Are Oral Contraceptives a Significant Contributor to the Estrogenicity of Drinking Water?†

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Recent observed feminization of aquatic animals has raised concerns about estrogenic compounds in water supplies and the potential for these chemicals to reach drinking water. Public perception frequently attributes this feminization to oral contraceptives (OCs) in wastewater and raises concerns that exposure to OCs in drinking water may contribute to the recent rise in human reproductive problems. This paper reviews the literature regarding various sources of estrogens, in surface, source and drinking water, with an emphasis on the active molecule that comes from OCs. It includes discussion of the various agricultural, industrial, and municipal sources and outlines the contributions of estrogenic chemicals to the estrogenicity of waterways and estimates that the risk of exposure to synthetic estrogens in drinking water on human health is negligible. This paper also provides recommendations for strategies to better understand all the potential sources of estrogenic compounds in the environment and possibilities to reduce the levels of estrogenic chemicals in the water supply.

Introduction

The recent increase in examples of intersex fish and organisms found in global waterways has led people to be concerned about estrogenic compounds in the environment (*1*−*13*). Often, oral contraceptives (OCs) are blamed, as they are an easily identifiable source of estrogen, with 11.6 million women of reproductive age using OCs in the U.S. (*14*). Use of OCs allows women a significant level of reproductive freedom and additionally has societal and global ramifications on population levels. However, after wastewater treatment, low levels of the main estrogenic ingredient in OCs, 17 alpha-ethinylestradiol (EE2), have been detected in some surface waters (*15*−*18*), and this has caused some concern about drinking water contamination. The Endocrine Society recently published a position statement expressing concern that low level, chronic exposure to such environmental endocrine disruptors cause or contribute to adverse human health effects (*19*). Intersex fish have been observed near sewage treatment plants in the U.S., across Europe, and in Japan (*5, 11, 20*). There is growing concern that a connection exists between estrogenic surface water, the occurrence of intersex fish in these rivers, lakes, and streams, and the rise in human reproductive problems (*7*). The peer-reviewed literature and popular media have pointed to EE2 from OCs as a major estrogenic endocrine disrupting chemical contributing to these phenomena (*6, 21*−*23*). We review the scientific literature to qualitatively assess the contribution of other estrogenic chemicals to the estrogenicity of waterways, to evaluate the pathway of EE2 from ingestion to drinking water, and to explore what is known about the effects of EE2 exposure in drinking water on human health. Studies from western Europe and the U.S. are highlighted because they have similar industrial practices and contraceptive use. We conclude with possible solutions to reducing the presence of estrogenic compounds, including EE2, in water.

Sources of Estrogens and Estrogenic Compounds. Many chemicals found in our waterways, both natural and synthetic, have the ability to mimic or disrupt the natural estrogens found in humans and animals (*11, 12, 18, 24*−*47*). Estrogenic chemicals of varying potency and persistence originate from agriculture, industry, humans, household products, and other pharmaceuticals. Figure 1 diagrams the various points of entry into waterways for estrogenic chemicals. The following sections will outline in more detail some of the different sources and their contribution to estrogenic contamination.

Background on Human Estrogens and OCs. The human body has three naturally occurring steroid estrogens: estrone

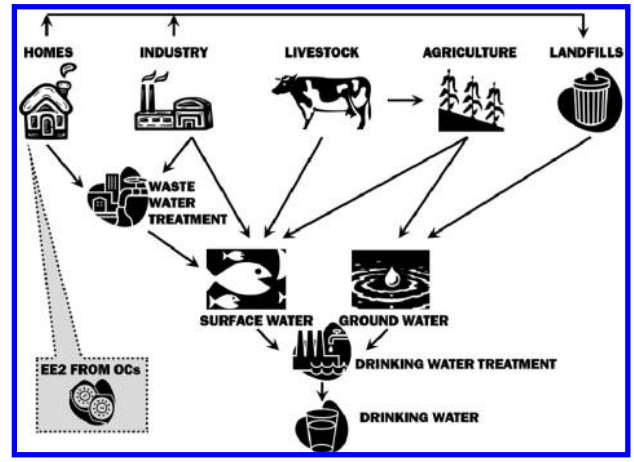

FIGURE 1. Simplified diagram outlining points of entry of estrogenic chemicals into the water supply, adapted from Velicu et al., 2009 (*12*).

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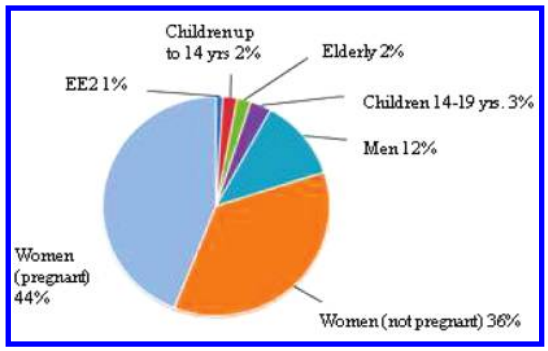

FIGURE 2. Estimated contribution of total natural estrogens (E1+E2+E3) and EE2 excretion to total estrogen excretion in the Dutch population. OCs are estimated to be 1% of total. Figure adapted from ref *51*. This figure does not account for the higher potency of EE2 compared to natural estrogens.

TABLE 1. Daily Estrogen Excretion by Humans (per Person) in μg/Day, from Johnson et al., 2000 (*52*)

	E2	E1	E3	total estrogens
males	1.6	3.9	1.5	7
menstruating females	3.5	8	4.8	16.3
menopausal females	2.3	4	1	7.3
pregnant women	259	600	6000	6859

(E1), 17-β estradiol (E2), and estriol (E3), with the synthetic estrogen EE2's structure being most similar to that of E2. The potency of these estrogens are typically measured in relation to E2 (having a value of 1) and are estimated to have the following relative potencies: EE2: 2.0; E2: 1; E1: 0.2−0.4; E3: 0.024−0.026. These values have been determined by estrogen receptor binding *in vitro* assays or vitellogenin induction in male juvenile fish (*6, 12, 48, 49*). It is important to note that *in vitro* responses have been known to underestimate *in vivo* responses from more complex wastewater (*50*), and potencies can vary somewhat depending on the determination method.

While there are many different versions of oral contraceptives, most OCs are a combination of an estrogen and a progestin, and the most widely used synthetic estrogen is 17-α-ethinyl estradiol (EE2) with an average daily dose of 30−35 μg of EE2 per pill. The progestin in combination pills is typically present in higher concentrations (≥1 mg per pill).

Human Sources. Human urine is often cited as the main source of natural and synthetic estrogens in the aquatic environment (*5, 29*). Humans excrete the natural estrogens, E1, E2, and E3, and estimates of the amount per day are shown in Table 1. In addition, several synthetic estrogens, ingested through pharmaceuticals, can be also be excreted and enter wastewater. The Dutch Central Bureau of Statistics evaluated the proportion of estrogen excretion, including EE2 by different groups based on the total population figures of 2001 and estimated that EE2 accounts for approximately 1% of the total human excretion of estrogens in The Netherlands (*51*). In 2001, 43% of Dutch females of reproductive age used OCs (*51*), compared to 28% in the U.S. (*14*). Therefore, the excretion of EE2 as a fraction of total estrogens in the U.S. is likely less than 1%.

Natural estrogens are also used in other prescribed drugs (*53*). We identified one study that provides estimates of the contribution of natural endogenous estrogens (E1, E2, E3), prescription form estrogens (E1, E2, E3), and prescribed synthetic estrogen (EE2) to drinking water estrogen levels (*53*). The estimates are derived from the Pharmaceutical Assessment and Transport Evaluation (PhATE) model, and the results are summarized in Table 2. (*53*). PhATE modeling integrates data from source emissions and migration in the environment to model and predict environmental concentrations of chemicals (*54*). EE2 has the lowest predicted environmental concentration in U.S. drinking water compared to natural estrogens in the human diet (such as from intake of dairy or soy), and is generally lower than naturally produced and prescription endogenous estrogens. It is still lower than E2 after considering relative potency.

TABLE 2. Estimated Values for Estrogen Concentrations in U.S. Drinking Water Based on PhATE Modeling (*53*)[a]

category, compound	upper estimate (ng/L)	adjusted for E2 estrogenic equivalency (ng/L)	average estimate (ng/L)	adjusted for E2 estrogenic equivalency (ng/L)
Natural Estrogens from Diet and Naturally Produced Endogenous Estrogens				
E1	0.1	0.03	0.03	0.0009
E2	0.02	0.02	0.01	0.01
E3	0.02	0.0005	0.01	0.00025
Prescribed Endogenous Estrogens				
E1	0.02	0.0006	0.01	0.0003
E2	0.002	0.002	0.0006	0.0006
E3	0.000015	$3.7 \times 10-7$	0.000006	$1.5 \times 10-7$
Prescribed Synthetic Estrogens				
EE2	0.003	0.006	0.001	0.002

[a] Includes upper and lower Predicted Environmental Concentrations (PEC) (ng/L) and the estimated estrogenic equivelancy normalized to E2 potency.

Other estrogenic pharmaceutical drugs are excreted by users and can contribute to the estrogenicity of waterways (*1*). Of these are two highly prescribed pharmaceuticals, hormone replacement therapy (HRT) (the main active component is conjugated equine estrogens) and veterinary medicine pharmaceuticals, with greater usage in the U.S. in 1995 compared to EE2 (*1*). Comparison of the type of active estrogen used in each and the amount is shown in Table 3. Even though the number of women who take HRT has declined since 2002, the current values are estimated to be similar to 1998 levels (*55*). In the United States, between 10% and 25% of women between the ages of 50 and 79 are currently using HRT (*55*) and an estimated 28% of reproductive-age women use OCs (*14*). A report by Arcand-Hoy estimates both HRT and veterinary medicine pharmaceuticals represent more prescribed estrogens per year than OCs (*1*). We identified only one study from the UK in 2009 which evaluated the environmental impact of conjugated equine estrogens (CEEs) from HRT in four sewage treatment systems (*45*). Equine estrogens and their metabolites were present in all sewage influent samples, and 83% of the effluent samples had concentrations similar to EE2 (0.07−2.6 ng/L). The CEEs were taken up by effluent-exposed fish and induced estrogenic responses including hepatic growth and the production of the egg-yolk precursor protein, vitellogenin, at concentrations as low as 0.6−4.2 ng/L, and the potencies of these estrogens were found to be 2.4−3490% greater than the potency of E2. This study provides evidence that estrogens from HRT are discharged at measurable levels which can influence fish health into the aquatic environment (*45*).

Ingestion, Metabolism, and Excretion of EE2. The pathway of EE2 ingestion, metabolism, excretion, and environmental transformation can generally be described as in Figure 3, with ingestion occurring from prescribed usage of drug, followed by metabolism and excretion.

EE2 primarily enters the water treatment system as domestic sewage via excretion by women prescribed OCs (*29, 57*). It is estimated women on OCs fully metabolize 20−48% of the daily dose of EE2 (*58*). The rest of the daily dose is excreted in either its original form or as EE2 sulfate

TABLE 3. Pharmaceutical Uses of Estrogens in 1995 (*1, 56*)

pharmaceutical uses of estrogens	treatment	active estrogen	estrogen used in 1995 (kg/yr)
oral contraceptives	ovulation inhibition	EE2	88
hormone replacement therapy	menopause, osteoporosis	conjugated equine (CEEs)	1687.5
cancer therapy	breast and prostate	Tamoxifen, DES	unknown[a]
veterinary medicine	growth promotion	E2 and Zeranol	579.15

[a] DES use in cancer therapy is declining, but it is still prescribed in some cases for prostate cancer.

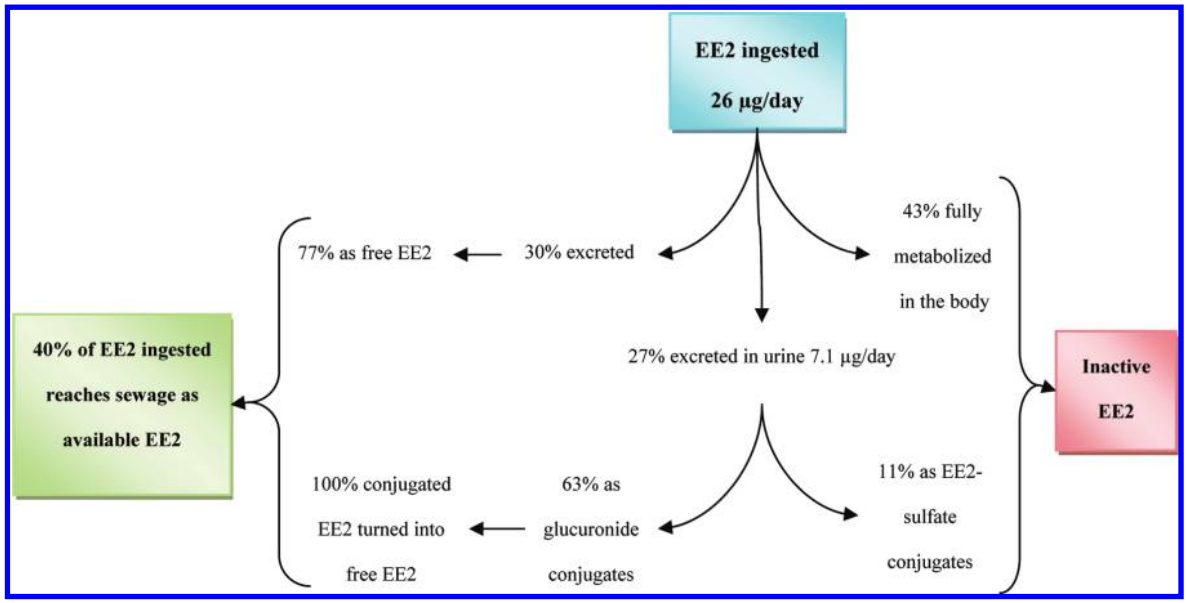

FIGURE 3. Estimates of fate and excretion of EE2 in the body, adapted from Johnson and Williams, 2004 (*57*). All numbers are approximate and final numbers do not add to 100% of the original ingested EE2, as a complete mass-balance analysis has yet to be performed.

or glucuronide conjugates (*58*). Figure 3 diagrams one model of EE2 excretion, using a starting dose of 26 μg/day (average upper amount of EE2 in a combination OC prescription is 35 μg/day of EE2 for 21 days per 28 day cycle). The model estimates 40% of the total EE2, about 10.5 μg/day of EE2, reach the sewage influent. About 60% of the ingested EE2 is excreted in urine or feces, primarily in the conjugated form, where most of it is deconjugated back to EE2 in the environment. It is assumed that EE2 glucuronides deconjugate to the original form in sewage treatment plants, water, or the environment (*57*).

Fate and Transport after Excretion. Levels of natural steroid estrogens and EE2 are higher in sewage influents than effluents, evidence that sewage treatment plants remove a portion of the synthetic hormone (*29*). The average concentration of EE2 in both influent and effluent is also less than the natural steroid estrogens, E1 and E2 (*59*).

Activated sludge and other effective methods of sewage treatment are sufficient in removing most estrogenic compounds, as shown by comparison of sewage influents and effluents (*26*). The efficacy of estrogen removal during sewage treatment depends on the specific process and conditions (*29*), with several wastewater treatment plants able to remove EE2 at ≥80−98% efficiency (*25*−*27*). Activated sludge has repeatedly been shown to remove estrogenic compounds (*26, 28, 37, 43*) consistently removing over 85% of EE2 and E2 (*60*). EE2 was also reported to be degraded completely by nitrifying activated sludge in six days, converting EE2 into hydrophilic compounds (*61*). Moreover, studies show that treatment of water with chlorine removes between 80−95% of EE2, and treatment with ozone removes 95−99% of EE2 (*62*−*64*).

Surface Water Studies. EE2 and other natural estrogens can enter surface water through wastewater treatment effluent and runoff from agricultural sources. Monitoring studies of surface water use a variety of EE2 detection methods find a range of values for EE2 and natural human and animal steroid estrogens (*35, 65*−*70*). Table 4 summarizes key studies of surface water levels of EE2 and the natural steroid hormones E1 and E2. In general, in studies where steroid hormones were detected above the detection limit, EE2 is detected with the lowest frequency and at the lowest concentration in comparison to E2 and E1 (*71*). Studies reviewed by the Environment Agency of the UK indicated that when EE2 was detected in surface waters it was generally found at concentrations less than 5 ng/L and often below 1 ng/L (*71*).

A 1999 US Geological Survey (USGS) of 139 U.S. streams identified as high risk for domestic and industrial pollution found EE2 was measured in 5.7% of these high risk regions (limit of detection = 5 ng/L). While this limit of detection is somewhat higher than other studies, the relatively low occurrence of EE2 detection is consistent with those of previously published investigations (*72*) that have lower limits of detection.

Drinking Water Studies. Monitoring Studies. There are a small number of reports that measure levels of EE2 in drinking water. The studies that report estrogen levels in source and finished drinking water, summarized in Table 5, primarily find that EE2 is below the limit of detection (LOD = 0.05 ng/L−1 ng/L) in drinking water in the UK and the U.S. With the exception of one study in Germany, the UK Environment Agency concluded from data available through 2004 that EE2 and natural steroid estrogens were not detected in drinking water above the LOD of 0.3 ng/L in a review of all available studies from Europe, the U.S., and Japan (*71*).

Studies that measure EE2 in drinking water in the U.S. suggest when present, it is typically at levels lower than the detection level used by the USGS (1 ng/L) (*16, 35, 71*).

TABLE 4. Key Studies Measuring Surface Water Levels of E1, E2, and EE2[a]

reference	location	study details	findings
Belfroid et al., 1999(*27*)	The Netherlands	11 samples from coastal estuarine and freshwater sources, LOD ranged from 0.1−0.6 ng/L	EE2 found in 3 samples (mean < LOD) E2 found in 4 samples (mean < LOD) E1 found in 7 samples (mean concn = 0.3 ng/L)
Williams et al., 2003 (*73*)	UK	28 samples from 2 rivers, LOD ranged from 0.1 ng/L-0.5 ng/L	EE2 found in 9 samples (mean concn = 0.7 ng/L) E2 was found in 14 samples (mean concn = 0.9 ng/L) E1 found in all samples (mean concn = 4.6 ng/L)
Kuch and Ballschmiter, 2001 (*35*)	Germany	31 samples from surface waters downstream of sewage treatment plants, LOD = 200 pg/L	EE2 found in 15 samples (concn range: <0.1−5.1 ng/L) E2 found in 14 samples (concn range: <0.15−3.6 ng/L) E1 found in 29 samplest (concn range: <0.1−4.1 ng/L) no detection of EE2 or E2
Ternes et al., 1999 (*59*)	Germany	15 rivers, LOD = <0.5 ng/L	E1 found in 3 rivers (concn range; 0.7−1.6 ng/L) Other studies have supported the absence of EE2 in Germany (*27, 63*).
Benotti et al., 2009 (*16*)	United States	19 surface waters used as drinking water sources before treatment. Method reporting limit was 0.2 ng/L for E1, 0.5 for EE2, and 1.0 for E2.	EE2 found in 1 sample (1.4 ng/L) E1 found in 15 samples (average = 0.3 ng/L) E2 found in 1 sample (17 ng/L)

[a] LOD = limit of detection.

TABLE 5. Estrogen Concentrations in Source and Drinking Water[c]

	U.S. Source Water[a] no. of samples = 19			U.S. Drinking Water[a] no. of samples = 18			German Drinking Water[b] no. of samples = 10		
estrogen	median (range) ng/L	no. of samples above LOD	LOD (ng/L)	median (range) ng/L	no. of samples a bove LOD	LOD (ng/L)	median (range) ng/L	no. of samples above LOD	LOD (ng/L)
E1	0.3 (<LOD-0.9)	15	0.2	<LOD	0	0.2	0.4 (0.2−0.6)	4	0.05
E2	17 (<LOD-17.0)	1	0.5	<LOD	0	0.5	0.3 (0.2−2.1)	5	0.1
EE2	1.4 (<LOD-1.4)	1	1	<LOD	0	1	0.35 (0.15−0.5)	4	0.05
nonylphenol	100 (<LOD-130)	8	80	97 (<LOD-110)	2	80	6.6 (2.5−16)	10	0.05

[a] Benotti et al., 2009 (U.S. data did not include a minimum detected level and only used samples above the LOD to calculate the median values). [b] Kuch and Ballschmitter, 2001. [c] LOD = limit of detection.

Model Estimates. A study published in early 2009 using PhATE to estimates EE2 levels in U.S. found that EE2 sewage effluent levels would range from 0.4 ng/L to 13 ng/L. The 13 ng/L is an upper estimate assuming no metabolism in the body and no removal by sewage treatment plants. The lower estimate of 0.4 ng/L assumes 50% metabolism and 82% removal by sewage treatment removal. EE2 concentrations in surface waters would be reduced further by in-stream dilution and degradation. The estimates also do not account for drinking water treatment. Treatment methods for drinking water vary in their effectiveness to remove estrogens, but a study by Benotti (2009) reports removal rates of 80−99% for EE2, E1, and E2 via chlorine or ozone oxidation (*16*). Thus, the PhATE model predicts less than 1 ng/L of EE2 in drinking water. This model is useful in identifying measurement outliers and suggests measurements greater than 1 ng/L may come from locally generated sources (e.g., downstream of a pharmaceutical manufacturing facility or agricultural waste stream) or that further evaluation of measurements is warranted to ensure accuracy.

Agricultural Sources. Livestock excrete the same natural estrogens (E1, E2, and E3) as humans, and there is a growing body of research showing elevated estrogen levels in surface and groundwater downstream of farms and agricultural land (*41, 74−77*). In the U.S., livestock produce 133 million tons of manure per year; 13-fold more solid waste than human sanitary waste production (*78*). Table 6 shows the estrogen excretion numbers for several types of livestock. An important source of agricultural effluents is from Concentrated Animal Farm Operations (CAFOs). The effluents are untreated, and their use as an agricultural fertilizer is growing. In addition to spreading manure for fertilizer, livestock waste can enter the environment when rain causes overflow, or from runoff and leaching into the soils near manure storage facilities (*32, 33, 39, 78, 79*). In addition to naturally excreted hormones, livestock are also given prescribed hormones. However, it is difficult to determine the contribution of natural versus pharmaceutical estrogen to total livestock estrogen excretion, as we found little information to distinguish the two. One study in 1995 found the use of veterinary estrogens was more than five times the use of OCs (*1*). A recent review by Combalbert and Hernandez-Raquet contains more recent data on various levels of hormones in human and animal waste. In human raw sewage effluents, E2 varies between 0.5

TABLE 6. Estimated Total Daily Estrogen Excretion of Different Livestock Species (μg/day)[a]

species	type	amount of total estrogens excreted in urine (μg/day)	amount of total estrogens excreted in feces (μg/day)	total estrogens excreted per day (μg)	million heads (U.S.)
	calves	15	30	45	17
	cycling cows	99	200	299	20
cattle	pregnant***	320−104,320	256−7300	576−111,620	43
	cycling sow	82	21	103	--
pig	pregnant	700−17,000	61		--
	cycling ewes	3	20	23	2.5
sheep	rams	3	22	25	0.6

[a] For cattle and sheep, data are estimated total of E1 + E2α + E2β + E3 excretions and includes hormones from veterinary treatment (*36*). Pig data and pregnant cattle data are from ref *81*. Number of animals were estimated by USDA in 2001 (*86*).

and 125 ng/L, while animal waste contains levels of E2 ranging from 30−2500 ng/L (*80*).

A number of studies in Europe and the U.S. have measured or estimated the contribution of livestock to total estrogens in water. In the UK, it has been estimated that if just 1% of the estrogens excreted by livestock reached water sources, this would account for 15% of all the estrogens in water (*81*). Another study estimates that animal waste contributes 90% of total estrogens in the environment (*82*). E2 concentrations were found to be as high as 3500 ng/L in surface runoff from grasslands in Arkansas where poultry litter was applied as fertilizer (*83*). U.S. aquifers under areas covered in animal manure were found to have an E2 concentration of 37.6 ng/L (*84*), and groundwater measurements of E2 ranged from 6 to 66 ng/L in five springs in northwest Arkansas where poultry litter and cattle manure were applied (*85*).

A study of 113 surface and groundwater samples in northern California found that only 5% contained concentrations of estrogenic compounds high enough to induce a vitellogenin response using an mRNA detection screen in juvenile rainbow trout (*87*). They determined that the few samples that induced vitellogenin response were from agricultural-dominated waterways.

The agricultural studies indicate that livestock effluents and runoff from manure are likely to be a significant source of natural estrogens in the waterways and have the potential to reach drinking water.

Pesticides. Many pesticides are known to have estrogenic or antiandrogenic properties in a variety of species. Some of them include the widely used atrazine (*88−90*), vinclozolin (*91−93*), and the organochlorine pesticides, such as DDT. Because of widespread agricultural use, these pesticides have been detected in surface water, groundwater, and drinking water at varying levels. Atrazine is especially mobile in the environment and is known to precipitate in rainfall (*90, 94*). Pesticides are present in streams in 97% of agricultural and urban areas, often at levels above quality benchmark doses and often more than one pesticide is present in any one location (*95*). In a USGS survey of U.S. drinking water wells, 70% of the samples contained at least one volatile organic compound, pesticide, or anthropogenic nitrate, with values ranging from 100 ng/L-100 μg/L (*96*). While the relative potencies of these pesticides are typically 100−1000 times less than E2, detected values are often 10−10,000 times higher than natural estrogens and adverse effects can be seen on aquatic amphibians at levels as low as 0.1 ppb (*88*) and at ppm levels for other wildlife. While it is beyond the scope of this paper to include an in-depth discussion of the estrogenic contributions of pesticides, it is clear the widespread detection and, in some areas, high levels of detection are cause for concern.

Plant-Based Sources. Phytoestrogens (estrogens produced by plants) are found in various plant matter, such as nuts and legumes and can be excreted by humans and livestock after eating (*97*). They are also present in high quantities near soy-processing facilities and other plant-based industries such as biodiesel plants. Their potency varies, with values ranging from 10^4−10^5 times less potent than E2 (*91*). Phytoestrogen entry into the environment is not as well studied, and they are largely ignored as a potential contributor to the estrogenicity of water. Yet, a recent study of 19 industrial wastewater streams in Minnesota and Iowa found plant estrogens at levels 250 times higher than the amount needed to cause feminization in fish (*38*). The elevated levels of these phytoestrogens were attributed to nearby industrial facilities including soy milk factories, biodiesel factories, and dairies. This study also measured elevated phytoestrogen levels downstream from wastewater treatment plants. While standard treatment can remove more than 90% of the phytoestrogens, industrial factories that process large amounts of soy, plant matter for biodiesel, and dairy products are often found in small towns which may not have the municipal treatment capabilities to remove phytoestrogens from the water. Thus, in certain locations, plant-based phytoestrogens may be an important contributor to the estrogenicity of the water.

Industrial Chemicals. Industrial chemicals can enter waterways through a variety of means, including point-sources like manufacturing facilities, domestic and industrial wastewater effluents, runoff from urban areas, and leaching from landfills. There is a great deal of uncertainty regarding the number of industrial chemicals with estrogenic activity since many chemicals are untested for this effect. There are many industrial chemicals present in the environment known to have estrogenic properties of varying potencies, including bisphenol A, polychlorinated biphenyls, brominated flame retardants, alkylphenols, and others (*19, 91, 98, 99*). One study of several classes of phenolic compounds found levels of octylphenol, nonylphenol, BPA, and other phenolic compounds from 0.13−3.6 μg/L in untreated wastewater and 0.16−0.36 μg/L in treated wastewater (*100*).

When studying estrogenicity of water samples, often a "total estrogenicity" is measured in addition to the concentrations of E1, E2, and less often, EE2. "Total estrogenicity" is usually determined in E2 equivalents by measuring *in vitro* binding to estrogen receptors. However, several studies measuring the total estrogenicity of wastewater report that samples contained more estrogenic activity than was predicted from the chemical analysis of E1, E2, and EE2 concentrations, indicating other contributing factors (*41*). Another study that investigated feminization of fish concludes that xenoestrogens and (as yet unknown) chemicals with

antiandrogenic properties also contribute to sexual disruption of aquatic species (*101*).

One specific example of industrial chemicals with the ability to disrupt the estrogenic hormone system is the alkylphenols. U.S. production of alkylphenol ethoxylates exceeds 500 million pounds per year (*102*), with nonylphenol's production alone estimated at 340 million pounds (*103*). The alkylphenols are used primarily for cleaning and sanitizing agents. The nonylphenol and octylphenol molecules, and their degradation products can independently induce vitellogenin production and inhibit testicular growth in fish at levels as low as 10 μg/L (*5*). Nonylphenol caused adverse reproductive effects in Medaka fish at levels as low as 17.7 μg/L (*47*), and octylphenol significantly increased vitellogenin production at levels as low as 3 μg/L.

The US Geological Survey reported that nonylphenol was one of the most frequently detected industrial chemicals in surface waters with a median concentration of 0.8 μg/L (*17*). It is estimated 60% of the alkyphenols produced end up in the aquatic environment after sewage treatment, either the original molecule, or as shorter chain alkyl degradation products (*64*). Effluents from domestic sewage treatment plants can contain the original alkylphenolic compounds at concentrations greater than 100 μg/L (*5*), and industrial effluents can contain significantly more (*104*). Additionally, other chemicals which can effect estrogenic activity, such as BPA or triclosan, have been found at 25 ng/L and 1.2 ng/L, respectively, in U.S. drinking water (*16*).

Alkylphenols are one example of the potentially thousands of chemicals used in industry with the ability to disrupt the estrogenic hormone system and also have the ability to contribute to the estrogenic activity in water (*3, 10, 11, 17, 105, 106*).

Public Health Implications. It has been suggested that environmental exposure to estrogenic chemicals are a risk factor for several human health outcomes including testicular dysgenesis syndrome, hypospadias, testicular cancer, breast cancer, endometriosis, and decreased sperm counts (*97, 107, 108*). However, uncertainty in the science remains about the nature and magnitude of risks that can occur from low-level exposures to estrogenic chemicals (*8*). The Endocrine Society has published a position statement stating "evidence for adverse reproductive outcomes (infertility, cancers, malformations) from exposure to endocrine disrupting chemicals is strong, and there is mounting evidence for effects on ... thyroid, neuroendocrine, obesity and metabolism, and insulin and glucose homeostasis" (*19*). The statement details the many EDCs, both estrogenic and those disrupting other hormones, which have shown adverse health effects in animal models. An additional concern with EDCs and other chemical exposures is that it cannot be assumed that there is a threshold for adverse health effects especially for vulnerable populations, which has also been echoed by the National Academy of Sciences (*109*).

A study published in early 2010, using the PhATE model, estimated exposure levels of natural and synthetic estrogens from drinking water and compared drinking water exposure to exposure from both milk intake for children and dietary intake for adults (*53*). The study used the upper estimate of the PhATE model of EE2 in drinking water and predicted that exposure to EE2 in drinking water is at most 82 times lower than background dietary exposure to estrogens for adults. The study estimated that a child's exposure to estrogens from drinking water was about 150−250-fold smaller than their exposure to estrogens from drinking milk. All comparisons of estrogenicity were made using E2-equivalents and thus accounts for the stronger potency of EE2 (*53*).

Discussion

While the overall contribution of EE2 to drinking water estrogenicity appears to be less than from other sources, EE2 is an additional endocrine disrupting chemical that could contribute to the feminization of aquatic species. Thus, there are still steps that could to be taken to reduce levels of EE2 and begin to address the larger issue of endocrine-disrupting chemicals in our environment.

Improved Wastewater Treatment. EE2 possesses the potential to adversely influence wildlife if local concentrations exceed levels of concern. Wastewater treatment and drinking water treatment vary in their abilities to remove EE2 and offer a point of intervention to reduce the levels of EE2 reaching animals and humans. While there is a range of effectiveness, many existing sewage treatment plants have been found to dramatically reduce EE2 concentrations in the influent (*29*). Updating to more effective methods and standardizing sewage treatment plants could contribute to reducing levels of EE2 and other estrogenic contaminants.

Improved Detection Methods and Monitoring Programs. Inexpensive, standardized methods for detection and determining concentrations of estrogenicity should be developed for use by city and state water departments to understand the extent of the issue. USGS data on EE2 in surface water use a method with a detection limit of 5.0 ng/L, which is much higher than the environmental risk level of concern of 0.1 ng/L determined by the European Agency (*71*). Until a more sensitive analysis of effluents, surface water, and drinking water is performed in the U.S., it is not possible to know the true distribution of EE2 in our water supplies. Local water departments already test their water regularly to monitor for contaminants. The addition of a centralized, nationwide reporting mechanism for reporting levels of a spectrum of the most prevalent and/or biological significant estrogenic contaminants (including EE2) would improve the ability to assess potential risks and the identification of at-risk regions. Standardized methods for detection will help ensure adequate removal of contaminants. Until there is routine screening for EE2 and other estrogenic compounds with appropriate limits of detection, it will be hard to fully characterize the contributors to the estrogenicity of U.S. waterways.

Improved Estrogenicity Tests. Estrogenicity tests give varying results on the estrogenicity of a compound. *In vitro* methods vary in their measurements of estrogenicity from each other and vary greatly from *in vivo* methods. Many *in vitro* methods only measure binding affinity to the estrogen receptor which does not account for the many possible biological pathways through which an endocrine disruptor may act. Improved testing assays and standards for measuring potency would improve the ability to compare results across studies. The EPA started an Endocrine Disrupting Chemicals Screening program in 1996 but has not screened any chemicals to date and has only recently released a list of EDCs for which to screen (*110*).

Chemical Policy Reform. There are tens of thousands of chemicals (US-EPA) for which we have little or no data on their potential to negatively influence health. Additionally, current regulatory structure has been identified as insufficient to require the necessary testing and subsequent regulation of harmful chemicals (*111*−*113*). Moreover, previous programs enacted by Congress to develop and implement testing protocols to identify endocrine disrupting chemicals have been inadequate due to significant delays in implementation and concerns about the sufficiency of testing. Requiring information on the potential toxicity for chemicals on the market is one critical step in identifying and reducing harmful chemical exposures. Efforts are underway to address this legislative gap with recent introductions of new legislation

in Congress (*114, 115*). New legislation and regulatory activities need to address both required testing and ensure that estrogenicity, as part of endocrine disruption, be adequately addressed in testing and implementation protocol.

Reducing Overall Use of EE2. Even though the limited evidence indicates EE2 is a relatively small contributor to the overall estrogenicity of drinking water, efforts to reduce the source of EE2, as part of an overall strategy for reducing sources of other estrogenic substances in general, should be pursued. These include low-dose OCs, which may reduce EE2 excretion by 28.5−43%, or reformulation to increase the efficacy of absorption and lower excretion. Additionally, there are nonhormonal methods of contraception including the copper intrauterine device (IUD), diaphragms, or condoms.

Further Research. There are many areas of research which would contribute to a better understanding of the risk of EE2 and other estrogenic compounds to the public's health. These research areas include the following:

• Investigating the role of agricultural sources such as CAFOs and fertilizer applications to the overall estrogenicity of surface, ground, and drinking water. Further efforts are need to identify effective intervention strategies, including policy approaches to reduce contribution to water contamination.

• Combined effects or additivity of endocrine disruptors should be accounted for to better characterize the threat posed by the combination and mixtures of the variety of chemicals found in surface and drinking water.

• Toxicological assessments of common estrogenic contaminants and mixtures should be done, especially the xenoestrogens like nonylphenols found in surface waters.

Conclusion

While the presence of estrogenic compounds in our environment has garnered attention, this review has found the contribution of OCs to overall estrogenicity in water is relatively small compared to other natural and synthetic estrogens. The risk of EE2 in surface waters to wildlife health may pose problems locally, but the risk to human health posed by EE2 at levels in drinking water appears to be minimal. However, more research is needed to understand how EE2 combines with the many other estrogenic sources to affect wildlife and human health. Removing EE2 from the market will have a negligible effect on the environment, aquatic life, and human health. However, removing OCs from the market would be detrimental to women's health and their ability to decide the timing and spacing of their children and would have societal and global implications. Future efforts to reduce the overall estrogenicity of water should take a broad approach to reducing the contribution from the multiple sources, particularly those that are unregulated or that are untreated.

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