Chapter 1

Managing the Effects of Endocrine Disrupting Chemicals in Wastewater-Impacted Streams

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Additional information is available at the end of the chapter

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1. Introduction

A revolution in analytical instrumentation circa 1920 greatly improved the ability to characterize chemical substances [1]. This analytical foundation resulted in an unprecedented explosion in the design and production of synthetic chemicals during and post-World War II. What is now often referred to as the 2nd Chemical Revolution has provided substantial societal benefits; with modern chemical design and manufacturing supporting dramatic advances in medicine, increased food production, and expanding gross domestic products at the national and global scales as well as improved health, longevity, and lifestyle convenience at the individual scale [1, 2]. Presently, the chemical industry is the largest manufacturing sector in the United States (U.S.) and the second largest in Europe and Japan, representing approximately 5% of the Gross Domestic Product (GDP) in each of these countries [2]. At the turn of the 21st century, the chemical industry was estimated to be worth more than $1.6 trillion and to employ over 10 million people, globally [2].

During the first half of the 20th century, the chemical sector expanded rapidly, the chemical industry enjoyed a generally positive status in society, and chemicals were widely appreciated as fundamental to individual and societal quality of life. Starting in the 1960s, however, the environmental costs associated with the chemical industry increasingly became the focus, due in part to the impact of books like “Silent Spring” [3] and “Our Stolen Future” [4] and to a number of highly publicized environmental disasters. Galvanizing chemical industry disasters included the 1976 dioxin leak north of Milan, Italy, the Love Canal evacuations in Niagara, New York beginning in 1978, and the Union Carbide leak in Bhopal, India in 1984 [2].

Understanding the environmental impact of synthetic compounds is essential to any informed assessment of net societal benefit, for the simple reason that any chemical substance
that is in commercial production or use will eventually find its way to the environment [5]. Not surprisingly given the direct link to profits, manufacturers intensely investigate and routinely document the potential benefits of new chemicals and chemical products. In contrast, the environmental risks associated with chemical production and uses are often investigated less intensely and are poorly communicated.

An imbalance in the risk-benefit analysis of any synthetic chemical substance or naturally occurring chemical, which presence and concentration in the environment largely reflects human activities and management, is a particular concern owing to the fundamental link between chemistry and biology. Biological organisms are intrinsically a homeostatic balance of innumerable internal and external chemical interactions and, thus, inherently sensitive to changes in the external chemical environment.

1.1. Environmental contamination: historical emphases

Much of the focus on environmental contamination in the decades since the institution of the 1970 Clean Air and 1972 Clean Water Acts in the U.S. and comparable regulations in Europe and throughout the world has been on what are now frequently referred to as conventional “priority pollutants” (so-called legacy contaminants). These include two primary groups: 1) wastewater nutrients and pathogens, and 2) a small subset of anthropogenic chemicals with relatively well-recognized toxicological risks, most notably “persistent bioaccumulative toxicants” (PBT) or “persistent organic pollutants” (POP). For example, the wastewater treatment infrastructure primarily reflects the early-recognized need to manage the environmental release of nutrients and human pathogens associated with human and animal waste. Likewise, the second driver of environmental regulation primarily concerns the relatively small number of known toxins or toxin-containing contaminant groups that, at least historically, were widely used in industry, frequently released accidentally or intentionally to the environment, are typically observed at part per billion (ppb) to part per million (ppm) concentrations, and are often well above recognized toxicological impact thresholds including carcinogenic thresholds. Managing the environmental impacts of these chemicals was the original motivation for and continues to be the primary focus of wastewater and hazardous waste regulations in the U.S.

1.2. Environmental contamination: expanding emphasis

The contaminants of historical environmental focus (conventional priority pollutants) are but a small fraction of the known and unknown chemicals that are potential environmental contaminants. As of September 2012, the Chemical Abstracts Service (CAS) has registered more than 68 million organic and inorganic chemical substances (not including proteins, etc.) [6]. While this chemosphere of known anthropogenic chemicals is impressive, the actual number of potential anthropogenic contaminants is incalculably larger, due to the continuing research, development, and marketing of novel chemical products and to the countless, unmanaged chemical transformations that occur following release to the environment [5].

The numbers and quantities of anthropogenic chemicals continue to increase rapidly [6]. In March 2004, the number of CAS registered organic and inorganic chemical substances was
approximately 23 million [5, 6]. Thus, the current estimate of approximately 68 million indicates a three-fold increase in the number of known chemicals between 2004 and 2012 [6]. To put this issue in perspective, Bohacek et al. [5, 7] provided a glimpse of the magnitude of the potential anthropogenic contaminant pool. Conservatively limiting the candidate atoms to C, N, O, and S and the total number of structural atoms to 30 or less, Bohacek et al. estimated over $10^{60}$ distinct possible structures [7]. Obviously, inclusion of additional common constituent atoms (e.g. phosphorous and halogens) or increasing the numbers of atoms per molecule would greatly increase this estimate [5].

The environmental impact of any anthropogenic chemical can be amplified due to the formation of numerous unidentified daughter products resulting from subsequent chemical and biological transformation processes in the environment [5]. A common example among the contaminants of historical focus is the reductive dechlorination of trichloroethene (TCE) and its intermediate daughter products (dichloroethenes, DCE) to form vinyl chloride (VC) [8]. Historically, TCE has been widely employed in dry cleaning and as a degreasing agent in industry. TCE has an MCL of 5 μg/L and a $10^{-4}$ Cancer Risk level of 300 μg/L [9, 10]. In contrast, VC is a demonstrated human carcinogen with an MCL of 2 μg/L and a $10^{-4}$ Cancer Risk level of 2 μg/L [9, 10]. An example among the contaminants of more recent concern is the transformation of 4-nonylphenol polyethoxylate compounds (primarily used as nonionic surfactants) to 4-nonylphenol (4-NP) and nonylphenol mono- and di-ethoxylates. The aquatic toxicity of 4-nonylphenol 16-ethoxylate (NP16EO) is 110 mg/L for fish, while that of 4-nonylphenol is 1.4 mg/L [11]. The 4-nonylphenol polyethoxylates are not estrogenically active. In contrast, 4-nonylphenol is a demonstrated xenoestrogen with a relative binding affinity of $2.1 \times 10^{-4}$ relative to the natural estrogen, 17β-estradiol (E2) [11].

Thus, considering just the inventoried substances, only about 0.4% (>295000) of the more than 68 million (as of Sept 08 2012) commercially available organic and inorganic chemical substances registered in CAS are government inventoried or regulated worldwide [6]. Thus, even considering only these registered commercial chemicals, each of which are or may become environmental contaminants, the vast majority are unregulated and largely unmonitored in the environment. Environmental contaminants, which are currently unregulated, are often referred to as a group as “emerging contaminants,” in an effort to distinguish them from the conventional priority pollutants (legacy contaminants).

### 1.3. Emerging concern versus emerging contaminants

The term, “emerging contaminant,” is misleading in the unintended implication that these chemicals are collectively new to the environment. In fact, large fractions of these emerging contaminants have been in use and, by extension, have been present in the environment for many years. However, many of these compounds occur in the environment at concentrations well below historical ppb to ppm analytical detection limits. The environmental threat associated with these contaminants has gone largely unrecognized or undefined, due to a lack of analytical methods of sufficient sensitivity and resolution to allow detection at environmentally relevant concentrations. Thus, while newly synthesized and produced commercial chemicals would in fact fit the perception; the “emerging” characteristic for the majority
of these unregulated compounds is not recent environmental release, but a nascent and growing appreciation of their real and potential impacts in the environment.

To illustrate the magnitude of the problem, consider just the pharmaceutical compounds, chemicals synthesized specifically to affect a biological impact. Pharmaceuticals were estimated to be approximately 23% of the global chemical production in 2000 [2]. More than 12000 approved prescription and “over the counter” (non-prescription) drug products and formulations are currently listed by the U.S. Food and Drug Administration, along with more than 5000 discontinued products [12, 13]. More than 80 new drug products or formulations were approved in 2011 [12, 13]. In contrast, analytical methods for detection and quantification in environmentally relevant matrices (e.g. sediment and water) exist for only a small fraction of the pharmaceuticals approved for use in the U.S. For example, the U.S. Geological Survey (USGS) has developed one of the more comprehensive analytical methods for the monitoring of pharmaceuticals in the environment [14]. However, the currently available USGS direct aqueous injection liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) method for filtered water includes only approximately 112 pharmaceutical compounds [14]. Similarly, the U.S. Environmental Protection Agency (USEPA) method for pharmaceutical and personal care products in water, soil, sediment, and biosolids by LC/MS/MS covers only about 60 pharmaceutical compounds [15].

Using these methods as a measure of the analytical coverage of pharmaceutical compounds in the environment and not including environmental transformation products, the vast majority of pharmaceutical chemicals, which have been in use and, consequently, may reasonably be expected to occur in the environment, are not currently monitored in the environment. From this perspective, these contaminants are more appropriately viewed as emerging concerns.

### 1.4. Contaminants of emerging concern

The potential impacts of contaminants of emerging concern (CEC) on the environment, in general, and on natural surface-water and riparian ecosystems, in particular, are a critical environmental management issue in the U.S. and Europe [11, 16]. CEC is a “catch-all” phrase that refers to a wide range of chemicals, which occurrence in and potential impacts on the environment have long been suspected but only recently validated with the advent of sensitive modern analytical capabilities. The CEC umbrella covers several broad classes of contaminants that are loosely categorized according to source, original intended use, and/or primary mode of ecological impact and which include: pharmaceuticals and personal care products, organic wastewater compounds, antimicrobials, antibiotics, animal and human hormones, as well as domestic and industrial detergents.

### 1.5. Endocrine disrupting chemicals (EDC)

Many CEC interact with animal endocrine systems and, consequently, are classified as endocrine disrupting chemicals (EDC). The endocrine system, sometimes referred to as the hormone system, is present in all vertebrate animals and consists of glands, hormones, and
receptors that regulate all biological functions including metabolism, growth, behavior, and reproduction [see for example, 11, 17, 18, 19]. Endocrine hormones include the estrogens, androgens, and thyroid hormones. The USEPA defines an EDC as:

"An exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior."[17]

Because the common conceptualization of “endocrine systems” is typically associated with vertebrates, much of the attention on environmental EDC has been focused on endocrine disruption impacts in vertebrate animals, particularly aquatic vertebrates [11, 18-24] and associated terrestrial food webs [25]. It is important to realize, however, that invertebrates (molluscs, insects, etc.) also have hormone systems that regulate biological function and maintain homeostasis [26-29]. Thus, many invertebrates are also susceptible to the impacts of EDC [26-29]. Because invertebrates account for approximately 95% of all animals on earth and are critical elements of freshwater environments, the potential impacts of EDC on these organisms cannot be overlooked [26].

EDC threaten the reproductive success and long-term survival of sensitive aquatic populations. The impacts of EDC in the environment are detectable at multiple ecological endpoints, including induction of male vitellogenin (egg yolk protein) expression [30], skewed sex ratios and intersex characteristics [31], degraded predator avoidance behavior [23, 24], as well as reproductive failure and population collapse in sensitive fish species [22]. All of these impacts have been observed at concentrations that have been widely documented in wastewater effluent and effluent-impacted surface-water systems [16, 23, 24, 30, 31]. The widespread co-occurrence of EDC [see for example, 16] and intersex characteristics in black basses (Micropterus species) [20, 21] in U.S. streams suggests endocrine disruption may be pervasive in aquatic populations and emphasizes the potential EDC threat to high value, sensitive surface-water and riparian ecosystems.

1.5.1. Natural and xenobiotic EDC

EDC can be divided into two general classes: endocrine hormones and endocrine mimics (xenobiotics including xenoestrogens, xenoandrogens, phytoestrogens, etc.).

Endocrine hormones are natural or synthetic chemicals produced specifically to interact with the hormone binding sites of animal endocrine systems. The release of endocrine hormones, including estrogens and androgens, is a particular concern owing to their high endocrine activity/potency and additive effects. These hormones have been identified as primary estrogenic agents in wastewater effluent [22, 32-39]. Examples of reproductive hormones that are commonly detected in effluent-affected ecosystems are 17β-estradiol (E2), estrone (E1), testosterone (T), and the synthetic birth control compound, 17α-ethinylestradiol (EE2).

Other endocrine disrupting chemicals share sufficient structural similarity with the endocrine hormones to interact with animal endocrine receptors sites and trigger organ- and or-
ganism-level endocrine responses. These endocrine mimics generally exhibit less endocrine reactivity, but are essentially ubiquitous in wastewater, are often reported at concentrations 3-5 orders of magnitude higher than the endocrine hormones, and have been detected in the majority of investigated surface-water systems. Examples of these structural analog EDCs include organic wastewater compounds like the ubiquitous detergent metabolite, nonylphenol and naturally-occurring phytoestrogens.

1.5.2. Environmental EDC sources

Numerous potential sources of EDC to the environment have been documented, including: pharmaceutical industry, other industry and manufacturing, land application of municipal biosolids, landfills and associated leachates, livestock and aquaculture operations, domestic septic systems, latrine and vault toilets, and municipal and industrial wastewater treatment plants (Fig. 1) [16].

![Toxicant Source Pathways to the Environment](image)

Figure 1. Potential sources of EDC in the environment (figure by E.A. Morrissey, USGS).

Among these, wastewater treatment plants (WWTP) discharge directly to surface waters and are often a particular concern for downstream surface-water and riparian ecosystems [11, 16, 23, 30, 40].

1.6. Chapter focus

Recent research indicates that a substantial and potentially protective capacity for in situ EDC biodegradation exists in the sediments and water columns of effluent-affected, surface-water systems in the U.S. However, the efficiency and circumstances of biodegradation can vary substantially between stream systems and between compound classes. Likewise, the potentials for in situ biodegradation of a large number of EDC remain untested. Improved understanding of the extent of contaminant occurrence and of the ten-
dency of surface-water receptors to degrade or to accumulate these wastewater contaminants is needed to support development of regulatory contaminant criteria and maximum load polices for the release of EDC to the environment. This chapter focuses on the impacts of wastewater EDC on downstream surface-water and riparian ecosystems and on the potential importance of the natural assimilative capacity of surface-water receptors as a mechanism for managing these EDC impacts.

2. EDC risk in wastewater-impacted surface-water and riparian ecosystems

The environmental or ecological risk associated with EDC can be defined in a number of ways. In one approach (Fig. 2), environmental EDC risk can be viewed as the net result of the interaction of three conceptual drivers:

- Environmental EDC occurrence and distribution
- EDC impact thresholds of species in downstream ecosystems
- EDC attenuation capacity of the surface-water receptor

The first two drivers are widely recognized and, currently, are the focus of a majority of investigations of environmental EDC risk. By comparison, relatively little is known about the environmental fate, transport and persistence of EDC.

![Managing EDC Impacts](image_url)

**Figure 2.** Interaction of occurrence and distribution, adverse impact thresholds and site-specific assimilative capacity as drivers of EDC environmental risk.
2.1. EDC occurrence and distribution

The risks of EDC are clearly predicated on their presence, concentration, matrix of occurrence, and bioavailability in the environment. Thus, developing analytical methods to detect and quantify EDC in water, sediment, and other environmental matrices has been a primary focus of field investigations over the past two decades. Current approaches to assessing EDC occurrence and distribution in the environment fall into two primary categories, selective and non-selective methods.

Selective methods have traditionally been the cornerstone of contaminant monitoring and this general approach has been critical to the documentation of EDC in the environment, identification of potential EDC sources, and the establishment of EDC as a fundamental environmental threat. Full scan, high-resolution Liquid Chromatography/Mass Spectrometry (LC/MS) is the mainstay of environmental EDC analysis, due primarily to the fact that many of these compounds are not volatile in the inlet of gas chromatography (GC) systems [41]. The complexities of environmental matrices and environmental EDC mixtures have led to wide use of LC/TOF/MS (time of flight, TOF) combined with isotopically labeled internal standards in order to achieve full spectral mass sensitivity, required analytical resolving power, and high mass-measurement accuracies sufficient to estimate elemental composition [41-43]. The fundamental limitation to these methods is the requirement for clean-up and separation methods tailored to selected target analytes and chemically-related unknowns. In essence, in analytical chemistry “what you see is largely dictated by what you look for.”

In light of the largely unknown nature of environmental EDC mixtures, using selective analytical methods to assess the total endocrine disrupting impact in a given environmental setting is not straightforward [32]. To address this general screening need, a number of biologically based assays (BBA) have been developed to assess the total amount of a specific endocrine activity (e.g. estrogenicity) that is present in the environment [32]. For example, a number of assays have been developed and successfully employed to assess total estrogenic activity, including the Yeast Estrogen Screen (YES) [44] and the bioluminescent version (BLYES) [45]. BBA are sensitive, cost-effective tools for assessing total estrogenicity of water samples. A priori knowledge of individual estrogenic compounds is unnecessary, because the assay measures target (estrogen) receptor binding. Thus, BBA can add considerable ecological relevance to selective analytical chemical results.

Current areas of active research include application of these analytical improvements to quantify the distribution of EDC between matrices. While a number of studies have demonstrated EDC impacts at concentrations observed in wastewater-impacted surface waters, the tendency of aromatic and polyaromatic contaminants to partition to the sediment phase is well recognized and sediment concentrations can exceed water concentrations by several orders of magnitude [46-48].

2.2. EDC environmental impact thresholds of aquatic populations

As noted earlier, the impacts of EDC in the environment involve multiple ecological endpoints. The adverse impact threshold for each of these ecological endpoints may differ sub-
stantially. Moreover, the threshold for each of these ecological endpoints can vary substantially among organisms within a specific setting and among environmental settings.

EDC present fundamental challenges to the traditional toxicological assessment approach. Historically, toxicological assessments have been based on a “dose alone determines the poison” maxim [49-51] and the use of a generalized monotonic dose response curve (threshold or linear nonthreshold models) for estimating adverse impact thresholds for individual toxins [51, 52]. However, a number of EDC, including several hormones, show nonmonotonic U-shaped and inverted U-shaped dose response curves for different biological endpoints [52-55]. In fact, the compelling argument has been made that threshold assumptions do not apply to EDC because these compounds are endogenous molecules or mimic endogenous molecules (like estrogen) that are critical to development. Thus, homeostatic balance is disrupted and the “threshold” is automatically exceeded with exposure to the EDC.

While the viewpoint that EDC do not have an acceptable “No Observable Effect Level” (NOEL) is compelling, practical management of EDC risk will depend on establishment of regulatory adverse impact thresholds (“acceptable risk” thresholds). The several challenges to a comprehensive understanding of environmental EDC risk and development of “acceptable risk” thresholds for EDC include the facts that: (1) these compounds generally occur in the environment as complex chemical mixtures, not single compounds, (2) many EDC exhibit trans-generational (epigenetic) impacts, (3) EDC impacts can vary substantially over the life-cycle of an organism and are often particularly severe during gestation and early development, and (4) EDC impacts can occur long after exposure. Development and implementation of appropriate methods for assessing EDC adverse impacts at multiple endpoints are environmental priorities.

In the U.S., regulatory adverse impact thresholds for EDC are under development and not currently available for implementation. Although thresholds for acceptable risk remain undefined, a number of studies have demonstrated that EDC concentrations currently observed in the environment often exceed levels known to cause adverse effects in aquatic populations. To illustrate, consider again E2, E1, and 4-NP.

Both E2 and E1 induce vitellogenesis and feminization in fish species [35, 39, 56-61] at dissolved concentrations as low as 1-10 ng/L [35, 39]. Municipal wastewater treatment plant (WWTP) effluent concentrations of 0.1-88 ng/L and 0.35-220 ng/L have been reported for E2 and E1, respectively. More common detections are in the range of 1-10 ng/L [see for review, 46]. E2 and E1 concentrations above 100 ng/L have been reported in surface waters [16], but are typically in the range of <0.1-25 ng/L [see for review, 46]. Because sensitive fish species are affected by concentrations as low as 1 ng/L and because the effects of reproductive hormone and non-hormonal EDCs are often additive [62], such dissolved concentrations are an environmental concern. Furthermore, estrogen concentrations in surface-water sediment can be up to 1000 times higher per volume than in the associated water column, ranging from 0.05-29 ng/g dry weight [see for review, 46].

Alkylphenol contaminants, like 4-NP, exhibit less estrogenic reactivity [36, 38] than E2, but are ubiquitous in WWTP effluent [11, 16], have been reported at concentrations up to 644
μg/L [40, 48] and have been detected in the majority of investigated surface-water systems [see for review, 63]. Nonylphenol-based compounds are the primary alkylphenol contaminants detected in WWTP-impacted stream systems [16], because nonylphenol ethoxylates constitute approximately 82% of the world production of alkylphenol ethoxylate [11]. The widespread occurrence of 4-NP in stream systems is attributable to WWTP effluents and microbial transformation of effluent-associated nonylphenol ethoxylates to 4-NP in anoxic, surface-water sediments [47]. Short-chain nonylphenol ethoxylates and 4-NP are produced within WWTP from biodegradation of ubiquitous, nonylphenol ethoxylate nonionic surfactants [47]. 4-NP that is released to the stream environment, rapidly and strongly adsorbs to the sediments suspended in the water column and to the bedded sediments [47, 48].

2.3. EDC attenuation and persistence

In contrast to the focus on assessment of EDC occurrence and distribution and EDC adverse impact thresholds, comparatively little is known about the environmental attenuation or persistence of EDC. Environmental persistence, however, is a fundamental component of contaminant environmental risk.

Persistence can be viewed as the resistance of the contaminant molecule to biological or chemical transformations. Pseudo-persistence may also result in settings where the contaminant molecule is continually replenished (e.g. wastewater-impacted systems). Because the longer a contaminant persists in the environment the greater the chance that the contaminant will reach and eventually exceed an adverse impact threshold, improved understanding of the fate of EDC in the environment is essential to a comprehensive assessment of EDC environmental risk.

Conservative mechanisms of contaminant attenuation like dilution and sorption have been the historical foundation of wastewater management in surface-water systems. However, the fact that EDC may trigger organ-, organism-, and community-level responses at ng/L concentrations raises concerns about the ultimate reliability of attenuation mechanisms that do not directly degrade endocrine function [64]. Endocrine disruption at hormone concentrations (1-10 ng/L) [35, 39, 60, 61], which have become detectable only with recent analytical innovations, illustrates this concern and emphasizes the importance of characterizing non-conservative, contaminant attenuation processes. In the following section, recent findings on the potential for EDC biodegradation are presented to illustrate the potential importance of this environmental attenuation mechanism and identify existing data gaps that need to be addressed in order to employ natural attenuation for the management of EDC environmental risk.

3. Biodegradation of wastewater EDC in surface-water receptors

This section focuses on EDC biodegradation as an example of the potential importance of the natural assimilative capacity of surface-water receptors as a mechanism for managing EDC impacts in aquatic habitats. Recent results demonstrating the potential for EDC biode-
gradation in wastewater-impacted streams are discussed along with several environmental factors known to affect the efficiency of EDC biodegradation.

3.1. Methods

The potential for EDC biodegradation was assessed in microcosms using $^{14}$C-radiolabeled model compounds [see for example, 46, 63] representing the two general classes of EDC: endocrine hormones and endocrine mimics. Each $^{14}$C-model contaminant compound contained a cyclic (aromatic) ring structure that is considered essential to compound toxicity and biological activity. Consequently, the $^{14}$C-radiolabel of each model contaminant was positioned within the aromatic ring such that recovery of $^{14}$C-radioactivity as mineralization products ($^{14}$CO$_2$ and/or $^{14}$CH$_4$) indicated ring cleavage and presumptive loss of endocrine activity [see for example, 46, 63].

Headspace concentrations of CH$_4$, $^{14}$CH$_4$, CO$_2$, and $^{14}$CO$_2$ were monitored by analyzing 0.5 mL of headspace using gas chromatography/radiometric detection (GC/RD) combined with thermal conductivity detection. Compound separation was achieved by isocratic (80°C), packed-column (3 m of 13x molecular sieve) gas chromatography. The headspace sample volumes were replaced with pure oxygen (oxic treatments) or nitrogen (anoxic treatments). Dissolved phase concentrations of $^{14}$CH$_4$ and $^{14}$CO$_2$ were estimated based on Henry’s partition coefficients that were determined experimentally as described previously [65, 66]. The GC/RD output was calibrated by liquid scintillation counting using H$^{14}$CO$_3^-$. To confirm the presence of oxygen (headspace [O$_2$] = 2-21% by volume) in oxic treatments or the absence of oxygen (headspace [O$_2$] minimum detection limit = 0.2 part per million by volume) in anoxic treatments, headspace concentrations of O$_2$ were monitored throughout the study using GC with thermal conductivity detection.

3.2. EDC biodegradation in surface water: environmental factors

While most investigations into the potential for EDC biodegradation continue to focus on WWTP, a growing number of studies address the potential for biodegradation of CEC, in general, and EDC, specifically, in a variety of environmental settings. For simplicity, we focus here on recent findings from USGS scientists, which illustrate that a substantial and potentially exploitable capacity for in situ biodegradation of a number of CEC, including known EDC, exists in the sediments and water columns of surface-water systems in the U.S. The efficiency and circumstances of biodegradation, however, vary substantially among stream locations, stream systems, environmental matrices, and EDC compounds. These findings illustrate the data gaps that need to be addressed in order to develop best management practices for individual surface-water systems and specific compound classes.

3.2.1. Between and within stream variation

Biodegradation of E2, E1, and testosterone (T) was investigated recently in three WWTP-affected streams in the U.S. [46]. Relative differences in the mineralization of [4-$^{14}$C] hor-
mones were assessed in oxic microcosms containing saturated sediment from locations upstream and downstream of the WWTP outfall in each system. The results for E2 are shown in figure 3.

Sediment collected upstream from the WWTP outfall in each of the three surface-water systems demonstrated substantial aerobic mineralization of [4-14C] E2 (Fig. 3), with initial linear rates of 14CO2 recovery ranging from approximately 1% d⁻¹ (percent of theoretical) for E2 mineralization in Fourmile Creek (Iowa) sediment (Fig. 3) up to approximately 3% d⁻¹ for E2 mineralization in Boulder Creek (Colorado) sediment. The recovery of 14CO2 observed in this study was attributed to microbial activity, because no significant recovery of 14CO2 (recovery less than 2% of theoretical) was observed in sterilized control microcosms. Recovery of 14CO2 was interpreted as explicit evidence of microbial cleavage of the steroid “A” ring and loss of endocrine activity, as demonstrated previously using the YES assay [67, 68]. The results are consistent with previous reports of microbial transformation and “A” ring cleavage of [4-14C] E2 in rivers in the United Kingdom [67] and Japan [69] and suggest that the potential for aerobic biodegradation of reproductive hormones may be widespread in stream systems.

Upstream sediment demonstrated statistically significant mineralization of the “A” ring of E2. This result indicated that, in combination with sediment sorption processes which effectively scavenge hydrophobic contaminants from the water column and immobilize them in the vicinity of the WWTP outfall, aerobic biodegradation of reproductive hormones can be an environmentally important mechanism for non-conservative (destructive) attenuation of hormonal endocrine disruptors in effluent-affected streams.

The E2 “A” ring mineralization was substantially greater in sediment collected immediately downstream from the WWTP outfall in the effluent-dominated Boulder Creek and South Platte River (Colorado) study reaches (Fig. 3). The recovery of 14CO2 in the immediate downstream sediment was approximately twice that observed upstream of the outfall in Boulder Creek and the South Platte River. Effluent may enhance in situ biodegradation of hormone contaminants by introducing WWTP-derived degradative populations or by stimulating the indigenous microorganisms through increased supply of nutrients and co-metabolites. The fact that no difference in E2 “A” ring mineralization was observed between upstream and downstream locations in the less effluent-affected Fourmile Creek suggested that the stimulation of E2 mineralization observed in the Boulder Creek and South Platte River study reaches was attributable to some characteristic of the WWTP effluent and may be concentration dependent. These observations illustrate the substantial variation in EDC biodegradation that may occur at different locations within a stream system and the need to account for location, particularly proximity to recognized sources, when assessing the potential for biodegradation of EDC in the environment.

These results also demonstrate that substantial variation in EDC biodegradation may occur between different stream systems. In Fourmile Creek, location relative to the WWTP had little effect on E2 biodegradation rates. However, location was a major influence on E2 biodegradation in Boulder Creek and in the South Platte River. Similarly, initial linear rates of 14CO2 recovery in sediment collected immediately downstream of the WWTP outfalls
ranged from approximately 4 % d\(^{-1}\) (percent of theoretical) for E2 mineralization in Fourmile Creek up to approximately 11 % d\(^{-1}\) for E2 mineralization in Boulder Creek.

**Figure 3.** Mineralization of \(^{14}\)C-E2 to \(^{14}\)CO\(_2\) in oxic microcosms containing sediment collected upstream (green), immediately downstream (red) and far downstream (blue) of the WWTP outfalls in Fourmile Creek, Boulder Creek and South Platte River. Black indicates sterile control.
3.2.2. **Effects of environmental matrices**

The effects of the environmental matrix on EDC biodegradation were evaluated for stream biofilm, sediment, and water collected from locations upstream and downstream from a WWTP outfall in Boulder Creek using E2, EE2, and 4-\textit{n}-NP (linear chain isomer) as $^{14}$C-mod-el substrates [70] (Fig.4). Initial time intervals (0-7 d) evaluated biodegradation by the microbial community at the time of sampling. Later time intervals (70 and 185 d) provided insight into changes in EDC biodegradation potential as the microbial community adapted to the absence of light for photosynthesis (i.e. shifted from photosynthetic based community to a predominantly heterotrophic community).

No statistically significant mineralization ($p < 0.05$) of 4-\textit{n}-NP or E2 was observed in the biofilm or water matrices during the initial time step (7 d), whereas statistically significant mineralization of 4-\textit{n}-NP and E2 was observed in the sediment matrices. Mineralization was not observed in autoclaved matrices; therefore, mineralization observed in all matrices was attributed to biodegradation. After 70 d, mineralization of 4-\textit{n}-NP and E2 was observed in the biofilm and sediment matrices, and after 185 d biodegradation of these compounds was observed in all matrices. Mineralization of EE2 was observed only in sediment treatments.

In this study [70], the sediment matrix was more effective than the biofilm and water matrices at biodegrading 4-NP, E2, or EE2. Biodegradation of all three EDC was generally least efficient in water only. These observations illustrate the substantial variation in EDC biodegradation that may occur in different environmental matrices from the same location within a stream system and the need to evaluate the potential for biodegradation of EDC in each.

3.2.3. **EDC compound effects**

The results of the study by Writer et al. [70] also demonstrated the substantial variation in biodegradation that may occur between different EDC compounds (Fig. 4). Biodegradation of EE2 typically is assumed to be slow in aquatic sediments, and limited direct assessments have been conducted [67].

Results from this study provided rare evidence that EE2 mineralization can occur in surface-water sediments, but EE2 mineralization was at least an order of magnitude lower than E2 or 4-\textit{n}-NP mineralization. Because the $K_{om}$ values for E2 and EE2 were similar and about an order of magnitude lower than for 4-NP [70], the relative recalcitrance of EE2, compared to E2, was not due to sorption differences. These results illustrate the need to evaluate the location-specific potential for biodegradation of each environmentally important EDC.

3.2.4. **Red-Ox effects**

Microbial mechanisms for degradation of historical environmental contaminants and, by extension EDC, are fundamentally redox processes. Consequently, in situ redox conditions are expected to control the efficiency of EDC biodegradation. Environmental endocrine activity is dependent on the presence of an aromatic ring structure with an extended carbon backbone. All natural and synthetic hormones are aromatic compounds and the endocrine mimic EDC are generally expected to share this characteristic.
Figure 4. Mineralization of EE2, E2, and 4-n-NP in microcosms containing sediment, epilithon, or water only collected from upstream and downstream of the WWTP outfall in Boulder Creek.
Because the energy available for microbial metabolism is a function of the potential difference between the electron donor and the terminal electron acceptor, the theoretical energy yield from the biodegradation of a contaminant serving as an electron donor is greatest when coupled to oxygen, and decreases in the order of oxygen-reduction > nitrate-reduction > Fe(III)-reduction > sulfate reduction > methanogenesis. Experience from the remediation of legacy contaminants over several decades has demonstrated that microorganisms can degrade aromatic contaminant compounds under a range of terminal electron accepting conditions. However, rates of aromatic contaminant biodegradation under oxic conditions are typically 1-2 orders of magnitude greater than under anoxic conditions.

Extending this experience to EDC, the efficiency of environmental EDC biodegradation would be expected to be greatest under oxic conditions and severely limited under anoxic conditions. The results of a recent assessment of the potential for 4-n-NP biodegradation in stream sediments are consistent with this expectation [63]. While substantial mineralization of 14C-4-n-NP was observed in sediment microcosms incubated under oxic conditions, the rate of mineralization under oxic conditions was inversely related to the sediment biological oxygen demand (BOD)(Fig. 5) and no evidence of mineralization was observed under anoxic conditions [63].

The importance of redox conditions on EDC biodegradation is also demonstrated by results (Fig. 6) of a recent investigation of E2 and E1 biodegradation potential in manure-impacted stream sediments collected from a small stream in northcentral Iowa. An accidental spill from a manure lagoon raised concerns about the effect of oxygen-limited conditions on the fate of manure-derived EDC contaminants in the stream. Specific questions concerned the potential for continued EDC biodegradation under anoxic conditions and the potential that manure-derived nitrate (NO3) amendment might stimulate EDC biodegradation by creating denitrifying conditions. Enhanced anaerobic biodegradation of environmental contaminants under denitrifying conditions has been reported previously [71].
The results (Fig. 6) demonstrated that significant biodegradation of E2 and E1 could occur in stream sediment under anoxic conditions. In general, biodegradation was substantially lower under anoxic conditions than under oxic conditions. However, comparable E2 biodegradation was observed in sediments collected upstream of the spill site under anoxic and oxic conditions (Fig. 6A). Somewhat surprisingly, rather than stimulating E2 and E1 biodegradation under anoxic conditions, the addition of NO₃ inhibited biodegradation in both E2 treatments and in the downstream sediment E1 treatment. These results and the results of the previous 4-n-NP biodegradation study (Fig. 5) [63] illustrate the substantial variation in EDC biodegradation that may occur in the same sediment under different redox conditions and the need to evaluate the potential for biodegradation of EDC under those redox conditions that predominate in situ.

4. Conclusion – Toward an integrated approach to EDC risk management in surface-water ecosystems

The risk associated with EDC in the environment may reasonably be viewed as the net result of the interaction of three conceptual drivers: (1) the occurrence and distribution of EDC in the environment, (2) site- and life-cycle-specific adverse-impact thresholds of EDC ecological endpoints, and (3) the potential for in situ EDC natural attenuation and persistence. The first two drivers are widely recognized and are the focus of numerous recent and ongoing investigations of environmental EDC risk. In contrast, environmental EDC persistence is a fundamental aspect of EDC environmental risk and relatively few studies have addressed the fate, transport and persistence of EDC in various environmental settings.
An understanding of the site-specific capacity for EDC attenuation is critical to EDC risk management. The longer contaminants or groups of contaminants persist in the environment the greater the chance that the contaminants will reach and eventually exceed an adverse impact threshold. The extent and rates of in situ contaminant biodegradation are key data needs for establishing total maximum daily load criteria for EDC, upgrading wastewater treatment infrastructure, and selecting protective treatment performance criteria. Ultimately, those EDC, which have little or no potential for biodegradation under environmentally relevant conditions, may need to be removed from commercial production, because any chemical substance that is in use will eventually find its way to the environment.

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