represent standard deviations (n = 3) of fiber uPSD samples. Based on linear regression, $R^2 > 0.94$ for all 8 uptake curves.

**Figure 2.** Correlations between sampling rates ($R_s$; mean (●) ± standard deviation) and octanol-water partition coefficient (log $K_{ow}$) for all steroid hormones in this study.

**Figure 3.** Elimination curves of two representative steroid hormones, one estrogen and one androgen: 17β-estradiol and epitestosterone, subject to three different flow velocities: 30 cm/s in channel 1 (●), 15 cm/s in channel 2 (○), and 7.5 cm/s in channel 3 (▼).

**Figure 4.** Comparison of mean concentrations of hormones measures from 7 filtered grab samples of water (○) to that estimated from triplicate fiber uPSD residues (●). Vertical lines represent standard deviations based on triplicate values.

**Figure 5.** Mean surface water estrogen concentrations (ng/L) measured from grab samples near a concentrated animal feeding operation, n = 15, (○) and estimated from fiber uPSD residues, n = 3, (●), with vertical bars representing standard deviations.
Figure 1. Uptake curves for three representative steroid hormones: 17α-estradiol, estriol, and androstenedione. The symbol, (●), represents mean values, and vertical lines represent standard deviations (n = 3) of fiber uPSD samples. Based on linear regression, $R^2 > 0.94$ for all 8 uptake curves.
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Figure 3. Elimination curves of two representative steroid hormones, one estrogen and one androgen: 17β-estradiol and epitestosterone, subject to three different flow velocities: 30 cm/s in channel 1 (●), 15 cm/s in channel 2 (○), and 7.5 cm/s in channel 3 (▼).
**Figure 4.** Comparison of mean concentrations of hormones measures from 7 filtered grab samples of water (○) to that estimated from triplicate fiber uPSD residues (●). Vertical lines represent standard deviations based on triplicate values.
Figure 5. Mean surface water estrogen concentrations (ng/L) measured from grab samples near a concentrated animal feeding operation, n = 15, (○) and estimated from fiber uPSD residues, n = 3, (●), with vertical bars representing standard deviations.
CONCLUSION

In this work, two novel PSDs were developed with the aim of creating sampling tools that provide chronic exposure estimates of hormones in surface waters. Both devices contain the same sequestration media, or sorbent, Oasis HLB®, yet differ in amount of sorbent available and membrane composition. The cartridge uPSD contains 200 ± 1 mg of sorbent filling about half its internal volume and is comprised of a thick, porous stainless steel membrane, resulting in an ~ 6.2 cm² internal surface area, while the fiber uPSD is packed full with ~ 30 mg of sorbent and is composed of a polyethersulfone membrane with a surface area of ~ 5.8 cm². Both devices underwent an initial uptake calibration experiment to determine sampling rates for all hormones, an elimination experiment to determine the effects of flow rate on elimination and establish the suitability of using PRCs in situ, and a final field deployment to validate the use of the devices in estimating chronic hormone exposure.

Over the course of the 30-day uptake calibration of the cartridge uPSD, all eight hormones remained in the linear uptake phase, indicating that cartridge uPSDs have the capacity to function as time-integrative devices. Resulting sampling rates ranged from 0.09 – 0.11 L/d. Differences in sampling rates among hormones varied by a factor less than 1.3; these slight differences were not correlated to any physicochemical difference, like molecular weight or log $K_{ow}$ of the analytes.

In the 30-day elimination experiment with cartridge uPSDs, flow rate did not have an effect on mass transfer kinetics. The lack of correlation between flow rate and elimination
rate suggest that performance reference compounds are not necessary to correct for differences in flow rate in laboratory calibration versus in situ.

Finally, cartridge uPSDs were validated in a 30-day field experiment sampling downstream of a wastewater treatment plant where uPSD-derived estimates closely mirrored observed averages, grab sample mean concentrations (ng/L range). So, cartridge uPSDs have proven useful in providing quantitative estimates of steroid hormone exposure.

Over the 30-day uptake experiment with fiber uPSDs, all hormones remained in the linear uptake phase with sampling rates ranging from 0.04 – 0.09 L/d. Sampling rates for each analyte were constant over the 30-day study, indicating independence of sampling rate from hormone concentration. Sampling rates have no clear correlation to log $K_{ow}$ values, however calibration of fiber uPSDs with an expanded suite of compounds differing in log $K_{ow}$ values would allow for more conclusions to be drawn.

In the 30-day elimination experiment with fiber uPSDs, generally no correlation was observed, with the exception of androstenedione and estrone. Further research is necessary to fully elucidate the effect of flow rate on elimination; however, results thus far indicate that the use of performance reference compounds (PRCs) to relate differences in field to calibration conditions may not be necessary for differences in flow rate. PRCs may still be useful in correcting for differences in uptake due to biofouling or temperature in situ.

Field deployments of fiber uPSDs in surface waters had time-weighted average (TWA) concentrations estimated using laboratory-derived sampling rates that were consistent with routine grab sampling. These results indicate that fiber uPSDs can be used as a quantitative tool for measuring steroid hormones in surface waters.
Certainly, the ability to estimate chronic exposure to steroid hormones in surface waters is very important to assessing ecological risk in future exposure assessments; many emerging or recently-emerged contaminants, like steroid hormones, have the potential to disrupt development and reproduction in certain aquatic species when exposure is low and chronic. In this study, two novel sampling devices were assessed as potential tools to estimate chronic exposure to steroid hormones in aquatic systems. Both uPSDs offer considerable advantages over traditional sampling methodologies, including reducing cost by decreasing number of samples necessary to achieve a TWA estimate, saving time by reducing sampling trips to the field, collecting and processing fewer samples, gaining knowledge of the bioavailable fraction of exposure, and capturing and quantifying transient or trace exposure that could otherwise be missed by grab sampling. Ultimately, both the cartridge uPSD and fiber uPSD can be used as tools to provide chronic exposure estimates to steroid hormones in surface waters, and both devices offer potential as monitoring tools to be used in a more holistic approach to chemical assessment.
Appendix A. Uptake curves for all steroid hormones into the fiber uPSD, and elimination of PRC from fiber uPSD. (●)s represent mean values, and vertical lines represent standard deviations (n = 3) of fiber uPSD samples. Based on linear regression, $R^2 \geq 0.94$ for all 8 uptake curves, and based on first-order exponential regression, $R^2 = 0.82$ for PRC elimination.
Appendix B. Elimination curves of all steroid hormones in this study from the fiber uPSD, including our PRC, subject to three different flow velocities: 30 cm/s in channel 1 (●), 15 cm/s in channel 2 (○), and 7.5 cm/s in channel 3 (▼), all modeled by first-order regression. Vertical lines represent standard deviations from triplicate fiber uPSD samples.