CAMBRIDGE JOURNAL OF HUMAN BEHAVIOUR

ENVIRONMENTAL SCIENCE

Water Contamination by Estrogenic Compounds: Review, Policy Gaps, and Recommendations for US Federal Regulation

Muskan Shrivastava[1]

[1] State University of New York, College of Environmental Science and Forestry, United States

Received January 16, 2023
Revision received March 8, 2023
Accepted March 17, 2023

Keywords:
estrogen,
Clean Water Act,
Safe Drinking Water Act,
regulation,
recommendations

E1, E2, E3, and EE2 are types of estrogens that need to be federally regulated in waterbodies in the United States. Absence of regulation has led to untreated releases from wastewater treatment plants of estrogen from natural human excretion and releases from animal feeding operations (AFOs) eluding proper waste management. Estrogens have been detected in drinking water, which has caused concerns over human health as there is little research evaluating the health risks. Due to there being no evidence supporting negative impacts on human health, there is no justification for regulation under current Safe Drinking Water Act provisions. However, as guided by the precautionary principle, current laws could be amended to utilize preventative measures since there is no certainty that exposure to estrogenic drinking water is harmless. Exposure also proves to be an issue for aquatic life and potentially other species. Thus, I argue that E1, E2, E3, and EE2 must be listed as pollutants under the Clean Water Act and increased regulations on AFOs must be implemented.

INTRODUCTION

Pollutants and contaminants released into waterbodies can dramatically impact the health of humans and aquatic wildlife (Bhandari et al., 2015; Wu & Janssen, 2011). These can include hormones such as estrogens and testosterones (Lopez, 2010). This has raised concern for human health when such hormones are present in drinking water (Wu & Janssen, 2011). Hormone releases into water have generally never been regulated which has caused problems for aquatic wildlife that are regularly exposed, such as changes in physical development and mating behavior (ibid.; Wright-Walters & Volz, 2009).

Endocrine-disrupting compounds (EDC) have been found in water supplies all over the world, where they bind to hormone receptors and/or alter expression of genes, thus interfering with several bodily processes like hormone synthesis and metabolism (Bhandari et al., 2015). Estrogens are EDCs that have increasingly become contaminants of concern due to their effects on aquatic wildlife and their lack of regulation.

The most common estrogens found in the environment are estrone (E1), estradiol (E2), estriol (E3), and 17α-ethynylestradiol (EE2; Gonsioroski et al., 2020). E1, E2, and E3 are naturally produced by humans and other animals, but can also be prescribed, while EE2 is synthetic and found in contraceptives (Caldwell et al., 2010). E2 is the most predominant estrogen in the human body (MohanKumar et al., 2018).

Since humans naturally produce these compounds, they are excreted in urine and enter wastewater treatment plants (WWTP) where conventional treatment processes are unable to completely remove them (Adeel et al., 2017; Caldwell et al., 2010; Gonsioroski et al., 2020; Kidd et al., 2007; McCullough et al., 2018; Rodriguez-Mozaz & Weinberg, 2010). Animal feeding operations (AFOs) are also a source of these estrogens as animal wastes contain estrogens, but are not treated before they reach surface waters (Adeel et al., 2017; Rodriguez-Mozaz & Weinberg, 2010).

There are concerns about the impacts of estrogenic water on humans, as exposure to xenoestrogens have been linked with altered reproduction, breast cancer, testicular cancer, and disruption of the endocrine system (Wright-Walters & Volz, 2009; Gonsioroski et al., 2020). Exposure to these exogenous estrogens is also considered to be responsible for intersex characteristics, alterations in mating behavior, and prevention of gonadal maturation in aquatic wildlife, which could destabilize populations due to lower reproductive fitness (Kidd et al., 2007; Wright-Walters & Volz, 2009). Despite the evidence that exogenous estrogens from WWTPs and AFOs are linked to negative effects on aquatic life and the concerns over human health, there are currently no standards set by the United States Environmental Protection Agency (shortened to EPA hereafter)[1] to measure or monitor these estrogens in water (McCullough et al., 2018).

Due to their potential impacts on human health and endangerment to aquatic populations, E1, E2, E3, and EE2 must be federally regulated. This paper will review the available information about estrogenic water pollution and its effects on humans and aquatic life and its sources. The central purpose is to propose policies to reduce the presence of the estrogens in the environment.

SOURCES

Estrogens that find their way into surface waters come from two main sources: wastewater treatment plants (WWTPs) and animal feeding operations (AFOs; Adeel et al., 2017). Neither of these sources are regulated for estrogen contamination in waterbodies by the EPA (2021; Wright-Walters & Volz, 2009). Thus, understanding where these estrogens originate will help contextualize impacts and provide the basis for possible regulation. Pathways of exposure are illustrated in **Figure 1** and information about each estrogen including source, structure, use, predicted no-effect concentration (PNEC) for fish, and acceptable daily intake for humans is summarized in **Table 1**.

GLOSSARY OF ABBREVIATIONS

AFOs	Animal feeding operations
AST	Activated sludge treatment
BPA	Bisphenol A
CAFOs	Concentrated Animal Feeding Operations
CCL	Contaminant Candidate List
CWA	Clean Water Act
EU	European Union
E1	Estrone
E2	Estradiol
E3	Estriol
EDC	Endocrine-disrupting compounds
EE2	17α-ethynylestradiol
EPA	(United States) Environmental Protection Agency
FDA	(United States) Food and Drug Administration
GAO	(United States) Government Accountability Office
NMP	Nutrient management plan
NPDES	National Pollutant Discharge Elimination System
NRDC	Natural Resources Defense Council
NWR	National wildlife refuge
PNEC	Predicted no-effect concentration
SDWA	Safe Drinking Water Act
TBEL	Technology-based effluent limitation
USDA	United States Department of Agriculture
WWTP	Wastewater treatment plants

WWTPs are inefficient in removing estrogens that are naturally produced in human wastes. As mentioned, humans naturally produce and can be prescribed E1, E2, and E3, while EE2 is entirely synthetic and is prescribed in contraceptives (Caldwell et al., 2010). These estrogens are excreted in urine and thus enter WWTPs where they are unable to be fully removed (Adeel et al., 2017; Bolong et al., 2009; Caldwell et al., 2010; Gonsioroski et al., 2020; Kidd et al., 2007; McCullough et al., 2018; Rodriguez-Mozaz & Weinberg, 2010). EE2, for example, has increasingly been detectable in sewage effluent in the last 20 years, ranging in concentration from <1 to 831 ng/L in effluents and surface waters (Roggio et al., 2014).

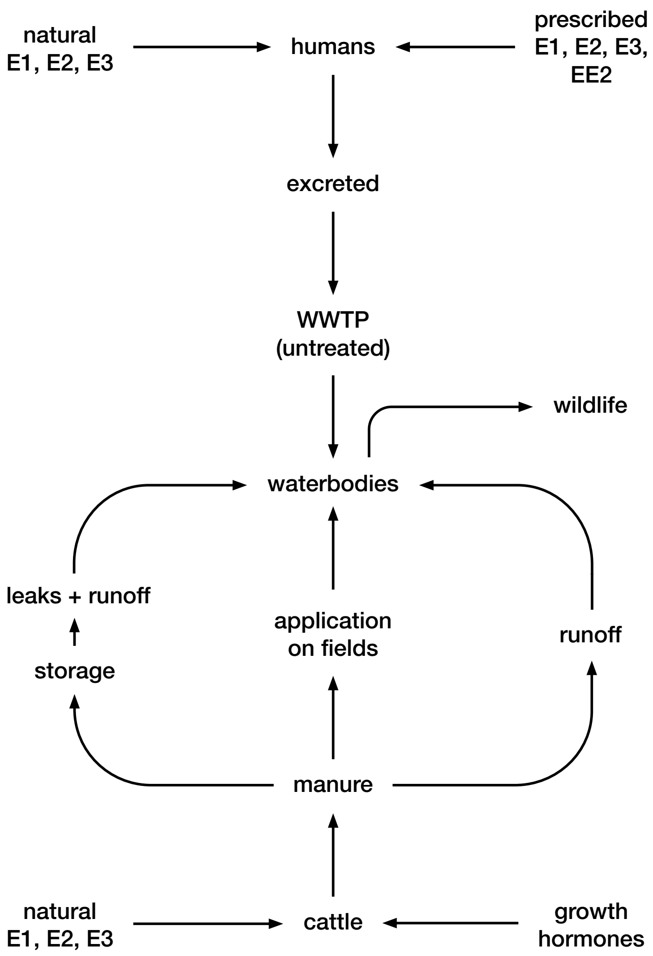
Estrogens have been detected in several studies of wastewater influents and effluents and have been found to be in waters at polluting levels close to WWTPs globally (Adeel et al., 2017; Caldwell et al., 2010). Atkinson and colleagues (2003) found that bodies of water had higher concentrations of E1 the closer they were located to sewage effluent. Kostich and colleagues (2013) modeled average influent concentrations to be 173 ng/L for E1, 92 ng/L for E2, 1491 ng/L for E3, and 6 ng/L for EE2, with removal rates varying and the potential for concentrations to exceed PNECs. Michelle E. Jarvie (2007) calculated that influent concentrations average 50.9 ng/L for E1 and 14.6 ng/L for E2, with an average removal rate of >75.1% and >79.9%, respectively. Effluent concentrations average <12.7 ng/L of E1 and <2.94 ng/L E2, which are above the PNEC (ibid.).

Even though effluent from WTTPs is a major source of estrogen release into waterbodies that have negative impacts on aquatic wildlife, there is no regulations or standardized way of measuring concentrations and removal (Wright-Walters & Volz, 2009; Rodriguez-Mozaz & Weinberg, 2010; Jarvie, 2007). In some cities, where effluent is concentrated due to population density, 50% of river flow can be discharged effluent and up to 90% of effluent in the winter (Wright-Walters & Volz, 2009). Cities can also experience overflows which forces plants to release untreated sewage into surface and drinking water (ibid.). This can lead to high concentrations of estrogens in nearby waterbodies.

However, the primary avenue for estrogens to enter aquatic environments is manure runoff from AFOs, mostly from cattle (Adeel et al., 2017; Caldwell et al., 2010; Gonsioroski et al., 2020). The US Food and Drug Administration (FDA) has stated that a number of synthetic hormones are approved to be used in cows raised for meat to improve growth rate and efficiency (FDA, 2018). These include synthetic E1, E3 and predominantly Zeranol which mimics E2 which is administered through an implant in the ear (ibid.; EPA, 2013). Estrogen administration is not approved in dairy cows, veal calves, pigs or poultry, but they still release natural estrogens in their feces (FDA, 2018).

Figure 1

Sources of E1, E2, E3, and EE2 to Waterbodies From Human Excretion and Manure Runoff From Cattle



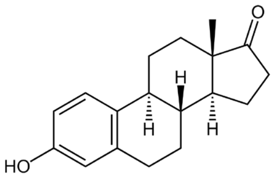
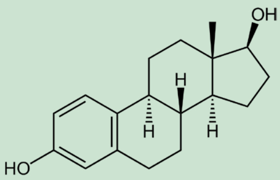
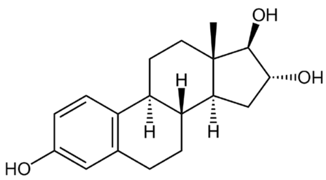
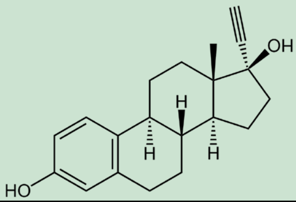
AFOs result in 2 trillion pounds of waste in the United States (Wu et al., 2009, p. 32). In 2002, farm animals produced 49 tons of natural and synthetic estrogen in their waste (Adeel et al., 2017). McCullough and colleagues (2018) have claimed that animal manure accounts for 90% of estrogens in the environment, and that if 1% of estrogens in their manure reached waterways, it would contribute to 15% of the estrogen released. The majority of estrogen is excreted by cattle, totaling 99,208 pounds of estrogen, which accounts for 92.7% of estrogen released by livestock (EPA, 2013).

The manure can reach waterbodies through runoff from pasture and rangeland, runoff from cropland fertilized with manure, leaks/overflows, and equipment failures from storage lagoons (Adeel et al., 2017; EPA, 2013). The EPA (2013), in their literature review, agrees that the estrogens cause endocrine disruption in fish. There are no sewage treatment requirements for animal manure (Miller et al., 2019). There are few studies examining the effects of estrogen in manure from AFOs on aquatic wildlife, and it is difficult to obtain at what concentrations they are being released at since there is no monitoring system in place (EPA, 2013).

Research shows that hormones are present in low but active levels in surface waters near AFOs (EPA, 2013). A study by Chen and colleagues (2010) showed that concentrations of E1, E2, and E3 of a river receiving discharge from livestock farms in Taiwan were significantly higher upstream (where discharge occurs) than those downstreams, especially in the winter where dilution and microbial activity was low. The site closest

Table 1

Summary of Common Estrogens Found in Waterbodies Including their Chemical Name, Short-Hand Name, Uses, Sources into the Environment, PNEC in Fish, and Acceptable Daily Intake in Humans

Full name	Short name	Structure	Uses	Source	PNEC (fish)	Acceptable daily intake (humans)
Estrone	E1		Excreted by all humans Pharmaceutcals Excreted naturally by livestock Supplemented in meat cattle for faster growth	WWTPs AFOs	6 ng/L	0.052 µg/person/day
Estradiol	E2		Excreted by all humans Pharmaceutcals Excreted naturally by livestock Supplemented in meat cattle for faster growth	WWTPs AFOs	2 ng/L	3 µg/person/day
Estriol	E3		Excreted by all humans Pharmaceutcals Excreted naturally by livestock Supplemented in meat cattle for faster growth	WWTPs AFOs	60 ng/L	0.084 µg/person/day
17α-ethynylestradiol	EE2		Pharmaceuticals (contraceptives)	WWTPs	0.1 ng/L	0.026 µg/person/day

to the livestock operation had a mean concentration of 398 ± 451 ng/L of E1, 84.3 ± 117 ng/L of E2, and 82.5 ± 69.6 ng/L of E3 (ibid.). The site furthest from the AFO had 46.4 ± 53.8 ng/L of E1, 9.6 ± 6.4 ng/L of E2, and 13.2 ± 12.5 ng/L of E3, whilst the reference site which did not receive any runoff averaged only 1.7± 4.1 ng/L for E2 (ibid.).

In general, EE2 is considered to be the most potent, but contributes less to water estrogenicity than other estrogens due to it only being excreted in urine by those who are prescribed it (Gonsioroski et al., 2020; Kostich et al., 2013; Thorpe et al., 2003). E1 has been shown to be generally more potent than E2 (although at times it can be equipotent) and is the most abundant estrogen in surface waters due to the fact it is excreted at higher levels by cycling women and can result from the breakdown of E2 (Jarvie, 2007; Thorpe et al., 2003). E3 is considered the least potent as it is 300 times less active than E2 and is not considered as responsible for estrogenicity as much as the other estrogens (ibid.).

There are two primary federal laws that protect water quality by regulating pollutant and contaminant discharges: the Clean Water Act (CWA) and the Safe Drinking Water Act (SDWA; EPA, 2004/2019; EPA, 2022 "Summary"). These laws protect waterbodies by limiting the amount of substances released into water bodies from particular sources, including WWTPs and certain AFOs (ibid.). E1, E2, E3, and EE2 discharges are not currently limited by either of these laws, but this paper will discuss them and the possibility for regulation (EPA, 2021; Wright-Walters & Volz, 2009).

HUMAN IMPACTS

Estrogens have been detected in drinking water and its presence has caused concern over human health (Caldwell et al., 2010; Rodriguez-Mozaz & Weinberg, 2010). In general, environmental exposure to EDCs during both development and adulthood can be a risk factor for diseases such as endocrine and reproductive cancers, diabetes, hypertension, and heart disease (Bhandari et al., 2015). Estrogen toxicity has increasingly gained attention from the scientific community, regulatory bodies, and the public as there has been a dramatic increase of endocrine and metabolic diseases since the 1970s by 2- to 3-fold (ibid.; MohanKumar et al., 2018).

Several studies show that an excess of estrogen is detrimental to human health. High levels of E2 have been associated with many disorders including several cancers, breast cysts, gallbladder disease, and thyroid disorders. It has also been associated with heavy menstruation, nervousness, irritability, mood swings, headaches, and sleep disturbances (Delgado & Lopez-Ojeda, 2022; Kumar et al., 2018; Watson et al., 2010). One study demonstrated that men, in particular, can experience infertility and swollen breast tissue, and postmenopausal women who were being treated with hormone replacement therapy with chronic exposure to estrogen demonstrated increased risk for dementia and cardiovascular disease (Delgado & Lopez-Ojeda, 2022). A review of several laboratory experiments in mice and rats by MohanKumar and colleagues (2018) suggests that chronic E2 exposure has an array of neurological, cardiovascular, and behavioral effects in addition to reproductive ones, including anxiety, ovulatory failure, mammary and pituitary tumors, and increase in blood pressure. However, these studies focus on effects from certain medications, controlled doses, and overproduction in the body, not from drinking water pollution.

Estrogens at polluting levels have been linked with cancer, such as breast and prostate, and reduced fertility (Adeel et al., 2017; Gonsioroski et al., 2020). But admittedly there are not many studies or much literature on estrogen toxicity in humans, and there is even less about estrogenic drinking water (Delgado & Lopez-Ojeda, 2022). Most exposures to estrogens occur at low, persistent levels, and effects of these chronic exposures have not been well studied in humans (MohanKumar et al., 2018).

Humans have no specific biomarkers of exposure to estrogenic EDCs and they do not follow linear-dose responses, making their impacts on humans harder to identify and predict (Bhandari et al., 2015; Jarvie, 2007). Some studies have claimed that current data supports that human exposure is low compared to effect levels and that there is little data supporting negative effects on humans (Caldwell et al., 2010; Kostich et al., 2013; Rodriguez-Mozaz & Weinberg, 2010). To the author's knowledge, there has been no specific study conducted that examined the specific correlation between estrogenic drinking water and health effects.

A study by Caldwell and colleagues (2010) suggests that people are exposed to E1, E2, and E3 in their diet, from products such as soy, dairy, and a wide variety of other foods, more than in drinking water. They found that total exposure in drinking water is at least 82 times lower than dietary exposure and 28 times less than acceptable daily intakes for sensitive individuals (ibid.). This suggests that regular dietary intake is the primary pathway of exposure to estrogens (apart from those naturally produced by our bodies and those that are prescribed) and that drinking water exposures are expected to have no adverse effects—at least according to current literature and relative to dietary exposure.

Therefore, while excess estrogens have the ability to negatively impact humans, there is no current evidence that suggests drinking water contains estrogens at polluting levels or that chronic exposures at current low levels is harmful. Because these human impacts are unknown and unsupported, the EPA does not view E1, E2, E3, and EE2 as a risk to humans under the SDWA (EPA, 2013). Nevertheless, humans are exposed to xenoestrogens from a variety of sources, including food, pharmaceuticals, and consumer products, hence a controlled study is needed to find relationships from estrogenic drinking water and human health (Kumar et al., 2018).

EFFECTS ON AQUATIC LIFE AND OTHER SPECIES

The unregulated release of estrogens by WWTPs and AFOs exposes aquatic life to additional doses of the hormone, causing an array of physiological, hormonal, biochemical, reproductive, and behavioral alterations. These health impacts, particularly on reproductive fitness, can lead to negative consequences for population stability. The majority of existing literature seems to focus on fish, while fewer studies focus on other species such as amphibians and invertebrates.

There is a wide consensus among the scientific community that estrogens in aquatic environments have the ability to feminize and alter fish physiology, notably of male fish (Adeel et al., 2017; Baynes et al., 2012; Bhandari et al., 2015; Bolong et al., 2009; EPA, 2013; Gonsioroski et al., 2020; Iwanowicz et al., 2016; Jarvie, 2007; Kidd et al., 2007; McCullough et al., 2018; Roggio et al., 2014; Orlando et al., 2004; Sumpter & Jobling, 1995; Thorpe et al., 2003; Wright-Walters & Volz, 2019). Every study that was examined in this review that mentioned E1, E2, E3, and/or EE2 supported their negative impacts on aquatic life.

Exposure to exogenous estrogens in fish has been observed to result in intersex individuals, reduced reproductive fitness, abnormal spawning behavior, altered oogenesis, skewed sex ratios, kidney damage, liver damage, and compromised immune systems (EPA, 2013). While there is no consensus on which estrogen is most responsible for these impacts, E1, E2, E3 and EE2 are of concern as they can impact stability of fish populations (Atkinson et al., 2003).

A commonly used biomarker of exogenous estrogen exposure is elevated levels of vitellogenin—an egg yolk precursor protein induced by estrogen found in adult females and intersex fish (Gonsioroski et al., 2020; Jarvie, 2007; Kidd et al., 2007; Sumpter & Jobling, 1995). Even low concentrations of estrogens in aquatic habitats can stimulate the production of vitellogenin, with some papers citing the no-effect concentration for fish to generally be 1 ng/L across the four estrogens (Baynes et al., 2012; Iwanowicz et al., 2016; Sumpter & Jobling, 1995). The presence of vitellogenin in males is associated with loss of secondary sex characteristics, such as reduced testicular size, and feminization, while elevated levels in females can lead to reduced ovary size and egg production (EPA, 2013; Gonsioroski et al., 2020; Jarvie, 2007). In a seven-year experimental lake study, investigators documented elevated vitellogenin levels and reproductive abnormalities post-exposure in fathead minnows, even resulting in the collapse of the experimental population prior to the completion of the study (Kidd et al., 2007).

The above impacts are not only seen in experimental contexts but are also prevalent in natural environments. A study by Iwanowicz and colleagues (2016) examining sites in national wildlife refuges (NWRs) in the northeast of the US recorded that intersex smallmouth bass males were present in all 12 sites across 7 NWRs with a composite of 85%. Out of 23 sites, intersex largemouth bass males were observed in 20 sites from 13 NWRs with a composite of 27%. The study also tested water samples at 45 sites across 19 NWR and observed that estrogenic activity was above the quantitation limit in 21 of these sites (ibid.).

Some studies have different PNECs: Caldwell and colleagues (2012) claim in their study that they are 6 ng/L E1, 2 ng/L E2, 60 ng/L E3, and 0.1 ng/L EE2, with Sumpter and Jobling (1995) agreeing with the PNEC for EE2, and Iwanowicz and colleagues (2016) reporting E2 PNEC ranges from 1–10 ng/L. It is important to note that some species of fish are more sensitive to estrogens than others, and there is no available composite study with PNEC for multiple species of fish (Iwanowicz et al., 2016).

Most studies examining the effects of estrogens on aquatic life focus on fish with less being known about other species (Bhandari et al., 2015). The lack of knowledge is exacerbated by the lack of quantification studies in aquatic environments (Atkinson et al., 2003). To take one brief example of the limited studies conducted on other species, in amphibians, exposure may distort sex ratios in favor of females and alter mating behavior (Bhandari et al., 2015). Several studies in different species of frogs demonstrate that exposure to EE2 during the developmental stage resulted in sex ratio imbalance, complete or partial feminization, male to female sex reversals, reduced fertility in males, and gonad alterations (ibid.). Exposure in male African clawed frogs of varying concentrations of EE2 reduced mating calls, sexual arousal, and the number of females willing to mate with them (ibid.).

CURRENT REGULATIONS

The Safe Drinking Water Act (SDWA) and the Clean Water Act (CWA) are the two primary federal laws that protect waterbodies in the United States. While no state water quality standards for estrogens and other hormones were found, some state governments, including New Jersey, Minnesota, and California, have conducted monitoring studies on emerging contaminants and EDCs whose reports included E1, E2, E3 and EE2 (Ferrey, 2011; Fischer et al., 2018; de Vlaming et al., 2007). However, this paper mainly concerns federal regulation. This section will discuss the basic components of the acts, the policy gaps in the regulations, and the potential for regulation of estrogen.

Safe Water Drinking Act

The SDWA passed in 1974, was amended 1986 and 1996, and aims to protect the public's health by regulating the drinking water supply, including rivers, lakes, reservoirs, springs, and groundwater wells. It enables the EPA to set health-based standards for natural and man-made contaminants (EPA, 2004/2019).

Every five years, the SWDA requires the EPA to publish a Contaminant Candidate List (CCL), which is a priority list of contaminants that are currently not regulated by the SDWA but are known or anticipated to occur in public water systems (EPA, 2014). Formal decisions, called regulatory determinations, are then made to determine whether the EPA should initiate the process to develop regulations for certain contaminants (ibid.). The SDWA requires the EPA to make regulatory determinations for at least five contaminants from the most recent CCL within five years of the previous round of determinations (ibid.). The EPA is also able to make regulations for contaminants currently not on the CCL if research shows that it poses a public health risk.

E1, E2 (specifically 17β-estradiol), E3, and EE2 were all listed on CCL 3 in 2009, but were not chosen to be regulated (EPA, 2009). They were also published in CCL 4 in 2016, but once again were not listed as regulated contaminants under the SDWA (EPA, 2021, 2022b). The EPA (2022c) published CCL 5 in November 2022 and none of the estrogens were listed as a candidate contaminant. This is likely due to the absence of research that supports the fact that estrogens are harmful to human health in drinking water.

The lack of regulations for estrogens in drinking water is unsurprising, but justified, as there is not enough research to quantify and prove adverse health effects of estrogenic drinking water. However, estrogenic compounds have received the most concern out of any other EDCs (Houtman, 2010). This not only includes the ones in this paper, but other human-made xenoestrogens that make their way into drinking water that are not regulated, such as bisphenol A (BPA; Kumar et al., 2018). The lack of research may not provide evidence of harm, but it also results in the lack of evidence of no-risk. Without proper toxicology and risk-assessment, there is no assurance that chronic exposure to E1, E2, E3, and EE2 in drinking water has no effect on humans. This uncertainty causes concern among the public. This may result in the need to use the precautionary principle to guide water quality policies to proactively protect human health not only from chronic exposure of the aforementioned estrogens, but other unregulated xenoestrogens as well.

The SDWA and the precautionary principle

The precautionary principle has been an increasingly compelling method for drinking water regulations (Crawford-Brown & Crawford-Brown, 2011). However, due to the current nature of the SDWA which requires a certain amount of proof, creating drinking water standards for E1, E2, E3, and EE2 becomes difficult and would require major changes to the law on a legislative level.

The precautionary principle exists as a method to proactively prevent negative consequences by avoiding an action entirely even in the face of uncertainty (Crawford-Brown & Crawford-Brown, 2011; Houtman, 2010). Despite evidence being far from definitive, the precautionary principle can be preferable when it comes to protecting drinking water from emerging contaminants (Houtman, 2010).

The European Union (EU) can serve as an example as to how to utilize precaution in drinking water. The EU has the precautionary principle written into its primary law in the 1992 Maastricht Treaty, which defines environmental policy in the EU in order to provide the highest level of protection (Dolan et al., 2013). The Drinking Water Directive, the EU's drinking water policy, uses the principle to justify a low maximum allowable concentration of 0.1 μg/L for most active pesticides substances, which the US either has higher allowable values for or no set standard at all (ibid.). A similar universal standard to regulate E1, E2, E3, and EE2 could be utilized in the United States.

When making the decision to regulate a contaminant in drinking water, the SDWA specifically states that it must meet three criteria (EPA, 2014): (1) the contaminant may have an adverse effect on the health of persons; (2) the contaminant is known to occur or there is substantial likelihood the contaminant will occur in public water systems with a frequency and at levels of public health concern; and (3) regulation of the contaminant presents a meaningful opportunity for health risk reductions for persons served by public water systems. In short, there must be strong evidence that the contaminant occurs in public drinking water and that it has the ability to harm human health.

If E1, E2, E3, and EE2 were to be regulated in drinking water under justification of the precautionary principle, the SDWA would have to be amended in Congress to allow for preventable measures. This would mean either amending the existing criteria or adding separate criteria for emerging contaminants. Not only would this allow the possibility of regulation of E1, E2, E3, and EE2 as a proactive measure to protect human health, but other xenoestrogens such as BPA and phthalates. (Kumar et al., 2018).

However, passing these amendments would be difficult as the precautionary principle is still poorly defined both in practice and in law, in the U.S. and internationally (Crawford-Brown & Crawford-Brown, 2011). Despite being written into EU law, it is not defined in the Maastricht treaty and relies on the European Commission for guidance (Dolan et al., 2013). Moreover, the use of the precautionary principle in the EU's drinking water does not seem to apply to EDCs as there are few standards and virtually none for xenoestrogens (Houtman, 2010). This could possibly indicate lack of application of the principle due to the absence of a concrete legal definition and general shortcoming in practice as it is still considered an underdeveloped concept. Without a solid legal definition and the sheer difficulty of creating environmental legislation in Congress, drinking water standards for estrogens seems unlikely for now.

Until more research emerges on estrogenic drinking water, there is no true justification for regulation under current SDWA laws. But while there are no specific recommended policies, we advocate for US laws to adopt the precautionary principle in the future to protect drinking water quality.

Clean Water Act

The CWA, enacted in 1948 and amended in 1972, provides the basic protection for surface waters by regulating pollution discharges and water quality (EPA, 2022a). It makes it illegal for point sources to discharge pollutants into navigable waterways without obtaining a National Pollutant Discharge Elimination System (NPDES) permit (ibid.).

Estrogens are not listed under the CWA as pollutants nor are they monitored for removal (General Provisions, 1974). The CWA's purpose is to "restore and maintain the chemical, physical, and biological integrity of the Nation's waters" with one of the goals being "protection and propagation of fish, shellfish and recreation in and on the water, wherever attainable" (Federal Water Pollution Control Act, 2002). Therefore, it is the federal government's duty to protect wildlife negatively impacted by water pollutants, including EDCs. Due to the widely-agreed upon evidence that E1, E2, E3, and EE2 impact aquatic wildlife, it is recommended that they be listed as pollutants under the CWA. This would require point sources that release estrogens into navigable waterways to obtain NPDES permits, be subject to technology-based effluent limitations (TBELs), and require compliance monitoring for the four estrogens (EPA, 2022a).

Municipal WWTP are considered point sources as they directly discharge into surface waters and thus are covered by the CWA and NPDES permitting (Jarvie, 2007; EPA, 2022a). WWTPs often fail to remove estrogens below PNEC for aquatic wildlife. The listing of E1, E2, E3, and EE2 as pollutants would force WWTPs to obtain NPDES permits for them, thus requiring their monitoring and removal in treated water before being released. It would require WWTPs to adhere to TBELs, hence the systems treating the water would need to be updated. Available technologies that show promise will be discussed later.

The lack of regulations: Concentrated animal feeding operations (AFOs)

Regulating estrogen from AFOs becomes complex since there are no treatment requirements for animal wastes and CWA laws only apply to certain operations (Miller et al., 2019). The AFOs that are regulated also tend to easily avoid any current requirements due to insufficient enforcement by the EPA. Therefore, even if estrogens were to be listed under the CWA, gaps in current regulations would prevent meaningful reduction of estrogens in the environment as AFOs are the primary source for contamination.

Most agricultural activity is not regulated by the CWA as they are classified as nonpoint sources, including AFOs (Copeland, 2010). However, a subset of AFOs, Concentrated Animal Feeding Operations (CAFOs), are explicitly a point source and thus regulated under the CWA (ibid.; Copeland, 2016). AFOs are considered CAFOs if they have more than 1000 animals and either discharge pollutants through a man-made ditch or similar man-made device, or discharge pollutants directly that "originate outside of and pass over, across, or through the facility, or otherwise come into direct contact with the confined animals" (Copeland, 2010, p. 3). "[AFOs] with 300–999 animals may be CAFOs depending on discharge characteristics; and those with fewer than 300 may be CAFOs in some cases" (ibid.). Only 5% of AFOs are CAFOs; however, they account for 40% of the livestock in the US raised in a confined facility (ibid.). AFOs not considered CAFOs are not subject to any standards as they are nonpoint sources (Copeland, 2016).

Since CAFOs are subject to CWA regulations, they require NPDES permits, but only if they discharge pollutants into waters of the United States (Copeland, 2010). CAFOs that do not discharge or propose to discharge do not need permits, therefore all CAFOs are not currently obligated to obtain NPDES permits (Copeland, 2016). Unplanned and accidental discharges from unpermitted CAFOs are illegal under the CWA (ibid.). NPDES requirements are mostly under a voluntary basis as a CAFO's "no discharge" certification is not reviewed by permitters or available for public comment (ibid.). A 2003 rule required that all CAFOs had a "duty to apply" for NPDES permits unless they proved they did not discharge (ibid.). This was then changed to the current rule in 2008 when the U.S. Court of Appeals for the Second Circuit vacated the rule in 2005 in *Waterkeeper Alliance et al.. v. EPA*, because the CWA only covers actual discharges rather than potential ones (ibid., p. 10; EPA, n.d.).

Many CAFOs remain unregulated and operate without a permit (Food & Water Watch et al., 2017; Miller et al., 2019). Even though 75% of CAFOs discharge into waters, only 40% have obtained the necessary NPDES permits to do so (Food & Water Watch et al., 2017). Most CAFOs remain unpermitted because the burden remains on the EPA to prove they are discharging (Kenyon, 2017). There is no collective database of CAFO size, location, and operations by any federal agency and states vary in enforcement and additional permit requirements with little guidance from the EPA (Food & Water Watch et al., 2017; Miller et al., 2019).

More than this, "agricultural stormwater discharge" (runoff as a result of precipitation events) are exempted from regulation as long as the manure has been applied in accordance to "site-specific nutrient management practices" (Copeland, 2016, p. 13; Food & Water Watch et al., 2017; Miller et al., 2019).

Here, nutrient management plans (NMPs)—which address the amount, source, timing, and placement of nutrients—can assist in preventing nutrient leaching and poor nutrient applications and practices. Such malpractices may lead to overapplication and runoff, for example. The US Department of Agriculture (USDA) recommends that NMPs should include measures to not apply nutrients to soils that are frozen, covered in snow, saturated (top two inches), on areas of concentrated flow, and/or steep slopes (USDA, 2019). It also encourages setback distances and consideration of weather, climate, soil characteristics, and amount of nutrients needed to be applied. While NMPs are required to be submitted with NPDES applications, the items are not enforceable and there is no federal oversight that ensures wastes are applied properly (Copeland, 2011; Food & Water Watch et al., 2017). Due to the lack of regulation, CAFOs are incentivized to over-apply wastes against NMP guidelines, which result in large amounts of runoff (Brotzman, 2015; EPA, 2013; Food & Water Watch et al., 2017).

Often, the amount of manure that is produced exceeds what can be applied to croplands, so manure is often overapplied and applied on saturated or frozen soils where nutrients can not penetrate the soil and be absorbed because it the cheapest method of disposal (Brotzman, 2015; EPA, 2013; Food & Water Watch et al., 2017; Miller et al., 2019). In 2014, *ALT v. EPA* made other discharges eligible under the current agricultural stormwater exemption definition, namely those associated from a "production area" defined as "the animal confinement area, the manure storage area, the raw materials storage area, and the waste containment areas" (CAFO Rule, 2003, 7266, cited in Kenyon, 2017, pp. 1199–1200) This made other aspects of AFOs other than land application become unregulated (Food & Water Watch et al., 2017; Kenyon, 2017).

If estrogens were listed as a pollutant under the CWA, the lack of regulation of AFOs, lack of permitting and enforcement for CAFOs, and the agricultural stormwater would render the law useless for estrogen pollution. As a result, additional policies would need to be put in place to reduce the amount of animal waste from reaching surface waters.

RECOMMENDED ADDITIONAL POLICIES UNDER CWA FOR CAFOs

Based on the current insufficient standards for manure from CAFOs under the CWA and NPDES permit system, additional policies must be put in place to protect aquatic wildlife from hormonal, physical, biochemical, and reproductive harm. While WWTPs are no doubt a source of estrogens that can affect aquatic life, due to the fact estrogen pollution is primarily from unregulated animal feeding operations, increased regulations should be focused in this area (Adeel et al., 2017; Caldwell et al., 2010; Gonsioroski et al., 2020). Therefore, it is essential that additional policies be implemented which close regulatory gaps, including adjustment to the definition of a CAFO and agricultural stormwater exemption, baseline manure management practices, NPDES and NMP enforcement, and creation of a comprehensive and accessible database.

The desire for increased regulations to limit discharges from CAFOs is nothing new. Wastes from CAFOs have been known to affect water quality as they contribute large amounts of phosphorus and nitrogen that cause increased algae blooms and fish kills, pathogens that can spread diseases among human populations, salts, trace elements, antibiotics, pesticides, and other hormones (Copeland, 2016; EPA, 2013; Miller et al., 2019). Therefore, implementing new rules on CAFOs in the frame of the multitude of these problems with water quality would reduce the amount of estrogen being discharged into surface waters.

Environmental organizations have advocated for increased regulations and new rules: they argue that current measures are too voluntary and fail to require improved technology rather than mandating strict compliance (Copeland, 2010). To date, few researchers have deeply explored this area of proposed policy changes. Suggested changes to CAFO regulation under the CWA in the last twenty years were searched for—six relevant works were found. It is important to note that while many environmental advocates and groups advocate for increased CAFO oversight, few relevant reports citing specific changes were found. Authors of recommendations include advocacy groups, law students, and government researchers.

The United States Government Accountability Office (GAO, hereafter) is an independent, nonpartisan agency that provides recommendations to Congress to improve its efficiency. They released two reports (GAO, 2003, 2008) on improvements in CAFO oversight to protect environmental quality: one in 2003 prepared on request from former Senator Tom Harkin over concerns on water quality and another in 2008 over similar concerns. Two papers were drafted by law students who acted as independent advocates to strengthen AFO and CAFO regulations (Brotzman, 2015; Kenyon, 2017). A large coalition of environmental groups submitted a petition to the EPA asking for several standards for CAFOs to be altered (Food & Water Watch et al., 2015). The Natural Resources Defense Council (NRDC) released a report (see Miller et al., 2019) detailing the negative impacts of CAFO manure on humans and the environment with a proposed permit system to increase transparency of farm practices.

The proposed rule changes proposed by the six authors were analyzed to find commonalities and aggregated into workable recommendations for new rules in this article. Concerns that need to be addressed include enforcement of NMPs, limiting the agricultural stormwater exemption, and enforcement of NPDES permits. **Table 2** summarizes what was proposed by each author and compares them to what this paper suggests. Proposed rules that would contradict court rulings or meet substantial and avoidable backlash from the farming industry are not included in the recommendations. For example, a petition item by Brotzman—proposing limiting the number of animals per acreage of sprayfields and NRDC—recommended that almost all CAFOs be required to have NPDES permits. These types of rules would likely cause backlash from farmers for being too restrictive and troublesome. Recommendations were limited to measures that seemed reasonable and achievable.

NMPs were frequently discussed, specifically enforcing their implementation or enforcing recommended practices associated with them (Brotzman, 2015; Food & Water Watch et al., 2017; Kenyon, 2017). It is crucial to reduce the amount of runoff of manure from fields, therefore it is recommended that these measures become enforceable, baseline requirements for all AFOs (not just permitted ones; ibid.). These should be universal standards for how much manure can be applied per acre to prevent over-application and runoff. NMPs should be increasingly scrutinized during the NPDES process and approved before the permit is issued. Violators of manure application standards and NMPs should be prosecuted and issued high monetary penalties. These measures would effectively limit the agricultural stormwater exemption by specifically banning certain practices and force farmers to comply with NMPs for

Table 2

Summary and Comparison of Recommended Policies by Various Groups, Including Advocacy Groups, Law Students, Government Researchers, and This Paper

Review authors	US GAO (2003)[1]	US GAO (2008)[2]	Brotzman, E. (2015)[3]	Food & Water Watch et al. (2017)[4]	Kenyon, E. (2017)[5]	NRDC (2020)[6]	Shrivastava (2023)[7]
Redefine CAFO definitions			x	x		x	x
Allow newly regulated CAFOs to apply for general permits			x				
Strengthening/enforcing NMP standards			x	x	x		x
Regulate waste management practices			x		x		x
Placing a cap on the number of animals per acre of sprayfield			x				
Establish more effective engagement with the public			x				x
Increase transparency in information reporting and encourage citizen enforcement			x			x	x
Institute monitory requirements to improve accountability				x	x		
Nationwide database of CAFO size, location, NPDES permit status, and operations		x				x	x
Limit/revise agricultural stormwater exemptions				x	x		x
Presume certain CAFOs will discharge thus requiring NPDES permits rather than CAFOs proposing or potentially discharging				x			
Apply Large CFO effluent limitations guidelines to all CAFOs				x			
Require all but the smallest CAFOs to obtain NPDES permits						x	
EPA and states develop and implement their own plans that identify how they intend to carry out their increase permitting, inspection and enforcement responsibilities within specified time frames	x						
Universal standard for manure application per acre							x

Note. [1,2] Government agency — Research and recommendation by nonpartisan government agency [Report]
[3] Independent environmental advocate [Petition]
[4] Coalition of environmental organizations [Petition]
[5] Law review [Journal article]
[6] NRDC — Environmental advocacy group [Online report]
[7] Environmental researcher [Journal review article]

"proper application" of wastes to their field.

The EPA must redefine and clarify the agricultural stormwater exemption to exclude the unregulated discharge from production areas that contribute to negative impacts on water quality (Food & Water Watch et al., 2017, Kenyon, 2017). Emily Kenyon (2017) suggested that the agricultural stormwater exemption be redefined so that it is limited to land application for crops that will be harvested. This recommendation should be followed so that all other discharges from other aspects of production are excluded from the exemption to protect surface waters from unregulated, unmonitored discharges.

Smaller AFOs that do not qualify as CAFO are not required to obtain NPDES permits, but still contribute heavily to water impairment (Brotzman, 2015). Out of 238,000 AFOs, only 20,000 are subject to regulations based on discharge characteristics (ibid.). Erika Brotzman (ibid.) has recommended that the number of animals on a farm to be considered a CAFO be lowered so that more farms are subject to NPDES permits. This adjustment would thus make more AFOs subject to CWA regulations for discharging, monitoring, and NMPs.

Many CAFOs remain unregulated despite their discharging of wastes into waterways. Action needs to be taken to enforce NPDES permits, which starts with increased monitoring of CAFOs themselves. While CAFOs that are permitted need to monitor, record-keep, and report discharges of CWA pollutants, unpermitted CAFOs do not. The NRDC has created a proposed permit system that would collect and distribute CAFO operations and data to the EPA, state, and public (Miller et al., 2019). However, this exact permit system is unlikely to be implemented due to the already present NPDES permit system. The GAO (2008) had proposed a nation-wide inventory in order to collect data on permitted CAFO to ensure compliance and protect water quality. This was implemented at a state level, but states vary in consistency and level of scrutiny in gathering data from permitted, unpermitted, and illegally unpermitted CAFOs and enforcing required permits (Food & Water Watch et al., 2017). It is recommended that a combination of the NRDC permit system and GAO recommendation be used to create a national database by the EPA for all AFOs, including size, location, animal type and number, NPDES permit status, NMP information, manure analysis, and other waste management practices. States should be required to gather the information of all AFOs and report it to the EPA to form a nation-wide CAFO database.

Tracking accurate information can be difficult for many states where CAFOs may slip under the radar and/or where states do not scrutinize their data-collection. Transparency to the public is crucial to maintain trust and keep citizens informed about the quality of their water. The NRDC found that most states have low transparency of CAFO data and operations. Brotzman (2015) has advocated for increased engagement with the public, and joined the NRDC in encouraging more transparency (see also Miller et al., 2019). The national database for CAFOs should be available online on a single website for the public to access.

The EPA generally has issues with enforcement of the CWA due to an insufficient budget (Feller, 1983). But the CWA allows for citizen enforcement measures by allowing individuals and organizations to sue violators of effluent standards or federal agencies for failing to enforce standards which helps fill the enforcement gap (ibid.). Under the CWA, citizens must provide the alleged violator with a 60-day notice of the intent to file suit (Federal Water Pollution Control Act, 2002; Ohio Environmental Council, n.d.). The suit may only be filed if during these 60 days the violator does not come into compliance or if another federal agency is taking action already (ibid.). The suit must be filed within the judicial district in which the violation occurred and a copy must be sent to the US EPA Administrator and the U.S. Attorney General (ibid.). Violators can be fined up to $25,000 per violation per day and the regulatory agency has the authority to order the violator to cease its operations, and revoke or refuse to renew the permit (Ohio Environmental Council, n.d.). If estrogens were listed as a pollutant, under current laws, citizens and organizations would be able to bring lawsuits against CAFOs who violate the effluent standards.

We recommend the EPA then take this one step further by allowing legal action against CAFOs for failing to follow reporting, permit, and farm practice requirements within the program for national database, such as reporting false information, failing to obtain NPDES permits when necessary, failing to follow baseline manure requirements, and failing to follow NMPs. These types of suits could follow the same requirements outlined by the CWA regarding a 60-day notice and $25,000 per violation per day fine to keep things standardized and simple. If citizens are unable to file suit due to financial or time commitment reasons, the online national database should have a form to report any violations either self-identified or anonymously. Citizens and independent organizations should also be allowed to submit supplementary information, such as AFOs that are in operation but there is no data on. The EPA would then be able investigate, collect information, and issue penalties as necessary. This method of citizen enforcement would not only help educate the public about CAFO operations in their proximity, but help supplement gaps and enforcement of CWA regulations, NPDES permits, and baseline farming practices, as well as lower costs associated with increased federal agency enforcement (Brotzman, 2015).

Due to the fact that CAFOs are the primary source for E1, E2, E3, and EE2 in the environment which threatens the health of aquatic wildlife, supplementary policies under the CWA, in summary, include: scrutinizing NMPs and making then enforceable by law; requiring standard manure management practices; limiting the agricultural stormwater exemptions to only land application of manure; lowering the numerical definition of CAFOs so that more farms require NPDES permits; and the creation of a comprehensive database of all CAFOs and their operations accessible by the public for citizen enforcement of NPDES permits.

METHODS FOR TREATMENT

WWTPs

With the addition of estrogens as a pollutant under the CWA, current WWTPs will need to be updated because current technology is not sufficient enough for estrogen removal to protect wildlife (Baynes et al., 2012). Many promising technologies have been explored for removal, but more research is needed to understand their use and improve efficiency before implementation.

Granular activated charcoal (carbon) adsorption has shown promise in studies for removing estrogens and preventing intersex induction in studies; however, disposal and high expenses may make this treatment method difficult to implement (Baynes et al., 2012; Bolong et al., 2009; Jarvie, 2007; Koh et al., 2008). Reverse osmosis has also been shown to be effective in experiments, but also may be too expensive (ibid.).

The effectiveness of sand filtration is up for debate: Jarvie (2007) claimed that it was effective whilst Baynes and colleagues (2012) demonstrated that the treatment method effectively prevented feminization in a controlled study. More research is needed, but it may prove to be a low-cost treatment method. Disinfection with sodium hypochlorite and ozonation have been shown to have the ability to remove up to 100% of estrogens, but more research is needed (EPA, 2013). Phytoremediation also has potential as sandbar willow, *Arabidopsis*, and curly leaf pondweed have demonstrated the ability to remove estrogens in controlled studies (Adeel et al., 2017; Trueman & Erber, 2013).

Bioremediation strategies have been explored, using selective microbes that can convert estrogens into different forms, degrade, or metabolize them (Adeel et al., 2017; Koh et al., 2008; Yu et al., 2013) Sources of bacteria include soils, activated sludge, dental plaque, and human intestines (Yu et al., 2013). Current knowledge of the chemical pathways are limited and 99% of potential bacteria are unculturable in a lab (ibid.). More research is needed to understand how the bacteria function to degrade, metabolize, or transform estrogens, and how to maximize their potential in treatment facilities (ibid.).

The most promising treatment method may be improvements in secondary activated sludge treatment (AST) that utilizes natural microorganisms that exist in the wastewater (Jarvie, 2007; Bolong et al., 2009; Koh et al., 2008). AST is the most common secondary treatment in WWTPs and it is where most estrogens are degraded, but not completely removed (ibid.). Longer sludge retention times and/or hydraulic retention times have been suggested to work as the longer times and increased aeration allow the bacteria to grow more and allow more contact time (ibid.).

Nitrifying AST tanks may also be an effective treatment option as it can remove more than 95% of estrogenic activity (Bolong et al., 2009; Koh et al., 2008). Effectiveness depends on sludge volume, pH, oxygen, and temperate conditions (Bolong et al., 2009). A study by Hicks and colleagues (2017) found that when the Kitchener WTTP in southern Ontario was upgraded to nitrifying AST with higher sludge retention times and aeration to better treat ammonia, there was a reduction in the incidence and severity of intersex male rainbow darter downstream from previous years. One site saw a decrease of 100% intersex incidence in fall 2012 (pre-upgrade) to 14% incidence in fall 2015, three years post-upgrade (ibid.).

CAFOs

Technologies have been explored and developed to treat manure as well, but expenses may be too high to implement them cost-efficiently (EPA, 2013). Proper storage of excess manure needs to be emphasized with structures like enclosures, piles, lagoons, and ponds should be updated to increase capacity and prevent leaks and spills (ibid.). Composting may be an effective method for reducing hormone concentration with one study finding that composting decreased E2 concentrations by 84% in chicken manure (ibid.), but more research is needed.

CONCLUSION

Waterbodies contaminated with estrogens as a result of WWTPs and AFOs have led to feminization of male fish, intersex males, reduced reproductive fitness, and damage to bodily systems which put stability of populations at risk. Research also suggests estrogens have an array of negative effects on other wildlife. Due to these impacts, E1, E2, E3, and EE2 should be regulated under CWA, particularly enforcing responsible practices on CAFOs. Recommended regulations include lowering the number of animals in the definition of a CAFO so that the operation is subject to NPDES permits, making NMP plans enforceable, requiring baseline manure management practices, limiting the agricultural stormwater exemption, creating a comprehensive database of CAFOs and their discharges, and allowing citizen enforcement. Limited research does not support impacts on human health, therefore there is no justification for any regulation under the SDWA. However, the precautionary principle remains as a possible future tool if lawmakers are willing to amend the SDWA or enact new legislation. This review was able to cover general impacts of estrogen contamination on fish from studies such as smallmouth and largemouth bass. However, we recommend more research be done on different PNEC levels on different species, as some species may be more sensitive to different concentrations of estrogens than other species. There also needs to be more research done for the effects on amphibians, reptiles, and other invertebrate life that may regularly be exposed to contaminated waters. Moreover, more studies are needed to quantify concentrations in different aquatic environments.

The link between the regular consumption of estrogenic water and human health needs to be further studied as no current research exists on the topic. Research should include the effects on young developing children as they may be more susceptible to lower concentrations. More research is also needed on estrogenic treatment methods for WWTPs and CAFOs, including the effectiveness of technologies like sand filtration and metabolic pathways of bacteria that can metabolize, degrade, or transform estrogens.

This review and policy recommendation will hopefully kickstart the discussion on comprehensive monitoring of not only estrogens, but other hormones, such as testosterone, in waterbodies. Many environmental organizations have advocated for increased regulations on AFOs, especially CAFOs, hence this paper can serve as one of the starting points for regulating CAFOs for the wide range of pollutants they discharge into waterbodies.

ACKNOWLEDGEMENTS

I am deeply grateful to my advisor, Dr. Sharon Moran, for her invaluable guidance and support. Special thank you to my sister as well, who helped forge the idea for this paper.

NOTES

1. EPA is abbreviated both in-text and citations for "United States Environmental Protection Agency".

Article references

Adeel, M., Song, X., Wang, Y., Francis, D., & Yang, Y. (2017). Environmental impact of estrogens on human, animal and plant life: A critical review. *Environment International*, *99*, 107–119. https://doi.org/10.1016/j.envint.2016.12.010

Atkinson, S., Atkinson, M. J., & Tarrant, A. M. (2003). Estrogens from sewage in coastal marine environments. *Environmental Health Perspectives*, *111*(4), 531–535. https://doi.org/10.1289/ehp.5233

Baynes, A., Green, C., Nicol, E., Beresford, N., Kanda, R., Henshaw, A., Churchley, J., & Jobling, S. (2012). Additional treatment of wastewater reduces endocrine disruption in wild fish: A comparative study of tertiary and advanced treatments. *Environmental Science & Technology*, *46*(10), 5565–5573. https://doi.org/10.1021/es204590d

Bhandari, R. K., Deem, S. L., Holliday, D. K., Jandegian, C. M., Kassotis, C. D., Nagel, S. C., Tillitt, D. E., vom Saal, F. S., & Rosenfeld, C. S. (2015). Effects of the environmental estrogenic contaminants bisphenol A and 17-ethinyl estradiol on sexual development and adult behaviors in aquatic wildlife species. *General and Comparative Endocrinology*, *214*, 195–219. https://doi.org/10.1016/j.ygcen.2014.09.014

Bolong, N., Ismail, A. F., Salim, M. R., & Matsuura, T. (2009). A review of the effects of emerging contaminants in wastewater and options for their removal. *Desalination*, *239*(1–3), 229–246. https://doi.org/10.1016/j.desal.2008.03.020

Brotzman, E. (2015). *Rulemaking petition to lower the threshold that qualifies animal feeding operations ("AFOs") as concentrated animal feeding operations ("CAFOs") and thereby "point sources" under §402 of the Clean Water Act ("CWA")* [Petition]. https://www.epa.gov/system/files/documents/2021-09/cwa_cafo_ebrotzman_9_2015.pdf

Caldwell, D. J., Mastrocco, F., Anderson, P. D., Länge, R., & Sumpter, J. P. (2012). Predicted-no-effect concentrations for the steroid estrogens estrone, 17-estradiol, estriol, and 17-ethinylestradiol. *Environmental Toxicology and Chemistry*, *31*(6), 1396–1406. https://doi.org/10.1002/etc.1825

Caldwell, D. J., Mastrocco, F., Nowak, E., Johnston, J., Yekel, H., Pfeiffer, D., Hoyt, M., DuPlessie, B. M., & Anderson, P. D. (2010). An assessment of potential exposure and risk from estrogens in drinking water. *Environmental Health Perspectives*, *118*(3), 338–344. https://doi.org/10.1289/ehp.0900654

Chen, T.-S., Chen, T.-C., Yeh, K.-J. C., Chao, H.-R., Liaw, E.-T., Hsieh, C.-Y., Chen, K.-C., Hsieh, L.-T., & Yeh, Y.-L. (2010). High estrogen concentrations in receiving river discharge from a concentrated livestock feedlot. *Science of The Total Environment*, *408*(16), 3223–3230. https://doi.org/10.1016/j.scitotenv.2010.03.054

Copeland, C. (2010). *Animal waste and water quality: EPA regulation of concentrated animal feeding operations (CAFOs)* (CRS Report for Congress No. RL31851; pp. 1–21). Congressional Research Service. https://nationalaglawcenter.org/wp-content/uploads/assets/crs/RL31851.pdf

Copeland, C. (2016). *Animal waste and water quality: EPA's response to the Waterkeeper Alliance court decision on regulation of CAFOs* (CRS Report for Congress No. RL33656; pp. 1–18). Congressional Research Service. https://crsreports.congress.gov/product/pdf/RL/RL33656/27

Crawford-Brown, D., & Crawford-Brown, S. (2011). The precautionary principle in environmental regulations for drinking water. *Environmental Science & Policy*, *14*(4), 379–387. https://doi.org/10.1016/j.envsci.2011.02.002

de Vlaming, V., Biales, A., Riordan, D., Markiewicz, D., Holmes, R., Otis, P., Zander, R., & Lazorchak, J. (2007). Screening California surface waters for estrogenic endocrine disrupting chemicals (EEDC) with a juvenile rainbow trout liver vitellogenin mRNA procedure. *Science of The Total Environment*, *385*(1–3), 66–79. https://doi.org/10.1016/j.scitotenv.2007.06.026

Delgado, B. J., & Lopez-Ojeda, W. (2022). *Estrogen*. In StatPearls. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK538260/

Derouiche, L., Keller, M., Duittoz, A. H., & Pillon, D. (2015). Developmental exposure to ethinylestradiol affects transgenerationally sexual behavior and neuroendocrine networks in male mice. *Scientific Reports*, *5*(1), 17457. https://doi.org/10.1038/srep17457

Dolan, T., Howsam, P., Parsons, D. J., & Whelan, M. J. (2013). Is the EU drinking water directive standard for pesticides in drinking water consistent with the precautionary principle? *Environmental Science & Technology*, *47*(10), 4999–5006. https://doi.org/10.1021/es304955g

Federal Water Pollution Control Act, 33 U.S.C. 1251 *et seq.* (2002). https://www.epa.gov/sites/default/files/2017-08/documents/federal-water-pollution-control-act-508full.pdf

Feller, D. A. (1983). Private enforcement of federal anti-pollution laws through citizens suits: A model. *Denver Law Review*, *60*(4), 553–571. Available at: https://digitalcommons.du.edu/cgi/viewcontent.cgi?article=2922&context=dlr

Ferrey, M. (2011). *Wastewater treatment plant endocrine disrupting chemical monitoring study* (Monitoring Study Results lrp-ei-1sy11). Minnesota Pollution Control Agency. https://www.pca.state.mn.us/sites/default/files/lrp-ei-1sy11.pdf

Fischer, J., Wilson, T., & Lippincott, R. L. (2018). *A reconnaissance of contaminants of emerging concern in wastewater and sludge from three publicly owned treatment works in New Jersey* (Draft Report No. SR10-016; pp. 1–36). Division of Water Quality, NJDEP Bureau of Pretreatment and Residuals. https://www.nj.gov/dep/dsr/Reconnaissance%20of%20Contaminants%20of%20Emerging%20Concern%20in%20Wastewater%20and%20Sludge.pdf

Food & Water Watch, Arkansas Rights Coalition, Assateague Coastal Trust (Maryland), Association of Irritated Residents (California), Buffalo River Watershed Alliance (Arkansas), Center for Biological Diversity, Center for Food Safety, Concerned Citizens Against Industrial Cafos (Maryland), Dakota Rural Action (South Dakota), Dallas County Farmers and Neighbors (Iowa), Des Moines Water Works (Iowa), Dodge County Concerned Citizens (Minnesota), Don't Waste Arizona, The Environmental Integrity Project, Grand Riverkeeper (Oklahoma), Helping Others Maintain Environmental Standards (Illinois), Illinois Citizens For Clean Air & Water, Institute For Agriculture And Trade Policy, Interfaith Worker Justice (New Mexico), … Rio Valle Concerned Citizens (New Mexico). (2017). *Petition to revise the Clean Water Act regulations for concentrated animal feeding operations* [Petition]. https://www.biologicaldiversity.org/programs/population_and_sustainability/pdfs/Citizens_CAFO_CWA_Petition.pdf

General Provisions, 33 U.S.C. 1251 *et seq.*, §401 (1974). https://www.ecfr.gov/current/title-40/chapter-I/subchapter-N/part-401

Gonsioroski, A., Mourikes, V. E., & Flaws, J. A. (2020). Endocrine disruptors in water and their effects on the reproductive system. *International Journal of Molecular Sciences*, *21*(6), 1929. https://doi.org/10.3390/ijms21061929

Hicks, K. A., Fuzzen, M. L. M., McCann, E. K., Arlos, M. J., Bragg, L. M., Kleywegt, S., Tetreault, G. R., McMaster, M. E., & Servos, M. R. (2017). Reduction of intersex in a wild fish population in response to major municipal wastewater treatment plant upgrades. *Environmental*

Science & Technology, *51*(3), 1811–1819. https://doi.org/10.1021/acs.est.6b05370
Houtman, C. J. (2010). Emerging contaminants in surface waters and their relevance for the production of drinking water in Europe. *Journal of Integrative Environmental Sciences*, *7*(4), 271–295. https://doi.org/10.1080/1943815X.2010.511648
Iwanowicz, L. R., Blazer, V. S., Pinkney, A. E., Guy, C. P., Major, A. M., Munney, K., Mierzykowski, S., Lingenfelser, S., Secord, A., Patnode, K., Kubiak, T. J., Stern, C., Hahn, C. M., Iwanowicz, D. D., Walsh, H. L., & Sperry, A. (2016). Evidence of estrogenic endocrine disruption in smallmouth and largemouth bass inhabiting Northeast U.S. national wildlife refuge waters: A reconnaissance study. *Ecotoxicology and Environmental Safety*, *124*, 50–59. https://doi.org/10.1016/j.ecoenv.2015.09.035
Jarvie, M. E. (2007). *Regulation issues and prediction of natural estrogen loads for wastewater treatment plants* [Doctoral dissertation, Michigan Technological University]. https://doi.org/10.37099/mtu.dc.etds/710
Kenyon, E. (2017). Enough of this manure: Why the EPA needs to define the agricultural stormwater exemption to limit the 'runoff' from the ALT Court. *New York University Law Review*, *92*(4), 1187–1224. Available at: https://www.nyulawreview.org/issues/volume-92-number-4/enough-of-this-manure-why-the-epa-needs-to-define-the-agricultural-stormwater-exemption-to-limit-the-runoff-from-the-alt-court/
Kidd, K. A., Blanchfield, P. J., Mills, K. H., Palace, V. P., Evans, R. E., Lazorchak, J. M., & Flick, R. W. (2007). Collapse of a fish population after exposure to a synthetic estrogen. *Proceedings of the National Academy of Sciences*, *104*(21), 8897–8901. https://doi.org/10.1073/pnas.0609568104
Koh, Y. K. K., Chiu, T. Y., Boobis, A., Cartmell, E., Scrimshaw, M. D., & Lester, J. N. (2008). Treatment and removal strategies for estrogens from wastewater. *Environmental Technology*, *29*(3), 245–267. https://doi.org/10.1080/09593330802099122
Kostich, M., Flick, R., & Martinson, J. (2013). Comparing predicted estrogen concentrations with measurements in US waters. *Environmental Pollution*, *178*, 271–277. https://doi.org/10.1016/j.envpol.2013.03.024
Kumar, A., Banerjee, A., Singh, D., Thakur, G., Kasarpalkar, N., Gavali, S., Gadkar, S., Madan, T., Mahale, S., Balasinor, N., & Sachdeva, G. (2018). Estradiol: A steroid with multiple facets. *Hormone and Metabolic Research*, *50*(05), 359–374. https://doi.org/10.1055/s-0044-100920
Lopez, J. (2010). Endocrine-disrupting chemical pollution: Why the EPA should regulate these chemicals under the Clean Water Act. *Sustainable Development Law & Policy*, *10*(3), 48–49. Available at: https://digitalcommons.wcl.american.edu/cgi/viewcontent.cgi?article=1045&context=sdlp
McCullough, A., George, J., & Jiru, M. (2018). Detection and quantitation of estrogen in watersheds. *International Journal of Scientific and Research Publications*, *8*(7). https://doi.org/10.29322/IJSRP.8.7.2018.p7930
Miller, D. L., Muren, G., Devine, J., & Baron, V. (2019). CAFOs: *What we don't know is hurting us* (Online Report No. 19-06-A). Natural Resources Defense Council. https://www.nrdc.org/sites/default/files/cafos-dont-know-hurting-us-report.pdf
MohanKumar, S. M. j., Balasubramanian, P., Subramanian, M., & MohanKumar, P. s. (2018). Chronic estradiol exposure: Harmful effects on behavior, cardiovascular and reproductive functions. *Reproduction*, *156*(5), R169–R186. https://doi.org/10.1530/REP-18-0116
Ohio Environmental Council. (n.d.). *Watershed watchdog: Guide to Clean Water Act citizen suits*. California Water Boards. https://www.waterboards.ca.gov/water_issues/programs/swamp/docs/cwt/guidance/112a1.pdf
Orlando, E. F., Kolok, A. S., Binzcik, G. A., Gates, J. L., Horton, M. K., Lambright, C. S., Gray, L. E., Soto, A. M., & Guillette, L. J. (2004). Endocrine-disrupting effects of cattle feedlot effluent on an aquatic sentinel species, the fathead minnow. *Environmental Health Perspectives*, *112*(3), 353–358. https://doi.org/10.1289/ehp.659
Rodriguez-Mozaz, S., & Weinberg, H. S. (2010). Meeting report: Pharmaceuticals in water—An interdisciplinary approach to a public health challenge. *Environmental Health Perspectives*, *118*(7), 1016–1020. https://doi.org/10.1289/ehp.0901532
Roggio, M. A., Guyón, N. F., Hued, A. C., Amé, M. V., Valdés, M. E., Giojalas, L. C., Wunderlin, D. A., & Bistoni, M. A. (2014). Effects of the synthetic estrogen 17-ethinylestradiol on aromatase expression, reproductive behavior and sperm quality in the fish Jenynsia multidentata. *Bulletin of Environmental Contamination and Toxicology*, *92*(5), 579–584. https://doi.org/10.1007/s00128-013-1185-2
Sumpter, J. P., & Jobling, S. (1995). Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. *Environmental Health Perspectives*, *103*(suppl 7), 173–178. https://doi.org/10.1289/ehp.95103s7173
Thorpe, K. L., Cummings, R. I., Hutchinson, T. H., Scholze, M., Brighty, G., Sumpter, J. P., & Tyler, C. R. (2003). Relative potencies and combination effects of steroidal estrogens in fish. *Environmental Science & Technology*, *37*(6), 1142–1149. https://doi.org/10.1021/es0201348
Trueman, R. J., & Erber, L. (2013). Invasive species may offer advanced phytoremediation of endocrine disrupting chemicals in aquatic ecosystems. *Emirates Journal of Food and Agriculture*, *25*(9), 648–656. https://doi.org/10.9755/ejfa.v25i9.16393
United States Department of Agriculture. (2019). *Nutrient Management* (590-CPS-1) [Conservation practice standard]. Natural Resources Conservation Service. https://efotg.sc.egov.usda.gov/api/CPSFile/15355/590_MD_CPS_Nutrient_Management_2019
United States Environmental Protection Agency. (n.d.). *Summary of the Second Circuit's Decision in the CAFO Litigation*. https://www3.epa.gov/npdes/pubs/summary_court_decision.pdf
United States Environmental Protection Agency. (2009). *Fact sheet: Final third drinking water contaminant candidate list (CCL 3)* (Fact Sheet No. 815F09001). Office of Water (4607M). https://19january2021snapshot.epa.gov/sites/static/files/2014-09/documents/fact_sheet_final_third_ccl.pdf
United States Environmental Protection Agency. (2013). Literature review of contaminants in livestock and poultry manure and implications for water quality (Literature Review 13_002; pp. 18–137). Office of Water. https://nepis.epa.gov/Exe/ZyNET.exe/P100H2NI.TXT?ZyActionD=ZyDocument&Client=EPA&Index=2011+Thru+2015&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5Czyfiles%5CIndex%20Data%5C11thru15%5CTxt%5C00000008%5CP100H2NI.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x&ZyPURL
United States Environmental Protection Agency. (2014, May 5). *Basic information on the CCL and Regulatory Determination*. Contaminant Candidate List and Regulatory Determination. https://www.epa.gov/ccl/basic-information-ccl-and-regulatory-determination
United States Environmental Protection Agency. (2019). *Overview of the Safe Drinking Water Act* (No. 816-F-04–030). Office of Water. https://www.epa.gov/sdwa/overview-safe-drinking-water-act (Original report published 2004)
United States Environmental Protection Agency. (2021). *Announcement of final regulatory determinations for contaminants on the Fourth Drinking Water Contaminant Candidate List*. Contaminant Candidate List and Regulatory Determination. https://www.federalregister.gov/documents/2021/03/03/2021-04184/announcement-of-final-regulatory-determinations-for-contaminants-on-the-fourth-drinking-water
United States Environmental Protection Agency. (2022a, July 6). *Summary of the Clean Water Act*. Office of Water. https://www.epa.gov/laws-regulations/summary-clean-water-act
United States Environmental Protection Agency. (2022b, October 31). *CCL 4 chemical contaminants*. Contaminant Candidate List and Regulatory Determination. https://www.epa.gov/ccl/ccl-4-chemical-contaminants
United States Environmental Protection Agency. (2022c, November 22). *Contaminant Candidate List 5 (CCL 5)*. Contaminant Candidate List and Regulatory Determination. https://www.epa.gov/ccl/contaminant-candidate-list-5-ccl-5
United States Government Accountability Office. (2003). *Livestock agriculture: Increased EPA oversight will improve environmental program for concentrated animal feeding operations* (Reports and Testimonies GAO-03-285). United States Government Accountability Office.
United States Government Accountability Office. (2008). *Concentrated animal feeding operations: EPA needs more information and a clearly defined strategy to protect air and water quality from pollutants of concern* (Report to Congressional Requesters GAO-08-944). United States Government Accountability Office.
Watson, C. S., Jeng, Y.-J., & Kochukov, M. Y. (2010). Nongenomic signaling pathways of estrogen toxicity. *Toxicological Sciences*, *115*(1), 1–11. https://doi.org/10.1093/toxsci/kfp288
Wright-Walters, M., & Volz, C. (2009). Municipal wastewater concentrations of pharmaceutical and xeno-estrogens: Wildlife and human health implications. In E. Nzewi, G. Reddy, S. Luster-Teasley, V. Kabadi, S.-Y. Chang, K. Schimmel, & G. Uzochukwu (Eds.), *Proceedings of the 2007 National Conference on Environmental Science and Technology* (pp. 103–113). Springer New York. https://doi.org/10.1007/978-0-387-88483-7_15
Wu, M., & Janssen, S. (2011). Dosed without prescription: A framework for preventing pharmaceutical contamination of our nation's drinking water. *Environmental Science & Technology*, *45*(2), 366–367. https://doi.org/10.1021/es104032a
Wu, M., Atchley, D., Geer, L., Janssen, S., Rosenberg, D., & Sass, J. (2009). *Dosed without prescription: Preventing pharmaceutical contamination of our nation's drinking water* [White paper]. Natural Resources Defense Council. https://www.nrdc.org/sites/default/files/hea_10012001a.pdf
Yu, C.-P., Deeb, R. A., & Chu, K.-H. (2013). Microbial degradation of steroidal estrogens. *Chemosphere*, *91*(9), 1225–1235. https://doi.org/10.1016/j.chemosphere.2013.01.112