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# Effects of 17 $\beta$ -estradiol (E2) on aqueous organisms and its treatment problem: a review

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**Abstract:** Natural estrogens, estrone (E1), 17 $\beta$ -estradiol (E2) and estriol (E3) are endocrine disrupting chemicals (EDCs) that are discharged consistently and directly into surface waters with wastewater treatment plants (WWTPs) effluents, disposal sludges and in storm-water runoff. The most common and highest potential natural estrogen that causes estrogen activity in wastewater influent is E2. This review describes and attempts to summarize the main problems involved in the removal of E2 from WWTP by traditional processes, which fundamentally rely on activated sludge and provide an insufficient treatment for E2, as well as advanced oxidation processes (AOPs) that are applied in tertiary section treatment works. Biological processes affect and play an important role in the degradation of E2. However, some investigations have reported that operations that rely on high retention times have low efficiencies. Although advanced treatment technologies are available, their cost and operational considerations do not make them sustainable solutions. Therefore, E2 is still being released into aqueous areas, as shown in this study that investigates results from different countries. E2 is present on the watch list of substances in the Water Framework Directive (WFD) of the European Union since 2013 and the minimum acceptable concentration of it is 0.4 ng/L.

**Keywords:** advanced oxidation processes (AOPs); aqueous area; degradation; endocrine disruption; 17 $\beta$ -estradiol (E2); wastewater treatment plant.

## Endocrine disruptors

The endocrine system releases hormones that act as chemical messengers that coordinate and regulate

communications among cells. These messengers interact with receptors in cells to trigger responses and prompt normal biological functions such as growth, embryonic development and reproduction.

Endocrine disrupting chemicals (EDCs) are substances that interfere with the normal communication between the messenger and the receptor in the cell so that the chemical message is not interpreted properly. The specific mechanisms by which EDCs disrupt the endocrine systems are very complex and are not yet completely understood. EDCs can interfere with normal cellular functions by (1) the following processes:

- They can mimic the action of naturally produced hormones, leading cells to have unwarranted responses and then producing hormones at the wrong time or to an excessive extent (agonistic effect).
- They can bind to receptors such that the presence of the chemical on the receptor will prevent the binding of natural hormones (antagonistic effect).
- They can bind to transport proteins in the blood, changing the amounts of natural hormones that are present in the circulation.
- They can interfere with metabolic processes in the body, affecting the synthesis and control of natural hormone concentrations.

Environment Canada (2) has reported that because the endocrine system plays a critical role in normal growth, reproduction and development, even small disturbances in endocrine function may have profound and lasting effects. This is also true because, during highly sensitive prenatal periods, even small changes in the endocrine system may cause effects in childhood or even much later in adult life. Thus far, scientists have defined five main categories of adverse effects that may be related to exposure to EDCs, which include cancer, reproductive and developmental alterations, neurological and immunological effects on humans. The endocrine systems that may be affected by EDCs include the thyroid, adrenal, pituitary and gonadal. There is some evidence that animals with small masses or body sizes have suffered from the following adverse effects of exposure to EDCs in the environment:

- Exposure to industrial chemicals has resulted in embryo deformation and mortality.

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- Exposure to effluents from pulp and paper mills has caused impaired reproduction and growth in fish.
- Unusual reproduction has been observed in snails that are exposed to antifouling substances applied to the exteriors of ships.
- Birds that have eaten fish exposed to EDCs have developed thyroid problems and weakened immune systems.
- Feminization and intersex behaviors in fish (males changing to females) have been observed near municipal effluent outlets.

At first, these issues have been identified in species exposed to relatively high concentrations of dioxin, organochlorine pesticides and PCBs, as well as synthetic estrogens for plants exposed to estrogens. At this time, it is not clear whether similar effects are occurring in the general human population from exposure to the same ambient environmental concentrations. Furthermore, EDCs may affect not only the exposed individual but also their children and subsequent generations. Some cases have indicated that adverse reproduction has occurred in individuals (or their offspring) accidentally exposed to high doses of EDCs, as, for example, the following reported effects from the National Science and Technology Council in 1996 (3–5):

- Boys in Yucheng (China), whose mothers ate contaminated rice oil, had shorter penises.
- Workers in a pesticide factory in Virginia (USA) who were exposed to Kepone suffered from reduced sperm counts.
- There was a high sex ratio of females to males at birth in Seveso (Italy) for pregnant women who were exposed to pesticides in 1976 while they were living near a pesticide plant in 1976.

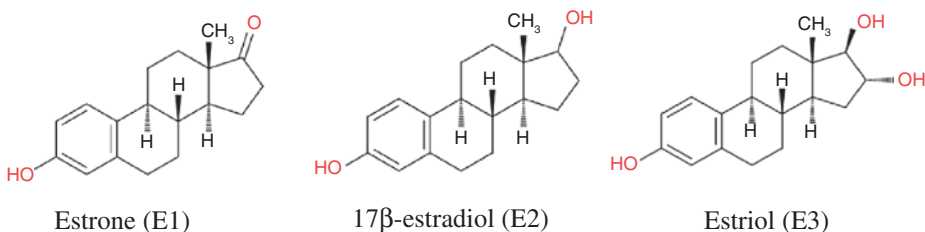
## Estrogen

Estrogens share a common root in the Greek term (oistros), which means “anything causing mad” or “sexual desire”,

and the suffix -gen means “producer of”. Estrogen is the primary female sex hormone and is responsible for the development and regulation of the female reproductive system and secondary sex characteristics. Three forms of human estrogens are estrone (E1), 17 $\beta$ -estradiol (E2) and estriol (E3) derive from cholesterol and readily diffuse across the membranes inside the cell and bind to and activate estrogen receptors (ER $\alpha$ ) to regulate expressions of many genes. They play a vital role in human health; however, when they are released into the environment, they become a concern. They are classified as endocrine-disrupting compounds (EDCs). Estrogen may also refer to any substance, natural or synthetic, that mimics the effects of the natural hormone. The steroid E2 is the most potent and prevalent endogenous estrogen, but several metabolites of E2 also have estrogenic hormonal activity. Synthetic estrogens are used as part of some oral contraceptives, in estrogen replacement therapy for postmenopausal women and in hormone replacement therapies for women (6, 7).

## Structure and properties of natural estrogenic compounds

Among the many different types of estrogens, only three are important to clinical practice and warrant environmental investigation. Their structures are shown in Figure 1. E2 is the most important biologically active and natural estrogen. In premenopausal women, E2 is the most abundant form of estrogen, and the amount of E2 in a woman’s blood varies throughout her menstrual cycle. The ovaries produce the estrogen E2, but E1 is mainly derived from the adrenal androstenedione, and E3 is a metabolite of E1 and E2. E3 levels are usually only measured during pregnancy. E3 is produced in large amounts by the placenta, which is the tissue that links the fetus to the mother. It can be detected as early as the 9th week of pregnancy, and its levels increase until delivery. It can also be measured in urine. E1 is the most abundant metabolite in postmenopausal



**Figure 1:** Chemical structures of three natural estrogenic compounds found in wastewater effluent. Source: European Molecular Biology Laboratory CHEBI (6).

**Table 1:** Physical-chemical properties of natural estrogens.

Physical-chemical properties	Units	Estrone (E1)	17 $\beta$ -estradiol (E2)	Estriol (E3)
CAS		53–16–7	50–28–2	50–27–1
Log Kow	[–]	3.1–3.4	3.1–4.0	2.6–2.8
M <sub>i</sub>	[g/mol]	270.4	272.4	288
C <sub>iw</sub> <sup>sat</sup>	[g/m <sup>3</sup> ]	6.6	8.6	8.25
P <sub>i</sub>	[Pa]	3 × 10 <sup>-8</sup>	3 × 10 <sup>-8</sup>	9 × 10 <sup>-13</sup>
Sw	[mg/L]	0.8–12.4	3.9–13.3	3.2–13.3
pKa	[–]	10.3–10.8	10.5–10.7	10.4

Source: Beecher et al. (6).

women. In menopause, the ovaries stop producing E2, and then E1 becomes the principal estrogen (8).

After menopause, E2 production drops to a very low but constant level, and it is the most potent estrogen of a group of endogenous estrogen steroids that includes E1 and E3. E2 is the predominant estrogen during reproductive years both in terms of absolute serum levels as well as in terms of estrogenic activity. Although E3 is the most plentiful of the three estrogens, it is also the weakest, whereas E2 is the strongest because E2 has the highest binding affinity for ER $\alpha$  and it binds to both ER $\alpha$  and ER $\beta$  receptors. Thus, E2 is the most important estrogen in non-pregnant females who are between the menarche and menopause stages of life. However, during pregnancy, the role shifts to E3, and in a postmenopausal woman, E1 becomes the primary form of estrogen in the body (9, 10).

In the natural environment, E1 and E2 are interconverted by the enzyme 17- $\beta$ -hydroxysteroid dehydrogenase (17 $\beta$ -HSD), light or biological activity before degradation. The physical-chemical properties of E1, E2 and E3 impact their environmental fates (Table 1). Low solubility and Kow values for the three compounds represent a lipophilic tendency, which means that the compounds tend to favor attachment to solid substrates instead of dissolving in aqueous solutions. Hydrolysis is not expected to be an important environmental fate process because these compounds lack functional groups that hydrolyze under environmental conditions (6, 7).

## 17 $\beta$ -Estradiol (E2) in males and females

Estrogens and testosterone are present in both males and females, although males have more testosterone and females have higher estrogen levels. Both sexes have receptors for each hormone, with a high concentration of  $\beta$ -receptors (ER $\beta$ ) in the hippocampus and hypothalamus. One reason for this in males is because testosterone is converted into E2. Testosterone can be broken down

into dihydrotestosterone via 5 $\alpha$ -reductase or to E2 via aromatase. A lack of testosterone will produce a lack of E2 as well.

Both hormones have an effect on the behaviors and brain organization of both sexes later in life. If either sex is castrated during a sensitive period, behavioral changes are observed in adulthood. Males require gonadal hormones early in development to induce male sexual behaviors. Females need a lack of gonadal hormones to develop female sexual behaviors and to produce luteinizing hormone later in life. Gonadal steroids act during a perinatal sensitive period to permanently alter the neural architecture and restrict the response to exposure of particular hormones. Castrating a male rat at birth will lead to more female behaviors, such as lordosis. They will also tend to perform more poorly on spatial and fear conditioning tasks. Administration of E2 or testosterone eliminates the difference between castrated and intact animals (11). E2 also plays a role in adults. As stated earlier, with the large concentration of ER $\beta$  in the hippocampus, E2 plays a role in spatial and non-spatial memory tasks such as water mazes, t-mazes and radial arm mazes. In women, E2 is the predominant sex hormone and is responsible for the growth of breasts and reproductive epithelia, maturation of long bones and development of the secondary sexual characteristics. E2 represents the major estrogen in humans and is produced from cholesterol, androstenedione and testosterone. The normal concentration of E2 in a healthy body is shown in Table 2. A decrease in E2 levels to less than 20 pg/mL causes the loss of its beneficial effects,

**Table 2:** The normal level of E2 in human blood (12).

Population	Reference range
Children	< 10 pg/mL
Males	10–50 pg/mL
Female premenopausal	30–400 pg/mL
Female postmenopausal	0–30 pg/mL

resulting in decreased calcium resorption, accelerated bone loss and an increase in triglyceride levels and in the cholesterol/HDL ratio (6, 7).

### Environmental health effects caused by the release of E2 in aqueous areas

Concern over steroid estrogens in our waterways has increased in the last decade with improvements in technology that can detect low estrogen concentrations. These contaminant loadings in wastewater treatment plants (WWTPs) cannot be removed efficiently or conveniently. Removal requires advanced technologies during treatment, which involve large financial costs. Therefore, the contaminant loadings are released into the environment in sludges and effluents (13, 14). In recent years, the presence of natural estrogens has been reported in both drinking water and surface water (15–17).

The observed risks by the exposure to E2 in the health of aquatic organisms have raised concerns regarding what damage and/or health-related conditions are being expressed in terrestrial wildlife, as well as the consequences for humans as a result of the lowest sensitive-concentration E2 exposure. In general, children and immature wildlife with lower body masses are at the greatest risk from elevated environmental E2 concentrations. Some studies in humans have shown that E2 exposure in pre-pubertal and pubertal children may lead to excessive rapid growth as well as the early onset of puberty in females and the late onset of puberty in males (18). In addition, in the post-puberty stages, E2 in especially high concentrations contributes to multiple disorders including testicular and ovarian cancer, as well as stimulated endometriosis, osteoporosis, cardiovascular diseases, neurodegenerative diseases, cognitive and behavioral alterations, hypertension, metabolic disorders (such as obesity and diabetes) and also immune disorders (19, 20).

Natural estrogens are generally quickly and well absorbed from the gastrointestinal tract, and there are few differences in uptake between E1, E2 and E3. They are inactivated by the liver, but a few studies have found accumulation and storage of E2, E1 and E3 after exogenous administration. All three natural estrogens are distributed to various target and non-target organs through systemic circulation, although they are also produced locally and stored in target tissues, particularly in fat (6, 7). Natural estrogens and their metabolites are excreted mainly in urine, and there are also small amounts in feces. The primary concern of steroid estrogens in the environment is related to their “endocrine disrupting ability” (EDCs) (21,

22). The most direct and clear evidence has been found in male fish swimming downstream from estrogen-impacted water sources exposed to estrogens (EE2-E2) even at low-dose concentrations (1–2 ng/L). Specifically, these fish have been found to have sexual characteristics of both male and female, such as partially developed eggs or ova in their testes (23). Table 3 shows some studies on the adverse effects of E2 concentrations in aqueous animals.

Not only can such sex-related damage to fish affect their populations, but these taxa can also be looked upon as a warning of potential dangers to humans (i.e. a canary in a coal mine). For example, E2 in our drinking water can interfere with sperm production and affect male fertility (4, 36, 37).

17 $\beta$ -estradiol, which is often called E2 because it has two hydroxyl groups in the structure of its molecules, is an essential form of estrogen in the body (38). Yeast estrogen screening assays use E2 as the primary standard to measure estrogenic activities. Most of the estrogenic activities in municipal wastewaters that are measured by yeast assay are thought to be due to E1 and E2 concentrations, whereas Matsui et al. (39) found in a study at three STPs in Japan that E2 is responsible for 34% of the whole estrogenicity of raw sewage and almost 100% in the final effluent. Steroidal estrogens have the highest estrogenic activity of known EDCs in wastewater, and E1, E2 and 17 $\alpha$ -ethinyl estradiol (EE2) are thought to be the priority EDCs to control in municipal WWPT (40).

The first step to reducing endocrine-active compounds in our waterways is to determine how our current WWT processes and associated environmental parameters affect the bioavailability of E2. To avoid adverse effects to the aquatic environment and human health, the transport and fate of steroid estrogens need to be better monitored and recognized. Steroid estrogens not removed during conventional WWT will be released into our environment. The efficiency of WWT processes needs to be quantified and better understood in terms of their ability to remove pharmaceuticals (41).

### Sources and fates of natural estrogen in the environment

#### Production from humans

Hormones produced by animals and humans are released into the environment continuously. Steroid hormones are very stable and are excreted either in an endogenous active form or as conjugates that are easily biotransformed into a free form. Human females excrete approximately 5  $\mu$ g/day

**Table 3:** The adverse effects of various E2 concentrations on fishes in aqueous areas.

Concentration ng/L	Environmental impact	Reference
During a 101-day study (after 100 days post-hatch)	– At 8–9 ng/L: reduced fertility and fecundity of Japanese medaka fish	(24)
Japanese medaka ( <i>Oryzias latipes</i> ) During 15 and 55 days	– 15-day exposure to 10 ng/L – Adverse effects on hatchability and spawning – Number of hatched females twice that of males – When hatched fish were exposed to 1, 10 and 100 ng/L for another 40 days – Increasing HSI in both males and females; the GSI increased in males and decreased in females – Sex reversal was found in fish exposed to 1 ng/L and above	(25)
Chinese rare minnow During 21 days	– At 5 ng/L – VTG induction in the adult – Sex ratio male/female = 36 : 62 for juveniles and 40 : 60 for larvae – At 25 ng/L: 80% of all fish were female – At 100 ng/L: there were no males	(26)
187-day study on <i>Oryzias javanicus</i>	– At 16 ng/L: effect on fertilization and egg production	(27)
14-day study on <i>Oryzias javanicus</i>	– At 379 ng/L: the production of eggs and the successful spawn rate decreased	(28)
During 21 days <i>Oryzias latipes</i>	– At 34 ng/L – Formation of ova-testis during the larval period – Sex transformation from male to female from larva to juvenile (approximately 14–20 days post-hatch)	(29)
Sheepshead minnow full life study for 280 days	– At 80 ng/L – Daily reproduction significantly reduced – Eggs infertile from F1 increase – At 40 ng/L – For F2 – effect on liver, kidney, gonadal tissue for F0_F1 at 200 ng/L – At 300 ng/L All F1 fish appeared to be female	(30)
Guppy juvenile, 90-day exposure study	– At 100 ng/L – Reduced gonopodium length, sperm cell number and sexual behavior in male – At conc. > 100 ng/L – Caused a reduction in the male ratio – Reduced the female gonad weight, number of oocytes and embryos	(31)
<i>Pomatoschistus minutus</i> in 8 months	– At 16 ng/L – No significant adverse effects	(32)
Fathead minnow during partial life stage study	– At 8 ng/L and above – After 92 days, growth was impaired – At 60 ng/L and above – The sex ratio almost changed to all female population – The 26 ng/L was the sensitive level on the number of eggs produced by the female	(33)
Zebrafish, partial life study	– At 87 ng/L: VTG production – At 272 ng/L (1 nM) – Decreased F1 survival – Increased F1 body length and weight – Inhibited spermatogenesis – 27 ng/L was the VTG effective level	(34)
Predicted and estimated no effect-concentration is 2 ng/L		(35)

F0, fish caught in the wild; F1, first generation of F0; GSI, gonadosomatic index; HIS, hepatosomatic index; VTG, Vitellogenin: an egg-yolk precursor protein expressed in females. Larva = 0–21 dph (days post-hatch), Juvenile = (21–42 dph), Adult = (150–171 dph).

each of E1 and E2. Daily excretion rates can be as high as 10 and 100  $\mu\text{g}$  by a cycling woman (Table 4), depending on the cycle phase (42). Although these natural estrogens are produced naturally in all vertebrate organisms, human

excretion is thought to be the primary source of elevated concentrations in our environment (38).

The amount of estrogen, mainly E3, excreted by pregnant women can be 1000 times higher than normal,

**Table 4:** Typical concentrations of estrogenic compounds produced by human females.

Steroid	Produced ( $\mu\text{g}/\text{day}$ )	Excreted in urine ( $\mu\text{g}/\text{day}$ )	Phase
17 $\beta$ -Estradiol	13	0	Pre-puberty
17 $\beta$ -Estradiol	82–695	0.3–5	Cycling
Estrone	41	0	Pre-puberty
Estrone	110–497	2–20	Cycling
Estriol	0	3–65	Pregnant

Source: Hoffmann and Evers (42).

**Table 5:** Estimated yearly steroid hormone excretion (tons/year) by farm animals in the USA.

Species	Heads (millions)	Estrogen (tons/year)
Cattle	98	45
Pigs	59	0.83
Sheep	7.7	0.09
Chickens	1816	2.7
Total	1981	49

Source: Lange et al. (45).

depending upon the stage of pregnancy. E1 and E2 are excreted in human urine at a rate of 4.4 kg/year/one million inhabitants. This estimate could account for 50% of the natural estrogen in the influents to WWPT (43).

### Estrogen production from livestock

Animal manure is a primary source of the natural estrogens that enter the environment (44). Lange et al. (45) measured the total output of domestic animals in a year and found that a large proportion of the produced natural estrogens are produced in the feces of cattle and chickens (Table 5). Gaulke et al. (46) stated that a major amount of the estrogen excreted in cow feces is from the last trimester of pregnancy, and almost all of the E1 and E2 excreted are in the free form.

Lange et al. (45) presented values ranging from 600 to 1600  $\mu\text{g}$  estrogen/kg total solids from dairy farm effluent slurries. The values for chicken manure were lower

than those for cattle; however, concentrations of estrogen excreted in chicken manure were still high. Chicken manure contains more than 533  $\mu\text{g}$  of estrogen/kg dry matter (46, 47). E2 and E1 are the main hormones excreted by chickens, whereas the excretion of estrogens in urine by laying and non-laying hens was approximately 3 and 2  $\mu\text{g}/\text{day}$  for E2 and E3, respectively, and 0.5  $\mu\text{g}/\text{day}$  for E1 (47, 48).

Large amounts of poultry litter and cattle manure are added as organic fertilizer amendments to fields and are emphasized as best management practices for manure management programs. These organic compounds are resistant to degradation in the soil; however, some degradation occurs in these estrogen compounds. Thus, during stormwater runoff, estrogenic compounds can be suspended in solution and enter surface water bodies, either directly or through the insufficient treatment of wastewater, where they adversely impact the health of organisms (49). To compare the production of estrogen by livestock and humans, results from an investigation conducted in Switzerland by Schoenborn et al. (50) are shown in Table 6. An estimate for Switzerland implies that the total annual amount of estrogen emission into the environment from livestock is at least 5 times higher than that excreted by humans. In addition, another study from the UK calculated and obtained the same factor of 5 (51).

### Hormones in food and feed

Average hormone consumption in food for an adult has been calculated to be 0.1  $\mu\text{g}$  estrogen/day, which is very

**Table 6:** Estimated annual estrogen load in Switzerland in 2007 from the excreta of humans and three common livestock animals.

Species	Excretion of natural estrogen (mg/individual/year)	Number of individuals	Annual estrogen loading (kg/year)	Share (%)
Cows	110 <sup>a</sup>	708,340	77.9	43
Pigs	43 <sup>a</sup>	1,573,090	67.6	37
Sheep	8.4 <sup>a</sup>	443,584	3.7	2
Humans	4.38 <sup>b</sup>	7,593,500	33.3	18

<sup>a</sup>After (cycling females), <sup>b</sup>Estimated from average excretion of 12  $\mu\text{g}/\text{person}/\text{day}$  (natural estrogen, without pregnant women).

small compared to the endogenous estrogen production in human bodies. The two main and usual sources of estrogenic compounds are meat and dairy products, and dairy products contribute a greater proportion of estrogen than meat. Meat contains small amounts of steroids ranging from 3 to 5 ng/kg (42). In comparison, for instance, bovine milk is rich in a variety of hormones. Steroid hormones are lipophilic and can concentrate in dairy products, depending upon their fat content (Table 7) (52, 53).

These high concentrations of estrogenic compounds in milk products cause concern and increased attention to the regular eating habits of humans, especially young children. The average consumption of bovine milk by children is approximately 300–700 mL/day (1–2 glasses), which translates into a potential ingestion of 40–100 ng/day of estrogen (E2, E1 and estrone sulfate), which is approximately the limit to the dose of estrogen consumed by adults when other dairy products are not included. Children are in the developmental stages of life, and this large consumption of estrogenic compounds may not produce clear effects in childhood and may contribute to cancer formation later in life (3, 53).

Moreover, estrogens are derived from plant compounds such as isoflavones (a type of phytoestrogen) in soy products or flavones, which are found in leafy green vegetables and herbs (54). As a result, the load of estrogen compounds is continuously increasing in the aquatic environment, where it affects aquatic animals that subsequently excrete the compounds into our drinking waters and contaminate them (55).

### Commercial use of estrogen

E2 is the most widely marketed of the three compounds and is available for use in many different forms: oral versions, transdermal patches, ointments, injection, vaginal ointments and vaginal rings. EE2, which is formed with the

addition of an alkyl group at the C3 position, is sometimes used to enhance uptake and distribution within the human body and is commonly used in birth control brands (56). Specifically, estrogens and related compounds are used for a wide array of therapeutic purposes, including contraception, menopausal syndrome, endometrial diseases, osteoporosis, prostate and breast cancer and heart disease. Steroidal hormones like E2 are sometimes used in pharmaceuticals to maintain non-pregnant hormone levels and prevent pregnancy. These estrogens interact with the ER $\alpha$  and readily diffuse across membranes (20, 47).

### How estrogens enter surface water

According to a study in 31 provinces in China, the average value of E2 emission was 342.0 kg/year, and for Jiangsu, the Yangtze River Delta and Zhejiang and Shanghai, the average values were 129.6, 238.4 and 24.62 kg, respectively (57).

Two sources of pollution are the main contributors of EDCs into surface waters: runoff from fields fertilized with manure and discharge into streams from sewage treatment plants (2). By considering the first prominent source waste, WWTP effluent, when human populations are increasing, WWT facilities near growth areas must increase their treatment rate to accommodate the increased waste production. However, traditional treatment trains (coagulation, settling, plain sedimentation and sometimes disinfection with chlorine) do not effectively remove pharmaceuticals, EDCs or their metabolites, and elevated concentrations of hormone metabolites and pharmaceuticals are then directly transported to surface waters; subsequently, they cause downstream exposure to wildlife, including fish and other mammals (58, 59).

Runoff is the second source and occurs in precipitation, which can saturate soils and carry contaminant loadings from agricultural fields that are fertilized with manure. In addition, soils may be contaminated with estrogen from digested sludges applied to land. In total, the estrogens from these lands can directly reach streams and aquatic areas through stormwater runoff or from the insufficient treatment of WWTP effluents (2, 47). The levels of E2 and E1 in freely flowing streams are not higher than 5 ng/L, but these concentrations are of the same magnitude as the lowest observed effects limit (LOEL) and are potentially harmful to aquatic organisms (60).

Another entrance route into the environment is facilitated by the improper disposal of pharmaceuticals. Expired or excess estrogenic pharmaceuticals, such as contraceptives and hormone replacement therapy treatments, are

**Table 7:** Estrogens (E2, E1) in dairy products in pg/mL or pg/g.

Source	Estradiol (E2)	Estrone (E1)	Estrone + Estrone-sulfate
Milk from cows			
Estrus	84	58	
Late pregnant	49	45	200–1000
Luteal	29	45	
Cream	< 30		260
Store milk	10	55	500
Cheese	10	55	170
Butter	82	539	1470

Source: Koldovsky and Thornburg (52).

flushed and rinsed down drains rather than disposed of in landfills, and they enter the environment via wastewater effluent in an active form (61). Increased exposure is a concern because these compounds are transformed and become EDCs. Trophic transfer and/or direct uptake of EDCs from the aqueous environment are mechanisms by which these bioavailable forms may cause disruption in the normal hormone signaling mechanisms of wildlife and reduce population fitness (62). Table 8 shows some studies on concentrations of E2 and other estrogens in the aqueous areas of some countries.

## E2 concentrations in aqueous areas in Malaysia

Water surface sampling was conducted in selected aquatic ecosystems (rivers, estuaries and lakes) from the north to the south of Peninsular Malaysia. Ismail et al. (17) collected surface water samples in triplicates from 99 stations, which are shown in Figure 2. The study revealed that the concentrations of E2 in the aquatic environment of Peninsular Malaysia varied among localities. As shown in Table 9, the average E2 concentrations based on zonation ranged from not detectable (ND) to almost 3700 ng/L. The average concentration in the southern area was 15.66 g/L. The central region had the greatest average concentration of E2, which was almost 150 ng/L. The northern region showed the highest average concentration of E2 in Malaysia, which was 95.04 ng/L, and the maximum concentration was 3677.4 ng/L. The lakes had an average concentration of 11.83 ng/L. Although the lake area did not have the lowest average concentration, it had the lowest maximum concentration, 27.7 ng/L, among the other maximum concentrations. The minimum value of E2 concentration ranged from approximately 2.1 ng/L to ND.

The E2 concentration ranges in the aquatic environment define the activities that exist around the water bodies and their sources. The highest E2 concentration in this study was in the northern area. There are still many villages in the northern area that do not have systematic water management. Therefore, the wastes are not well managed, and most of the waste enters the rivers. The E2 concentrations in the central area and in the Klang Valley area, which are primarily urban areas with planned sewage systems, are not as high as those in the northern area. Most of the lakes show non-detectable values of E2, possibly because most of the lakes chosen were actually control areas, such as Tasik Titiwangsa and Tasik Perdana. Typically, lakes are well managed, so when a

lake is actually under control, no wastewater will be channeled into the lake to preserve the quality of the water.

In Malaysia, Koyama et al. (68) reported that the rivers and estuarine waters around Kuala Lumpur contained E2 concentrations ranging from 6.1 to 284 ng/L, whereas Ismail et al. (17) reported that the values ranged from ND to 27,700 ng/L in rivers around the Klang Valley, which was also found in a previous study in 2007. Mustafa et al. (96) studied EDCs in several states in Malaysia and found that the maximum levels of E1 and E2 in Perak and Melaka wastewater were 228 ng/L and 44.5 ng/L, respectively, and the maximum concentration of E2 in Klang was 4.25 ng/L. If all of the E2 data in the present and previous studies are compiled together, it can be said that Malaysia has a very high E2 in its aquatic environments. E2 in the aquatic environment is reportedly derived from wastewater. This is because E2, and estrogen in general, is found in many personal care and pharmaceutical products (PCPP). For example, E2 is used by women to help reduce symptoms of menopause (such as hot flashes, vaginal dryness). These symptoms are caused by the body making less estrogen, and certain products may also be used by women after menopause to prevent bone loss (osteoporosis) (56, 72).

## Estrogen in ground water

The potential for estrogen contamination of ground water is minimal because estrogen remains bound to the upper crust of the soil, aided by the binding of the phenolic group to soil particles (97). Although extensive surveys have indicated that estrogen can be detected in springs and wells used for drinking water, the levels are usually well under 1 ng/L (98). In addition, Arnon et al. (99) detected estrogen to a depth of 32 m in the soil beneath a waste pond in Israel, and estrogen was detected at very low concentrations in the groundwater.

## Problems with the removal of E2 and other estrogens in WWTP

Human excretion and activities are significant sources of estrogens in the environment; therefore, WWT is the first line of defense for reducing and treating the concentration of these chemicals (100). Activated sludge has become the standard of WWTs and is typically applied at most levels of sewage treatment (treatments larger than the lagoon level). The manipulation of physical parameters



**Table 8:** The concentration of E2 and other estrogen compounds in surface waters, sediments and drinking water.

Location Water (ng/L)	Level of estrogen compounds			Method analysis	Reference	
	E1	E2	E3			EE2
Brazil, campinas and Sumare drinking water	0.03–0.06	0.93–1.32	0–72	91.2–154	LC-MS/MS	(15)
USA, drinking water	0.9	17	–	1.4	GC-MS	(63)
USA, New Orleans surface water	3.71	3.76	–	–	LC-MS/MS	(64)
China, Liao River	55.8	7.4	–	–	GC-MS	(65)
Spain, Lobregat River	0–560	0–130	0–170	–	GC-MS	(66)
Brazil, Velhas River	4.6–62.4	62.6	–	5.6	LC-MS/MS	(67)
Vietnam, Long Xuyen surface water	6.8–77.5	8.1–10.2	–	26.8–28.6	GC-MS	(16)
Indonesia, Cikamasan surface water	2.9–26.1	6.4–6.2	–	6–9.1	–	–
Thailand, Khong River	3.8–15.4	6.1–7.5	–	7.2–10.4	–	–
Cambodia, Siem Reap River	4.5–4.9	4.6–7.4	–	19.7–20.7	–	–
Laos, Ton River	5–38.6	4.6–6.0	–	19.4–22.4	–	–
China Fenhe River	1.3–19.8	8.8–9.5	–	21.8–24.4	–	–
South Korea, Yeongsan and Seomjin Rivers	4.4–6.5	3.5–4.3	–	n.d	–	–
Malaysia, Tuaran, Salut River	–	2.3–3.2	–	4.3–8.6	–	–
Malaysia, river and estuarine around Kuala Lumpur	–	6.1–284	–	–	ELISA	(68)
Malaysia around Klang Valley in 2007	–	n.d–27.700	–	–	ELISA	(17)
Peninsular	–	n.d–115.9	–	–	ELISA	(17)
Malaysia	–	n.d–3677.4	–	–	–	–
River estuary	–	2.1–58.3	–	–	–	–
Malaysia lakes	–	n.d–27.7	–	–	–	–
USA, Acushet River estuary	0.73–1.20	0.73–1.2	–	3.01–4.67	GC-MS	(69)
Iran, Hamadam surface water	9.00–2.00	10.00–3.00	–	2.00–0.01	SPE-HPLC	(70)
USA, South Florida	0.88–5.20	n.d–1.80	–	–	GC-MS	(71)
Turkey Buyukcekme watershed, Istanbul	1.40–5.74	1.10–5.39	2.15–5.2	11.70–14.0	LC-MS/MS	(72)
Australia, South East Queensland	0.55–20.91	0.39–3.77	–	0.00–0.52	ELISA	(73)
Czech Republic and Germany, River Elbe and tributaries	<0.20	<0.20	–	<0.20	GC-MS	(74)
Brazilian surface water	n.d–39.0	n.d–7.30	n.d–2.30	n.d–25.00	LC-MS	(75)
Italy, River Lambro	0.05	0.00	0.05	0.00	LC-MS/MS	(76)
Netherlands, surface water	<0.3–7.2	<0.8–1.0	–	<0.3–0.4	ELISA	(77)
Venice Lagoon, bay of the Adriatic Sea	1.20–10.0	1.00–175.0	–	<0.8–34.0	LC-MS/MS	(78)
Dan-Shui River, Taipei, Taiwan	22.4–66.20	1.40–33.90	12.40–73.60	7.53–27.4	LC-MS	(79)
Germany, Würm River	<0.30–2.0	<0.3–0.7	–	<0.03–0.7	GC-MS	(80)
China, Jiaozhou Bay, Qingdao	14.00–180	n.d–134.0	4.0–94.0	7.00–24.0	GC-MS	(81)
South China, Guangzhou	n.d–50.0	n.d–2.0	n.d–1.00	n.d	GC-MS	(56)
China, Tiajin area, Northern	0.64–55.3	n.d–21.20	n.d–46.40	n.d–24.40	GC-MS	(25)
China, Yundang Lagoon	n.d–5.34	n.d–1.56	–	n.d–0.43	GC-MS	(82, 83)

Table 8 (continued)

Location	Level of estrogen compounds				Method analysis	Reference
	E1	E2	E3	EE2		
China, Southern Jiangsu Province	0.02–1.87	1.28–23.22	0.01–0.8	–	LC-MS	(84)
China, Dianchi Lake	n.d	1.90 $\pm$ 0.3	n.d	n.d	LC-MS/MS	(85)
China, Northern Taihu Lake	2.1–28.8	40.0–117	n.d–22.4	n.d–33.5	LC-MS/MS	(86)
China, Yellow River	n.d–15.60	n.d–2.30	–	–	GC-MS	(87)
Sediment ( $\mu$ g/kg dry weight)						
European river	<0.50	<1.00	<0.05	<0.10	LC-MS	(88)
UK, River Ouse	0.40–3.30	<0.03–1.2	–	n.d	LC-MS/MS	(89)
Brazil, Itacorubi mangrove	0.71–50.75	0.87–40.96	–	133.64	SPE-HPLC	(90)
North of France	0.30–1.28	0.18–1.58	n.d–1.26	n.d	GC-MS	(91)
Australia, Sydney, Malabar	0.16–1.17	0.22–2.48	–	<0.05–0.5	GC-MS	(36)
Northern China Tianjin area	0.98–21.60	n.d–9.70	n.d–7.29	n.d–9.26	GC-MS	(25)
Japan, Tokyo Bay	0.05–3.60	n.d–0.59	–	n.d–0.34	LC-MS/MS	(92)
China, Pearl River Delta	<1.3–10.9	<0.9–2.6	–	n.d	GC-MS	(93)
China, Licun River	3.0–10.80	n.d–1.20	1.00–7.60	n.d–5.10	GC-MS	(81)
China, Xiamen Bay	n.d–7.38	n.d–2.35	–	n.d–2.18	GC-MS	(94)
China, Yundang Lagoon, China	4.61–11.22	n.d–3.71	–	n.d–2.48	GC-MS	(82, 83)
China, sediment Northern Taihu Lake	5.49–164	9.78–151	9.19–203	4.32–184	LC-MS/MS	(86)
China, Biota Northern Taihu Lake	6.36–364	39.4–562	n.d–456	22.6–417	LC-MS/MS	(86)
China, Yellow River	n.d	n.d	–	–	GC-MS	(87)
Central-southern Chile, Nine locations	0.06–4.61	0.06–16.81	10.26–53.21	4.18–48.14	GC-MS	(95)

n.d, no detection.

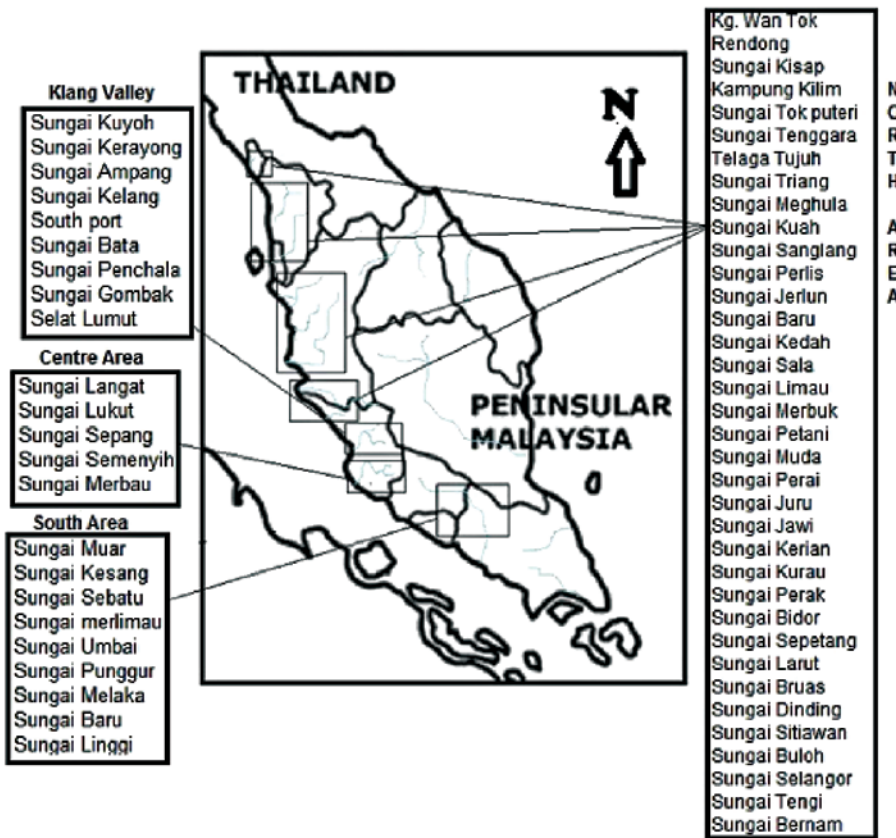


Figure 2: Location of the water sampling sites from north to south in Peninsular Malaysia.

Table 9: Mean Concentrations of E2 around Peninsular Malaysia based on regions.

Zonation	Number of site in each zone	17β-estradiol (ng/L)		
		Minimum	Maximum	Average
South	13	2.1	58.3	15.66
Center	10	ND	3677.4	149.19
North	57	ND	115.9	95.04
Lakes	8	ND	27.7	11.83

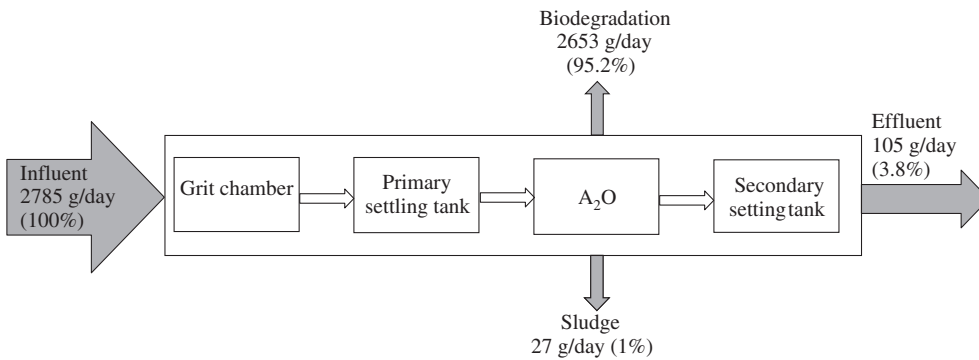
ND, non-detectable; Source: Ismail et al. (17).

could possibly influence the efficiency of removing contaminants of concern in our wastewaters; however, this is unlikely to occur because these parameters, such as pH, DO and (T) temperature, often need to be maintained and controlled within a regulatory range set by a governing agency (e.g. USEPA) to ensure healthy waters. Although pH has been shown to have a significant interaction effect in treatment, the range (7.1–7.4) is most likely not biochemically meaningful. Many other studies have focused their investigations on new or advanced technologies to reduce pharmaceutical and EDC concentrations; although, very

few have focused on improving the efficient removal of EDCs in WWT processes already in practice (22, 101).

Ma et al. (102) investigated the largest WWTP in China (Gaobeidian WWTP), which is located in Beijing. The plant treats an average flow of 1,000,000 m<sup>3</sup>/day and services a population of approximately 2.4 million people. The treatment process includes an aerated grit chamber, a primary sedimentation tank, an A<sub>2</sub>O [anoxic, anaerobic and oxic (aerobic)] tank and at the end a secondary sedimentation tank. The WWTP receives industrial and domestic wastewater in equal measures. Some effluent from the secondary sedimentation tank is treated with flocculation and then filtration for reuse, and the surplus water is discharged. If the influent mass of estrogens (2785 g/day) is taken as 100% of the input, the masses of the estrogens in the excess sludge (27 g/day) and in the effluent (105 g/day) evaluated for 1% and 3.8% of the total mass balance output. Therefore, the biodegradation amount is 95.2% of the total output. The mass balance is shown in Figure 3.

For the reasons discussed above, wastewater treatment techniques must be improved via the revision or modification of existing facilities to successfully remove organic microcontaminants from wastewater (47).



**Figure 3:** The mass balance of influent estrogen in a WWTP.

Source: Ma et al. (102).

## Main tradition (secondary section) WWTP process for the removal of E2

### Adsorption

The adsorption of EDCs is performed by the interaction of the compounds with settleable solids. The adsorption process can effectively remove estrogens from aqueous systems, but these contaminants are only transferred from the water phase to the solid phase without mineralization or decomposition (103). Estrogen molecules are moderately hydrophobic and do not ionize at normal environmental pH range (7–7.5); therefore, they are extensively absorbed by sludge particles and organic matter and remain in sludge. The unconjugated steroidal estrogens E1 and E2 have low solubilities in water and are relatively lipophilic, as indicated by their log Kow values, 3.1 and 4, respectively. Because of its low vapor pressure, the volatility of E2 can be neglected. Because the pKa of E2 is 10.5, the undissociated form of E2 reacts with hydroxyl radicals at a pH of 6.5–7 (104, 105). Koh et al. (106) examined the adsorption rates for steroid estrogens in two biological wastewater processes and found that differences in removal were related to the Log Kow values. The E1 and E2 removal rates were between 20%–30% and the E3 removal rates were lower (10%). Snow et al. (107) showed in their study that an increase in organic carbon increases the removal of E2 from the aquatic environment.

### Degradation

Degradation is a biological process in all water treatment plants and is the primary method for removing nutrients (phosphorus and nitrogen) from domestic wastewater. Studies on wastewater have also demonstrated the effective degradation of many organic compounds (45). The

degradation occurs in 70%–80% of EDC removal in wastewater in the activated sludge section of the treatment plant and is mainly an aerobic process (108).

A major question regarding the degradation of EDCs in sludge relates to whether degradation is rapid or slow, and Carballa et al. (109) suggested that short sludge retention times insufficiently degrade E2. Kreuzinger et al. (110) concluded that E1, E2 and E3 were degraded at higher sludge retention times related to the waste energy in the WWTP. Only 16% of the estrogen removal was achieved when sludge was retained for 1 day, whereas if the retention time was extended to 10 and 24 days, the amount of degradation was 66% and 98%, respectively.

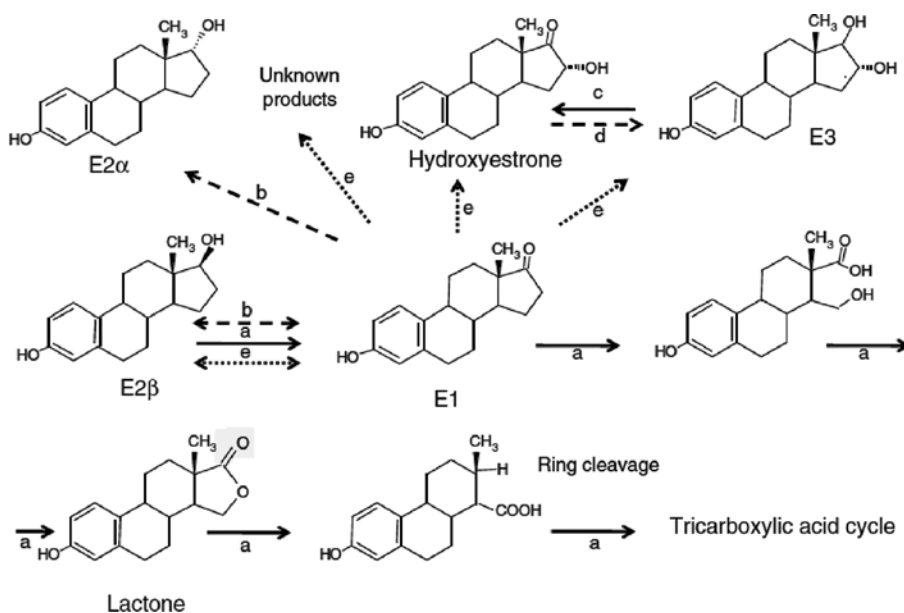
Koh et al. (111) described that the solid retention time (SRT) parameter influenced the removal of estrogens from a sewage treatment work (STW) because microorganisms prefer to degrade other organic compounds rather than estrogens. When low sludge loads due to biochemical oxygen demand (BOD) occur, the microorganisms are forced to mineralize poorly degradable organic compounds. An increase in the SRT may enhance the biodegradative and sorptive capacity of the activated sludge and furthermore allows for a more specialized microbial culture to grow, including slow growing microorganisms that are essentially used to remove estrogen. Another parameter that affects the removal efficiencies of the biological process is the hydraulic residence time (HRT). A long HRT allows more time for adsorption and biodegradation. Note that the minimum required time to observe degradation in E2 and in other estrogens is approximately days. Temperature changes that are related to seasonal variations affect the removal of estrogens from sewage treatment works (STWs). Generally, decreasing temperature reduces STW treatment efficiencies because of the metabolic rates of microorganisms. It has been reported that effluent E2 concentrations are higher in winter, and the efficiency of treatment is lower.

Some studies have found that increasing sludge ages can diversify bacterial groups in the treatment facility process, allowing for the selection of specific organisms to reduce targeted EDCs. Instead, degradation is simply facilitated by strains of bacteria innate to the wastewater facility, and microbial foraging for carbon sources causes the reduction. Other researchers have hypothesized that contaminant degradation is not bacterial-strain specific (45, 47). Other studies also have suggested that the competition between nitrifying and heterotrophic bacteria for carbon resources while extending sludge retention times increases degradation rates; the heterotrophic bacteria drive the majority of degradation because they are associated with slow growth and aged sludge (47). Hernandez-Raquet and Combalbert (112) proposed the degradation pathway for natural estrogens by bacteria, as shown in Figure 4.

The estrogenic activity of sludge increases during anaerobic digestion due to the reduction of E1 relative to the more abundant estrogenic E2 (105). No E3 was detected in the sludge during anaerobic digestion, and most of the E1 and E2 partitioned into a solid phase and remained there during digestion (105). Holbrook et al. (113) also indicated that estrogenic activity nearly doubled during mesophilic aerobic and anaerobic digestion, but 51%–67% of the estrogenic activity in the influent wastewater was biodegraded during the process of the WWTP.

de Mes et al. (114) reported that no significant decrease in the totals of E1 and E2 were observed over 205 days of anaerobic sludge digestion in pilot scale batch experiments. However, Carballa et al. (109) found that more than 85% of the estrogens (E1 and E2) could be removed using either mesophilic (37 °C) or thermophilic (55 °C) anaerobic digestion in pilot plants fed mixed sludge collected from a municipal WWTP. These studies imply that there are conflicting results for anaerobic digestion. In conclusion, the biological degradation of estrogens is mostly employed in sewage treatment processes (STPs) and is not suitable for drinking water treatment due to its slow reduction rates and incomplete decomposition (111). There are some studies on the degradation of E2 with other natural estrogens in activated sludge in waste water treatment that show the removal dependency to HRT of biodegradation and also the effectiveness of different microorganisms that are summarized in Table 10.

According to the study by Slater (3) on the removal of E2 and E1 in a traditional WWTP, the WWTP reduced 54% of E1 and E2, 27% of the estrogenic activity and 38% of androgenic activity in the waste (Figure 5). The significant reduction occurred in the secondary clarifier, which indicates that E1, E2, the estrogenic activity and the androgenic activity were adsorbed to solids and the removal was through solids settling. Therefore, he concluded that the wastewater processes that reduced total suspended

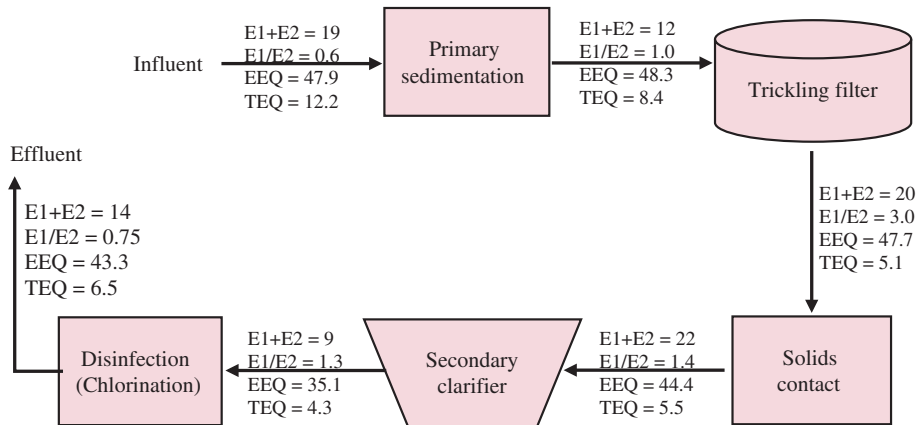


**Figure 4:** The degradation pathway of estrogens by bacteria under aerobic (solid line), anoxic or anaerobic conditions (dashed line) and by algae (dotted line).

Source: Hernandez-Raquet and Combalbert (112).

**Table 10:** Biological degradation in E2 and other estrogens in (activated sludge) in a WWTP.

Operating condition	Organism	Compound	Method analysis	Removal %	Reference
AS of an STP in Yokohama and Tokyo, Japan	<i>Novosphingobium tardaugens</i> (ARI-1)	E1, E2, EE2	GC-MS NMR	E3 and E1 (99%, 40% in ARI-1, 20 days, 10 days) EE2 (0%)	(115)
Biodegradation of E3, E2 and E1 in a STP Roma	Bacteria	E2, E1 and E3	GC-MS/MS	E2 (60%–99% in 14–30 days) E3 (97%), E2 (85%), E1 (61%) in activated sludge treatment plant	(116)
AS and mixed culture aerobic	<i>Ralstonia Pickettii</i> and <i>A. xylooxidans</i>	E2, EE2	HPLC-DAD/FLD	EE2 and E2 (0%, 100% in membrane AS in 3 h)	(117)
AS of three WWTPs, the College station, Houston and Austin	Strain KC8, ARI-1, ammonia-oxidizing bacteria (AOB), amoA genes	E1, E2	GC-MS	EE2 and E2 (Low%, 100% in conventional activated sludge in 7 h) E2 (62%–99%) E1 (100%) in 72 h	(118)
AS in a WWTP in Japan	<i>Rhodococcus zopfii</i> and <i>Rhodococcus equi</i>	E1, E2, E3 and EE2	GC-MS	E2 (80%–99%), E1 (80%–99%), E3 (80%–95%), EE2 (70%–80%) in 1 day	(119)
Photodegradation, Aerobic & Anaerobic degradation in UK river	Not stated	E1, EE2	HPLC	EE2 and E2 (0% and 100% in anaerobic degradation in 28 days) E2 and EE2 (complete in aerobic degradation in 1.2 and 17 days)	(120)
AS in a Rome SWT	Bacteria	E1–E2	LC-MS/Ms	EE2 and E2 (40% in Photolysis) 88% of E2, 74% of E1 HRT: 15–16 days	(43)
Biodegradation in a WWTP in the UK	Not state	E1, E2 and E3	LC/ESI-MS/MS	E1, E2, E3 78%–80% in 13 days	(106)
Digestion in pilot plants fed mixed sludge from a WWTP	Mesophilic 37 °C or thermophilic 55 °C anaerobic	E1–E2	Not state	E1, E2 85% In 10 days = mesophilic 6 days = thermophilic	(121)
Anaerobic sludge digestion of a WWTP in Canada	Mesophilic anaerobic (35–40 °C)	E1–E2	GC-MS	E1 = 12%, E2 = 63% and total E1 + E2 = 26% in 29 days	(3)



**Figure 5:** In a WWTP, showing concentrations (ng/L) of the total E2 and E1 + E2; ratio of E1 to E2; estradiol equivalent concentration (EEQ); and testosterone equivalent (TEQ).

Source: Slater (3)

solids in the wastewater stream were associated with the reduced total E1 + E2.

In the primary process of the settling tank, 38% of the E1 + E2 was removed, which implies a high reduction. The ratio of E1/E2 was significantly increased in the trickling filter and was perhaps due to biological processes in the trickling filter that degraded some of the more estrogenically potent E2 into the less estrogenic by-product, E1. The increase in total E1 + E2 was also likely due to their release from particulate compounds and suspension in higher solid contents and occurred more in the trickling filter and solids contact effluents.

The next process in the secondary clarifier removed 59% of the E1 + E2 from the trickling filter/solids contact and was the most effective process for removing the target E1 + E2 from the wastewater. In total, the wastewater plant removed 69% of the E2, the most potent estrogen, by solids removal (the primary mechanism) and biodegradation.

In the final process, chlorination increased the E1 + E2 concentrations, it seems by releasing them from the solid phase and the converting/degrading of non-target compounds by oxidation as well as increasing estrogenic and androgenic activity (3).

### Removal of estrogen in tertiary section by advanced processes

Recently, after the secondary treatment, which in traditional treatments was the endpoint of WWTPs, a wide range of water treatment technologies have been successfully used to remove steroid estrogens in the WWT process. The technologies include physical treatments such as coagulation, activated carbon and membrane

separation or chemical treatments identical to advanced oxidation processes (AOPs). Although these advanced treatment techniques will surely reduce the discharges of these contaminants, they represent a large financial cost in addition to environmental impact caused by increasing energy consumption and CO<sub>2</sub> emissions. Some of the AOPs employed to remove estrogens in the wastewater are presented in this section (111).

### Physical function

#### Activated carbon

Activated granule carbon is used as a sorbent in WWT for removing tastes and odors that are produced from organic compounds in drinking water; it can also remove organic contaminants (122). Activated carbon easily removes estrogen compounds by sorption both from the surface and within the granules or substrate matrices. The effectiveness of activated carbon is affected by the surface area, surface pH, porosity and surface charge (123). The hydrophobicity of estrogenic compounds, more commonly called the octanol-water coefficient (Log K<sub>ow</sub>), determines which and how much of a compound will adsorb to the substrate (106). In fact, activated carbon effectively removes estrogenic compounds from wastewater. However, some physical factors such as high levels of the organic component in wastewater can reduce the capacity of the target compound and the efficiency by competing for sorption sites and preventing access to openings due to pore blockages within the structure. It has been found that the reduction amount of adsorbed E2 was about 1000 in municipal STW and river water by the large amount of copresent substances that compete with E2 for adsorption

(111, 123). The saturation and inactivation of the binding sites are serious disadvantages and concerns for treatment efficiency using activated carbon because it must be replaced to promote continued removal once these occur, and it is an expensive process. Domestic and industrial surfactants affect the performance of activated carbon (AC) because they produce negative effects on the adsorption capacity of the AC for estrogens; when the concentration of surfactants increases, the E1 adsorption rapidly decreases. Because surfactants are a good solvent of sparingly soluble organic compounds, they have an effect on the separation of such compounds (124). Thus, even though it is effective at reducing EDC concentrations in wastewater effluent, activated carbon is not an economically feasible option for many WWT facilities.

Biologically active carbon (BAC) is a granular activated carbon (GAC) that is covered with a biofilm. The dual mechanisms of microbial degradation and GAC adsorption make BAC filter a potential water treatment technology to remove low-level estrogens during municipal water treatment. BAC filters performed better than GAC to remove intermittently spiked E2 in water. To assess and effectively treat estrogen-contaminated water, BAC filter should operate under a continuous flow mode and be monitored and controlled to remove estrogen that is usually present in the influent. The removal efficiency of BAC filters should be systematic, and experiments have shown that GAC has a higher adsorption capacity and efficacy to remove E1 than E2 (57).

### Membranes

Membrane efficiencies for removing micropollutants depend on compound sizes, the chemical conditions of the influent and the membrane material. Removal of estrogen by membrane size exclusion is important, and with tight and small pore-size membranes [nanofiltration (NF) and reverse osmosis (RO)], up to 90% removal can be achieved. In contrast, large pore-size membranes [ultrafiltration (UF) and microfiltration] do not perform well. An investigation of the removal of 52 steroid estrogens by applying UF and NF membranes revealed that most estrogens are retained on the NF membranes due to both size exclusion and hydrophobic adsorption, whereas the UF membrane typically retained only hydrophobic estrogen due to mainly hydrophobic adsorption (111, 125). The important role adsorption plays in removing estrogen and many researchers have detected significant concentrations of accumulated E1 on hydrophobic hollow fiber microfiltration membranes. It results in lower retention with a potential breakthrough. The nonpolar

and hydrophobic nature of estrogens indicates that these compounds will potentially absorb onto sediments and sludge. Although estrogens and most organic micropollutants have small molecular sizes in the range from 150 to 500 Da, only those compounds that join and form with particles or colloidal organic matter will be physically removed during UF and MF (111).

NF and RO technologies are too expensive and produce a concentrated reject stream that needs further treatment. Moreover, fouling is a disadvantage of the membranes, which causes insufficient filtering and is less efficient, and the need for regular cleaning arises. Several studies have undertaken to reduce fouling (126). However, this still does not solve the problems of removing EDCs by membranes, and these processes only transfer EDCs from one source to another, such as from the aqueous area to the solid phase. Adams et al. (127) stated that NF and RO systems are usually not an economical option at WWPTs, which would imply that they are not expected to be economical at STW plants either.

### Chemical coagulants

Chemical coagulants such as aluminum and ferric salts are being used to remove organic compounds. An investigation that compared common adsorbents used in the WWT industry found that MIEX<sup>®</sup> and FeCl (applying resin to remove natural organic material by ion exchange) are not very suitable or used for removing the most of the contaminants (111). Bodzek and Dudziak (128) found that polyaluminum chloride was the most suitable and reliable coagulant for removing natural and steroidal sex hormones, and removal efficiencies more than 30% were observed for E3, diethylstilbestrol and mestranol. However, ferric sulfate used as a coagulant was shown to be less efficient for removing and degrading estrogens, with the exception of E2, where the removal rate was same as that achieved with a polyaluminum chloride coagulant. In addition, aluminum sulfate has been shown to negligibly remove E1 and E2 in WWT (129).

The removal of EDCs is minimal during coagulation because, in the previous process, the large and hydrophobically favorable matter is removed. Another investigation has also shown that the removal efficiency is poor (18%) for estrogens in STWs with chemical precipitation using ferric or aluminum salts without biological treatment. Furthermore, the use of coagulants, such as aluminum and ferric salts, is usually impractical due to high costs and the frequent environmental impact issues (111).



## Advanced oxidation process

### Ozone

Ozone ( $O_3$ ) has been effectively used as a disinfectant and as an oxidant. This process uses a strong oxidant ( $O_3$ ) to treat or chemically convert estrogenic compounds into less harmful materials that pose little risk to the environment (122). During the conversion, free oxygen radicals are formed; this highly reactive radical is short-lived. The level of degradation that can be obtained with ozonation is related to pH, water chemistry and temperature. For EE2, E1 and E2 compounds, an  $O_3$  exposure of  $2 \times 10^3$  mg-min/L could achieve more than 95% removal efficiency (130).

Huber et al. (131) investigated the use of  $O_3$  to degrade organic compounds and hormones in wastewater effluent from an activated sludge unit and from a membrane reactor. The effective concentration of ozone for the transformation of the organic compounds was 2 mg  $O_3$ /L. The advantages of  $O_3$  include its effectiveness over a wide pH range, its strong oxidizing potential over a short reaction time and the lack of toxic by-products from  $O_3$  splitting after treatment.  $O_3$  treatment can be very costly due to equipment and maintenance expenses. The impact of transformed by-products is still being evaluated (58). Because of the selective nature of ozone, it requires coupling with other AOPs, such as UV/ $H_2O_2$ ,  $O_3$ / $H_2O_2$  and  $O_3$ /UV (105). Sarkar (105) also investigated the removal of estrogen using AOPs technologies and reported that applying  $O_3$  degraded more than 90% of the E1, as well as 99% of the estrogenicity at a concentration of 0.3 mg/L during less than 14 min in a sample of wastewater. He concluded it was the cheapest technology when compared to other AOPs.

### Ultraviolet degradation

High energy ultraviolet (UV) light has been used widely for the microbial disinfection of water and wastewater and can degrade estrogenic compounds via direct photolysis. For UV treatment to be effective, the compound must be a chromophore (i.e. capable of absorbing light energy), and the energy of the light must be adequate to break chemical bonds in the compound structure. UV degradation of estrogenic compounds is mostly used in drinking water treatment and is very rarely used in WWT due to the complexity of the wastewater matrix and the effluent that contain higher levels of organic compounds and other light-scattering and absorbing constituents that reduce the treatment efficacy. In drinking water, a monochromatic low-pressure lamp and a polychromatic medium pressure UV lamp had limited success in reducing E2

and EE2. Even in water with lower turbidity, the limited removal (<20%) of EE2 and E2 was aided by UV photo transformation. Another study proposed that filtration to reduce organic carbon content before the application of  $O_3$  as a tertiary treatment would increase treatment efficacy and reduce UV treatment costs to reasonable levels; this design could be successful in reducing the concentrations of E1 and E2 released into the aquatic environment (47, 132). Sarkar (105) demonstrated that UV with  $O_3$  was more effective than using  $O_3$  only. Applying both UV and  $O_3$  decreased  $O_3$  consumption and transformation times, and it was shown that EE2 and E2 were more effectively degraded using UV/ $H_2O_2$  advanced oxidation compared to direct UV photolysis treatment.

### Manganese oxide

The high dependency of the reaction rate is related to the effect of pH on the adsorption of E2 to the oxide surface and on the electron-transfer reaction. The ideal pH for the reaction is pH=4. The effect of  $MnO_2$  on the reaction was investigated by Jiang and co-workers (133), who found that the best ratio of E2 :  $MnO_2$  was 1 : 100, and if the concentration of  $MnO_2$  was increased or decreased, the effect was lessened. Thus, an ideal  $MnO_2$  concentration should be chosen for any contaminant concentration. It was found that during an 8-h reaction at a pH=6.8 and with a ratio E2 :  $MnO_2$ =100, the oxidation was approximately more than 80%, and there was more than 95% oxidation at 15 min with a pH=4. Therefore, this advanced treatment technique will be cost effective in the long run. In addition, the estrogen activity of by-products should be tested (111).

### Ferrate

Ferrate [Fe (VI)] in the form of salts, usually potassium ferrate ( $K_2FeO_4$ ), in WWTPs is often considered as an alternative oxidant in WWT because it can be used in a dual process of coagulating and oxidizing. In acidic conditions (pH < 2), the redox potentials of ferrate (VI) ions are greater than  $O_3$ , and they are able to oxidize amines, phenol and alcohols. To remove estrogens in real wastewater, a higher dose of Fe (VI) was required because competition for these ions occurred between the organics and estrogens. For example, to remove 99% estrogen in a wastewater with DOC 5.3 mg/L, more than 1 mg/L ferrate was needed; whereas for another sample with DOC 1.6 mg/L and the same concentration of E1 and EE2, only 0.5 mg/L was used. Nevertheless, there is potential for this chemical at low doses (mg/L) to degrade EE2 and E2 in both wastewater and natural water that contain high concentrations of natural organic matter. However, at that concentration in

WWT, a tertiary reactor is needed to separate the Fe ions after use and prior to discharge into the aqueous environment (134, 135).

### Chlorination

Chlorination has observed widespread use as a water oxidation and disinfection agent to reduce inorganic species such as S(II), Fe(II) and Mn(II) in municipal water and sewage WWT processes. However, disinfection byproducts (DBP) are often generated, and some of these have mutagenic and carcinogenic properties such as 4-chloro-EE2 and 4-chloro-E2 (136). Due to EU regulations and policy the conservation of aquatic wildlife in the environment, chlorination is only applied as a disinfection agent to drinking water supplies and not to the discharge of wastewater effluent (111).

In a study by Hu et al. (137), an E2 solution was chlorinated for different times (0, 10, 30, 60, 120 and 180 min) and exhibited  $\beta$ -galactosidase activity, which indicates estrogen activity, as shown in Figure 6. Although the maximum  $\beta$ -galactosidase activities appeared by the chlorinated sample at 10, 30 and 60 min were similar or slightly lower than those before chlorination, the activities of the chlorinated solutions at 120 and 180 min were approximately 40% of those before chlorination.

### Titanium dioxide (TiO<sub>2</sub>)

TiO<sub>2</sub> is the most favorable photocatalyst due to its high photocatalytic efficiency. In addition, it is commercially available in various crystalline forms and particle characteristics and is photochemically stable. TiO<sub>2</sub> photocatalysis has been employed to degrade a mixture of E1 and E2

in model aqueous solutions to study the effects of operating conditions (i.e. radiation wavelength, pH, catalyst and H<sub>2</sub>O<sub>2</sub> concentration) on its kinetics (138). However, it has been shown that the by-products of the degradation still have estrogenicity. Frontistis and co-workers (139) showed that a greater than 90% degradation of 100  $\mu$ g/L of single compound E1, E2 and EE2 was achieved by 250 mg/L TiO<sub>2</sub> during 90 min in wastewater. However, they also showed that the by-product of the degradation still presented estrogenicity.

### Fenton

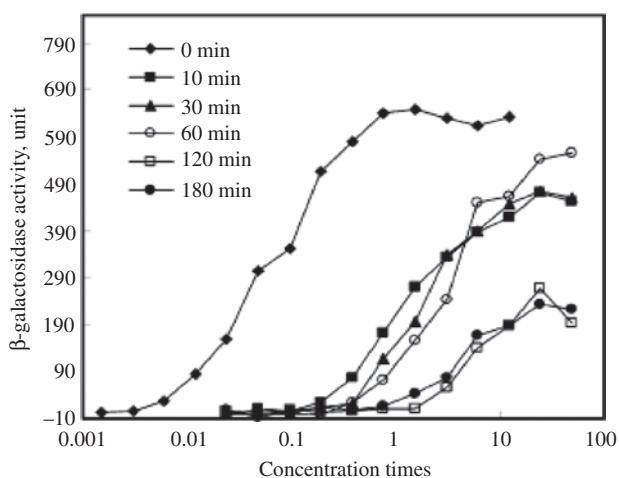
Fenton, or FeOOH-coated resin ( $\alpha$ -FeOOHR), has been made and used for the photodegradation of natural estrogen in the presence of H<sub>2</sub>O<sub>2</sub> under weak UV irradiation. The continuing loading of ferric oxide on resin was achieved by in situ hydrolysis of Fe<sup>3+</sup> in alkaline solution. The Fenton process has its own unique advantages as an oxidizing process because its reagents are inexpensive, environmentally benign and relatively easy to transport and handle. The homogeneous Fenton process is efficient only in the pH range of 2–4 and is usually most efficient at approximately 2.5–3; however, it is rather inefficient in the pH range of most natural waters (pH 5–9) (139).

Yaping and co-workers (140) have shown that Fenton catalysts under weak UV illumination (0.3 mW/cm<sup>2</sup>) and H<sub>2</sub>O<sub>2</sub> can degrade E2 approximately 90% during 8 h with [E2]: 272 mg/L; [H<sub>2</sub>O<sub>2</sub>]: 9.7 mmol/L; [goethite]: 1.43 g/L (0.5 g Fe<sup>3+</sup>/L); and [ $\alpha$ -FeOOHR]: 5 g/L (0.5 g Fe<sup>3+</sup>/L). For a pH=3, a 90% degradation in E2 occurred in 3 h, and the degradation in the dark Fenton test under the same conditions was approximately 30%.

Naimi and Bellakhal applied an Electro-Fenton treatment with acetonitrile-water (30/70, v/v), E2=5 mg/L, Fe<sup>2+</sup>=0.1 mM, Na<sub>2</sub>SO<sub>4</sub>: 50 mM, V: 250 mL, I: 200 mA, V: 250 mL and pH=3 to achieve a greater than 90% degradation in more than 15 min; interestingly, when they added 5 times more catalysts, the degradation rate drastically decreased. They explained that the excess ferrous ions were able to react with  $\cdot$ OH, leading to utilize the hydroxyl radicals with a high oxidative potential, causing a decrease in the ferric reduction efficiency, which affected the rate of hormone degradation during the Electro-Fenton process.

### Sonolysis

Most of the AOPs generate highly reactive and nonselective hydroxyl radicals ( $\cdot$ OH), which are capable of oxidizing nearly all toxic organic pollutions and nonbiodegradable compounds. Among the AOPs producing  $\cdot$ OH, ultrasound is a new method in which water molecules undergo



**Figure 6:** Dose-response curve of aqueous chlorinated E2 at various reaction times.

molecular fragmentation and release  $\cdot\text{OH}$  owing to high-frequency acoustic cavitation. For many years, ultrasound has been used extensively for the degradation/removal of organic compounds from water or wastewater. The main disadvantage of ultrasound is the insufficient production of  $\cdot\text{OH}$ . Thus, water sonolysis is often used with other AOPs, such as  $\text{H}_2\text{O}_2$ ,  $\text{O}_2$  for developing hybrid techniques and potential to increase the degradation efficiency and also to lower treatment time (141–143). The advantages and disadvantages of several treatment technologies for estrogen compounds are shown in Table 11.

## Cost of treatment by AOPs

Sarkar (105) has estimated the cost of the removal of E1 by some AOP technologies. According to that review and the almost similar structure and activity of E1 and E2, E1 is an E2 by-product in aerobic degradation. Furthermore, with regard to the results of a study by Pereira et al. (147), which showed that the formation by-products of the oxidation of E1 and E2 by ozonation follow the same degradation pathway because their corresponding by-products, like E1 and E2, differ by two mass units, the data for the removal cost of E1 may be useful to also estimate the cost of removing E2 using AOP technologies. The result for the costing of AOP technologies for removing 90% of E1 in wastewater in a WWTP influent at 1000 L/min and an E1 concentration = 1  $\mu\text{g/L}$ , with  $\text{pH} = 6.5$ , implies high capital costs. Assuming that the total operating cost was in Operations and Maintenance (O&M) and amortized, the cost was estimated for removing 90% of E1 at an initial concentration of 1  $\mu\text{g/L}$ , while assuming that the wastewater flow rate in the plant was 1000 L/min. The required time was calculated as  $t_{90} = 13.54$  min. In the experiment,  $\text{H}_2\text{O}_2$  was used at concentrations of 20, 40 and 60 ppm/L, and the  $\text{O}_3$  concentration was applied at 0.3, 0.56 and 1.31 mg/L with 254 nm UV radiation; the results were that ozonation was the best option for the removal of both E1 and estrogenicity (99%) compared with other tested technologies and that UV system had the lowest efficiency. Table 12 shows the cost estimation of the AOPs for degrading E1 that were tested in the experiments.

## Commission implementing decision 2015/495

On August 12, 2013, Directive 2013/39/EU of the European Parliament and Council was adopted, and that document

partially replaced the provisional directives regarding priority substances in the area of aquatic policy. Three substances, diclofenac (DCF), EE2 and E2, as well as E1, a breakdown product of E2, were included for the first time in the watch list. The change was caused by considerations of the newest scientific knowledge and reports on the potentially harmful effects of those extant substances in the water. The proposed levels at which they should be monitored are exceptionally low: 10 ng/L for (DCF), 0.4 ng/L for E2 and E1 and 0.035 ng/L for EE2. According to the European Commission Implementing Decision 2015/495 on 20 March, 2015, the agency shall monitor each substance included on the watch list in Table 13 at a minimum of four selected representative monitoring stations over a minimum of 12-month period, and the monitoring period should commence on or before 14 September, 2015 (148). The author indicated here that the capability of many laboratories to measure those levels is not proven, and the cost of these measurements will be significant.

## Conclusions and recommendations for future works

In summary, this study was intended to identify the harmful effects of E2 on aqueous wild animals and humans. The adverse effects are now clear in low mass body animals in surface waters, which, in most countries, are contaminated with E2 compounds. However, the special effects on humans, such as when a pregnant woman or human infant is exposed to E2 through their diets, has not yet been completely recognized because the effects require time to manifest themselves and only appear in adulthood. Because the lifespans of fish are short and the mechanisms of the exposure to estrogen are clear, the influence of estrogen on fish bodies is easily detectable from larvae to adult fish. Because the effect on fish is feminization, even as little as 2 ng/L of a concentration of E2 over some weeks has received the same attention as the impact on human infants at higher doses and for longer exposure. However, even a small change in behavior has a worse effect on human cultures and reproduction. This is the case despite some reports on the adverse effects previously mentioned. Perhaps there is more evidence in the changing behaviors of men in South Asia due to the contamination of water and food by E2. Concerning water, at least regarding this review, surely it can be stated that it is contaminated by E2 at high levels in most areas.

**Table 11:** Advantages and disadvantages of treatment processes in WWTPs for estrogen compounds.

Treatment	Technique	Advantage	Disadvantage
Physical Treatment	a) Adsorption and sorption	<ul style="list-style-type: none"> <li>– Low removing EDCs, which has a high octanol-water coefficient, same as an estrogen (144)</li> <li>– Non-destructive removal technology</li> <li>– Low treatment costs by regenerated and reused MIPs</li> <li>– Strength against acid, base and organic and resistance to high T, P</li> <li>– Removal selectivity for large variety of pollutants (144)</li> <li>– Removing very low conc.</li> <li>– Able to remove micro-pollutant waste and smaller particles: microbes</li> <li>– Very Less sorption of micro-pollutants during the aged of MBR sludge (144)</li> <li>– Higher adsorption capacities</li> <li>– Suitable to remove organic chemical and heavy metals (144)</li> <li>– Removal efficiency of estrogen is very high because of the small-sized contaminants (144)</li> </ul>	<ul style="list-style-type: none"> <li>– Waste generation of used AC</li> <li>– Removal efficiency is low for estrogen</li> <li>– High O&amp;M of AC</li> <li>– Effect of organic matter on removal, and it is a non-selective removal of chemical compounds (144)</li> <li>– Cause hormones binding to the receptors</li> <li>– Waste generation (144)</li> </ul>
	i. GAC		
	ii. Powder activated carbon (PAC)		
	iii. AC		
b) Adsorption with MIP (molecularly imprinted polymers)	iv. Biomass waste		
c) Membrane	i. Membrane bioreactor (MBR)		
	ii. Reverse osmosis		
d) CNTs (carbon nanotube)	i. Multi-walled carbon nanotube		
	ii. Single-walled carbon nanotubes		
	e) Filtration (UF, NF)		
	i. Ultrafiltration		
	ii. Nanofiltration		
Biological treatment	a) Activated sludge (AS)		
	i. Tricking filter and AS		
	ii. Bacteria		
Chemical treatment	iii. Photodegradation		
	a) Chemical addition		
	i. MIEX		
	ii. Ferric chloride coagulant (FeCl <sub>3</sub> )		

Table 11 (continued)

Treatment	Technique	Advantage	Disadvantage
Tertiary treatment	a) Advance oxidation processes (AOPs) i. Ozonation,  ii. MnO <sub>2</sub> iii. Ferrate [Fe(IV)]  iv. Ultraviolet (UV) photolysis reactions (Fenton or TiO <sub>2</sub> )  v. Electrochemical  vi. Chlorination  vii. Sonolysis	<ul style="list-style-type: none"> <li>- Degrade almost all organic contaminants by producing hydroxyl radicals (-OH)</li> <li>- High ability to remove recalcitrant compounds, such as estrogens (144)</li> <li>- High removal of estrogen (144)</li> <li>- More effective and less detrimental than existing oxidative technologies such as ClO<sub>2</sub>, Chloramination and MnO<sub>2</sub></li> <li>- Not costly and no expertise required to operate comparing to ozone and ClO<sub>2</sub> (144)</li> <li>- UV + O<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> degrades E1 efficiently in a short time (105)</li> <li>- Photo-Fenton is used for commercial applications</li> <li>- Photo-TiO<sub>2</sub> is cheap and commercially available</li> <li>- Fast and complete degradation by photocatalyst (142, 143)</li> <li>- Electro-Fenton (Fe II) can be used for WWT (146)</li> <li>- Rapid and economical process to remove estrogen in aqueous solution (136, 137)</li> <li>- No requirement to use added catalysts or chemicals</li> <li>- Simple ultrasonic transducer is sufficient for the complete degradation</li> <li>- Effective mass transfer (143)</li> </ul>	<ul style="list-style-type: none"> <li>- Environmental risk due to the residual oxide from present estrogenic activity (144)</li> <li>- High costs and rarely used for large-scale WWTPs</li> <li>- Produce carcinogenic and mutagenic by-products (144)</li> <li>- Expensive raw material and utilize high dose Fe(IV) ion (135)</li> <li>- Too expensive at WWTP scale</li> <li>- Fenton Efficiency in pH=3</li> <li>- TiO<sub>2</sub> is used in high dose- High toxicity for plant and human</li> <li>- Need slurry reactor to filter solid</li> <li>- Extra economical issue too</li> <li>- Deactivated by adsorption of particles</li> <li>- Mass transfer limitation (142, 143)</li> <li>- Efficiency in pH=3</li> <li>- Must investigate the by-product</li> <li>- Higher dose of Fenton can have inverse effect of degradation (146)</li> <li>- Use high energy and effect by interference of radical scavengers</li> <li>- Generate chlorinated by-products: carcinogenicity, mutagenicity and estrogenic (136, 137)</li> <li>- Complete mineralization is too long</li> <li>- Insufficient producing OH to oxidize organic compound, more free radical needed, so mostly utilized with other oxidants</li> <li>- Too expensive for WWTP (143, 147)</li> </ul>

**Table 12:** Summary of the cost estimates of various AOPs for the degradation of E1.

Process	Total annual O&M cost (\$)	Amortized annual capital cost (\$)	Total annual operating cost (\$) [O&M+Amortization]	Cost (\$)/1000 gallon	Cost (\$)/gm of E1
UV (254 nm)	887,421.4	299,594.58	1,187,015.98	8.53	0.45
UV/H <sub>2</sub> O <sub>2</sub> (20 mg)	693,994.08	231,124.64	717,118.72	6.65	0.35
UV/H <sub>2</sub> O <sub>2</sub> (40 mg)	626,154.64	207,106.35	833,260.99	5.99	0.32
UV/H <sub>2</sub> O <sub>2</sub> (60 mg)	551,027.47	180,510.96	731,538.43	5.26	0.28
O <sub>3</sub> (1.31 mg)	44,389.86	3149.93	47,539.79	0.34	0.018
O <sub>3</sub> (0.56 mg)	43,874.43	1574.96	45,449.39	0.32	0.017
O <sub>3</sub> (0.3 mg)	43,874.43	1574.96	45,449.39	0.32	0.017
O <sub>3</sub> (0.3 mg)/UV	344,858.8	102,279.00	447,137.8	3.21	0.17
O <sub>3</sub> /H <sub>2</sub> O <sub>2</sub> (20)	44,413.86	3149.93	47,563.79	0.34	0.018
O <sub>3</sub> /UV/H <sub>2</sub> O <sub>2</sub>	294,067.8	84,364.03	378,431.83	2.72	0.14

Source: Sarkar (105).

**Table 13:** Watch list of substances for union-wide monitoring as set out in Article 8b of Directive 2013/39/EU.

Name of substance/group of substances	CAS number <sup>a</sup>	EU number <sup>b</sup>	Indicative analytical method <sup>c,d</sup>	Maximum acceptable method detection limit (ng/L)
17- $\alpha$ - ethinylestradiol (EE2)	57-63-6	200-342-2	Large-volume SPE –LC-MS-MS	0.035
17- $\beta$ -estradiol (E2), Estrone (E1)	50-28-2, 53-16-7	200-023-8	SPE – LC-MS-MS	0.4
Diclofenac	15307-86-5	200-348-5	SPE – LC-MS-MS	10
2-Ethylhexyl 4-methoxycinnamate	5466-77-3	226-775-7	SPE – LC-MS-MS	6000
2,6-Direct-butyl-4-methyphenol	128-37-0	204-881-4	SPE – GC-MS	3160
Methiocarb	2032-65-7	217-991-2	SPE – LC-MS-MS or GC-MS	10
Macrolide antibiotics			SPE – LC-MS-MS	90
Neonicotinoids			SPE – LC-MS-MS	9
Tri-allate	2303-17-5	218-962-7	LLE/SPE – GC-MS or LC-MS-MS	670
Oxadiazon	19666-30-9	243-215-7	LLE/SPE – GC-MS	88

<sup>a</sup>Chemical Abstracts Service. <sup>b</sup>European Union number not available for all substances. <sup>c</sup>To ensure comparability of results from the different Member States, all substances shall be monitored in whole water samples. <sup>d</sup>Extraction methods: LLE, liquid-liquid extraction; SPE, solid-phase extraction. Source: European Union Environmental Objectives (148).

Perhaps the reason can be found in WWTPs or municipal WTPs. The difference in these countries is high precipitation. When the precipitation is high, natural estrogens, especially E2, from animals or plants, enter the water treatment facilities where they cannot be treated sufficiently. E2 then enters through the WTPs or enters surface waters from stormwater runoff. It affects drinking water and food. It requires the attention of scientists and more analysis as soon as possible. However, in the field of water treatment, it is essential to consider different methods for treating water before more problems occur in our environment. The methods should be especially chosen based on the following considerations.

- The capability of many laboratories to measure estrogen in the range of the watch list of European regulation levels has not been demonstrated, at least in developing countries, and the cost of these measurements is significant. It is, therefore, necessary to develop a convenient and inexpensive method to

measure estrogen and estrogen activity (for example, a simple and accurate kit or biological assay) rather than very expensive equipment such as LC-MS/MS, which is also difficult to use.

- Despite economic issues, some technologies should be chosen that have already been checked and that have the ability to treat estrogen and estrogen activity in by-products. These technologies should be used as needed in WTPs, and countries should be informed and encouraged to apply these technologies despite their high cost. New, economical and effective technologies should be developed to treat E2 and estrogen activity in their by-products to standardized levels.
- Researchers working in other fields should attempt to find technologies or methods to degrade estrogen in soils, especially in animal wastes and dry sludge, and even in disposed solid wastes, to control the entry of natural estrogens directly into surface waters by stormwater transport.

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