

## E2, an Aquatic Hazard Worldwide

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Received: June 16, 2016

Accepted: August 8, 2016

Online Published: September 15, 2016

doi:10.5539/jas.v8n10p147

URL: <http://dx.doi.org/10.5539/jas.v8n10p147>

### Abstract

Endocrine disruptors are defined as exogenous agents that alter the function of endocrine system, which in turn, causes adverse health effects in an intact organism or its progeny. Of these compounds, 17 $\beta$ -estradiol is of primary importance, since it is physiologically present in both men and women, as well as, being produced synthetically as a component in some pharmaceutical products. Once it reaches the aquatic environment through domestic sewage, ground water and streams, it makes a serious threat to the aquatic life. The review tackles the biological significance of these compounds as well as the danger that they present to the surrounding environment, areas at which these compounds have been detected worldwide, the methods used in detection and fundamentally significant solutions to get rid of this hazard using different methods such as; the bioremediation process.

**Keywords:** 17 $\beta$ -estradiol (E2), pollution, aquatic organisms, bioremediation

### 1. Introduction

The presence and effect of pharmaceutical compounds in the aquatic ecosystems has been one of the emerging concerns to the environment. One of these EDCs is E2, which has been a matter of delving since the 30s (Cook et al., 1934; Tawfic, 2006). The major sources of aquatic contamination by E2 are excretion from female human bodies and live stocks (Narender & Cindy, 2009), and synthetic estrogenic chemicals. Reaching aquatic systems, it may lead to severe damaging effects to the aquatic organisms' reproductive system, and reduction of the total aquatic reproduction (Versteeg et al., 2005).

#### 1.1 Estrogenic Pharmaceutical Residues

Pharmaceuticals are in fact a group of a large list of rising contaminants that have been detected around the globe and used largely in up to tons per year (Boxall, 2004). They have been found in waste water, surface and groundwater, and drinking water. The most familiar source by which these compounds bust in the environment is via treated and untreated wastewater, or via urban or agricultural runoff (Shane et al., 2011).

The effect of these pharmaceuticals on the environment can be clearly demonstrated through the following examples; very tiny concentrations of 17 $\alpha$ -ethynylestradiol and fluoxetine were able to cause a remarkable decline in the growth rates for *Physa pomilia* snails (Luna et al., 2013), traces of Clotrimazole that are similar to those present in nature rendered the algal 14 $\alpha$ -demethylase unfunctional in laboratory trials (OSPAR-Commission, 2013) and diclofenac in low amounts (1 ug/L) caused deleterious effects on the kidney and intestine of the rainbow trout (Mehinto et al., 2010).

Most organisms that live in the sea have an innate phenomenon called "Smellscape"; where these organisms use the natural chemicals present in the sea for signalling and other functions. As a matter of fact, many pharmaceuticals were found to have a disruptive effect on this process, owing to their structural likeness to the original compounds (Klaschka, 2008).

Generally, pharmaceuticals and compounds derived from personal care products (PCPs), such as antibiotics, caffeine, contraceptives, chemotherapeutics, narcotics and painkillers, are all found in urban streams. In fact, once these compounds are released into the aquatic environment, they are diluted, to solids, degraded biologically or by photolysis. Some compounds can persist and can be available in drinking water even after treatment.

For instance, different groups of pharmaceuticals were detected in urban waste water in Spain (Gracia-Lor et al., 2012). It was also found that even when wastewater is being treated by waste water treatment plants (WWTP), there is a high possibility of potential transport of the compounds, as well as other organic wastewater compounds, to the groundwater and streams.

Different EDCs were added by the Environmental Protection Agency (EPA) to limit the levels of pharmaceutical residues in water, but only four of the compounds in the EPA list were pharmaceuticals, three of them belong to birth contraceptives and one is an antibiotic (EPA, 2009).

Natural estrogens exist naturally in human and animal bodies in certain amounts. Synthesis of natural estrogens occurs predominantly in the ovary (Yaghjian & Colditz, 2011) in premenopausal women and in peripheral tissues in postmenopausal women (Halm et al., 2004). Part of active estrogens is also manufactured from circulating estrone sulfate or  $17\beta$ -estradiol sulfate as a result of de-conjugation by sulfatase (Chetrite et al., 2000; Ogunleye & Holmes, 2009; Zhou et al., 2011). Local release of biologically active estrogens from conjugates and their further metabolism extend peripheral tissues' response to estrogen (Sawssan et al., 2015).

Unlike synthesized estrogens, natural estrogens remain in the blood for a short time; maximum few hours, then they are broken down in the liver by enzymes and are either extracted or used to build up molecules thereafter (Katie, 2008). On the other hand, synthesized estrogens are more stable in the bodies and take longer time to breakdown. So, they are emanated daily in urine and feces by fish, homo sapiens, and wild animals and then accumulated in domestic sewages (Kolodziej et al., 2003).

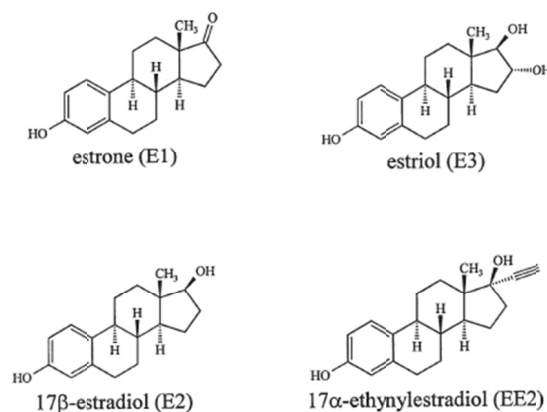


Figure 1. Structure of estrogens (Kuch & Ballschmiter, 2000)

There are 3 types of these estrogens which are commonly used in application for medicinal use, also known as bio-identical hormone replacement therapy (Holtorf, 2009). These types are estrone (E1), estradiol (E2) and estriol (E3) as shown in Figure 1. Estrone and estradiol are the two main types which cause most of the health problems associated with estrogen use.

It is worth noting that pharmaceutical residues are one of the dangerous residues which have not received proper attention in developing countries like Egypt for instance. Infact, Egypt heavily commercialized these medical compounds in early eighties, mainly due to the massive increase of population and the limited natural resources which is currently over 91 million citizens (CAPMAS, 2016) Unlike the case in other countries, only very few people in Egypt didn't use any contraceptive method in spite of sexual exposure and an expressed desire to avoid pregnancy (Sultan et al., 2010).

### 1.2 Role of E2

E2 is one of the powerful natural estrogens. It is found in men and women. It is believed to have a role in physiological activities and the reproductive process. For instance, E2 as well as other estrogens are responsible for the stimulation of the sprouting of sex organs and the development of secondary sexual characteristics, and they also influence the gonadotropin production (Chimento et al., 2014). In addition, estradiol concentration can be affected by equine chorionic gonadotropin (eCG) (Fu et al., 2014).

In female organisms, E2 is produced basically by the developing follicle of the ovary. But in male organisms, the role of E2 is not fully clear though it appears to be indulged in the control of gonadotropin secretion (Haring et al., 2012). E2 was believed to have an inhibitory effect on the pituitary gonadotropin hormone either by direct inhibition or by indirect inhibition of the Gonadotropin-releasing-hormone (GnRH) (Sandeep et al., 2011; Ten Kulve et al., 2011).

It was found that the antioxidant activity of E2 and other steroid hormones play a role in the neuronal protection in neuronal cells; hence, E2 may have implications for the prevention and treatment of Alzheimer (Xu et al., 2016). The latter indicated that the neuronal protection afforded by E2, and which may help in the prevention of Alzheimer's disease, was estrogen receptor-independent.

Estradiol, as well as, the other estrogens has an important influence on big animals' growth, therefore, it is added to progesterone or testosterone in cattle feeding to boost the cattle's mass.

In plants, E2 and esterone were found to be able to improve chickpea plant growth and play a key role in controlling its biochemical parameters to survive under harsh conditions (Erdal & Dumlupinar, 2011).

## 2. Extraction and Detection of E2

There are different methods to determine E2 and other similar compounds; chemical method used for identification and quantification, and bio analytical method for evaluation of the compounds' activity (Birkett & Lester, 2003) in Table 1. The most common bio monitoring method to identify the exposure of E2 in the aquatic systems is Vitellogenin (Vtg) (Seifert et al., 2003; Navas & Segner, 2006) which is a phospholipoprotein egg yolk protein found in juvenile and male fish (Denslow et al., 1999) and spiggin which is the androgen-induced glue protein in stickleback (Katsiadaki et al., 2002; Hahlbeck et al., 2004).

Analysis of estradiol and other estrogens is commonly achieved by HPLC or GC. However, LC-MS and GC-MS are more accurate techniques that have been used in recent years. Different extraction protocols, using Soxhlet extraction (Petrovic et al., 2001) sonication, supercritical fluid extraction (SFE), accelerated solvent extraction (ASE) (Petrovic et al., 2001) or microwave- assisted extraction (MAE), have been established for these compounds, before application of any of these analysis instruments as shown in Table 1. Generally, a complete chromatographic separation is achieved on a C18 column when applied on HPLC or LC-MS.

Table 1. Summary of common extraction and detection methods of 17 $\beta$ -estradiol

Source	Extraction and analysis technique	Reference
Water	ELISA	(Tanaka et al., 2004)
	Solid phase extraction/ELISA	(Matsumoto et al., 2005)
	molecularly imprinted polymer/HPLC	(Le Noir et al., 2007)
	Gas chromatography-mass spectrometry	(Kosjek et al., 2007)
	Pre concentrated on LiChrolut RP-18 cartridges/(SPE)/LC-MS	(Rodriguez-Mozaz et al., 2004)
	Pre concentration centrifugation/HPLC-UV	(Wang et al., 2006)
	Separation on Zorbax SB-CN/LC-UV	(Havliková et al., 2006)
	SPE with Oasis/(LC-(ESI)MS-MS)	(Pedrouzo et al., 2009)
	EDS-1 cartridge using Aqua Trace Automatic SPE system/LC-MS-MS	(Isobe et al., 2003)
	SPE/LC-ESI-MS	(Rodriguez-Mozaz et al., 2004)
	SPE/ELISA	(Hintemann et al., 2006)
	HPLC fluorescence	(Panter et al., 2006)
	Chip separation utilizing micellarelectrokinetic chromatography (MEKC)	(Collier et al., 2005)
Soil	Soxhlet extraction in methanol/GC-MS	(Petrovic et al., 2001)
	Dionex, Thermo-stated Column Compartment TCC-100/HPLC	(Scherr et al., 2009)
Fish	SEP/non-radioactive HPLC	(Delvoux et al., 2007)
	Pre-column TMS/GC-MS	(Zou et al., 2007)

The advantage of using HPLC over GC is avoiding two steps occur in GC; the enzymatic hydrolysis step and the derivatization step. The enzymatic hydrolysis step is significant in GC for the immunoassay analysis of conjugated

and unconjugated estrogens and progestogens, and the derivatization step that usually precedes a subsequent GC analysis is avoided (Petrovic et al., 2001).

However, the clean-up step for both GC and HPLC techniques is commonly dependent either on solid phase extraction (SPE) or on solid liquid adsorption chromatography in open columns using a miscellany of adsorbents. The most commonly used for column chromatography are modified Silica, Florisil, Alumina and different types of carbon are predominantly used for column chromatography whilst C18, NH<sub>2</sub> or CN modified are more widespread among SPE.

### 3. Global Detected Levels of E2 and Estrogenic Residues

E2 was detected in many locations worldwide; the investigation proved its presence in drinking water is very harmful to humans (Caldwell et al., 2009). Similar contamination was also reported in the west bank, within realm of possibility of pollution by sewage water, and along the Jordan River close to drainage (Barel-Cohen et al., 2006).

Different monitoring studies have detected different levels of a wide range of pharmaceuticals, including hormones, steroids, and antibiotics in soils, surface waters and groundwater (Hirsch et al., 1999; Kolpin et al., 2002). E2 and its derivatives were determined in marine environments, drinking waters and rivers in various streamlined countries even where tremendous safety measures of water and environmental safety are considered. For instance, in India, pharmaceutical residues were detected in the effluent in wastewater treatment plant (Larsson et al., 2007). Also in USA, 12 similar compounds and personal care products (PCPs) have been detected to discharge in the Mississippi river waters in New Orleans, Louisiana (Zhang et al., 2007).

Also, it was detected in the surface waters in Germany, Italy, Netherlands and the United Kingdom with levels ranged between 5.5 and 12 ng/L. The utmost of the reported concentrations so far range from the lowest quantity of a substance that can be detected to around 4 ng/L (Kolodziej et al., 2003). Yet, the metabolite of estradiol, estrone, was detected at concentrations up to 17 ng/L.

Although studies in the UK have did not detect estrogenic compounds in drinking water (Harries et al., 1996; Harries et al., 1997), they were detected in raw domestic sewage discharged into rivers (Desbrow et al., 1998; Rujiralai et al., 2011) and waste water in South Korea in ranges of 1.2-10.7 ng L<sup>-1</sup> (Ra et al., 2011), China (Liu et al., 2011; Lu et al., 2011; Zhou et al., 2011), The Netherlands (Belfroid et al., 2006), Italy (Pojana et al., 2004; Pojana et al., 2007), Germany (Körner et al., 2001; Matsumoto et al., 2005; Hintemann et al., 2006) and was also detected in the drinking water in some parts of USA (Caldwell et al., 2009), as summarized in Table 2.

Table 2. Selected worldwide detected levels of estrogens

Type	Conc.	Source
<b>Fish and Crustaceans</b>	4.57 µg/kg	Japanese mackerel (Zou et al., 2007)
	96 µg/kg	Snails (Elnwishy et al., 2012)
	36 µg/kg	Catfish (Elnwishy et al., 2012)
	Nd	Tilapia (Elnwishy et al., 2012)
	4.63 µg/kg	Crucian (Zou et al., 2007)
	0.08 mg/g	Tilapia (Jiang et al., 2009)
	4.7 µg/kg	Greasy-back shrimp (Zou et al., 2007)
	0.0783 mg/g	Prawn (Jiang et al., 2009)
	26.4-77.1 ng/L	Surface water (Hintemann et al., 2006)
	$4.1 \times 10^3$ ng/L	Sewage (Zhou et al., 2011)
	12 ng/L	Effluent from (STP) (Rujiralai et al., 2011)
	Nd	Waste water (Liu et al., 2011)
	1.2-10.7 ng/L	WWTP (Ra et al., 2011)
	75.2 ng/L	bottled mineral water, Germany (Wagner & Oehlmann, 2009)
	0.8-150 ng/L	Water, Netherlands (Vethaak et al., 2005)
0.06-67 pM	River water, Japan (Matsumoto et al., 2005)	
1-191 ng/L	effluents from sewage treatment plants (Pojana et al., 2004)	
<b>Sediment</b>	200 pg/g	Fresh water sediment (Petrovic et al., 2001)
	0.3 µg/kg	Lake Temsah (Elnwishy et al., 2012)
	0.9-2.6 ng/g	River sediment (Gong et al., 2011)
<b>Big animals</b>	3.1-289 µg/kg	Sediment (Pojana et al., 2004)
	4-28 ng/g	Cattle Manure (Andaluri et al., 2011)
	104-262 µg/kg	Dairy cattle feces (Wei et al., 2011)
	45-926 µg/kg	Beef cattle feces (Wei et al., 2011)
	40 pgm/L	Male bovine (Biddle et al., 2007)
44 pgm/L	Females bovine (Biddle et al., 2007)	

Also, it was detected in prawn and fish (Jiang et al., 2009), Japanese Spanish mackerel *Scomberomorus niphonius*, bivalves and snails (Zou et al., 2007), and in shellfish in France (Lagadic et al., 2007). Estrogens were detected in Llobregat catchment area in Spain in water samples at low levels between 2-5 ng L<sup>-1</sup> (Brix et al., 2010). Furthermore, in Spain, detection was reported in sediments of rivers (Petrovic et al., 2001; Lopez et al., 2002; Gong et al., 2011). Lately, it was detected for the first time in Egypt (Elnwishy et al., 2012).

#### 4. Impact of 17β-Estradiol and Other Estrogens on Aquatic Life

Despite the presence of these synthesized compounds in tiny amounts in the environment, the impact of their traces is very huge on marine animals and on homo-sapiens (Hansen et al., 1998). Synthesized estrogens have been suggested as one of the major groups of substances that cause endocrine disruption in wildlife (Lee & Liu, 2002), yet, there is not much information on the effect of these estrogens on the environment in spite of their ultimate impact on the ecosystem.

These estrogenic substances can affect the oestrous cycle and cause infertility and weak estrogenic activity that would affect the female reproductive tract of fish, rodents and livestock (Burton & Wells, 2002).

The presence of E2 with concentrations even lower than 1 ng/L in the aquatic environment can cause infertility, reduce estrogenic activity of fish females (Burton & Wells, 2002), induce hermaphroditism of aquatic organisms (Mills & Chichester, 2005) and change detectable habits which may change reproductivity of fish (Denslow & Sepúlveda, 2007).

Estrogens, including E2, are transferred to the aquatic life via direct discharge of wastewater into aquatic systems. The application “Agro-industrial effluent for microalgae cultivation used for feeding fish” (Cheunbarn & Cheunbarn, 2015), is also another method to transfer these pollutants to fish. When E2 reaches the marine environment, it may be bio-transformed, bio-concentrated (Lai et al., 2002a) and/or accumulate in marine animals

(Lai et al., 2002b; Gomes et al., 2004) through the food chain. In fact, feeding fish on algae cultured on wastewater can even increase the content of E2 level in fish by 3% (Meng-Umphun, 2009). Eventually, the environmental safety, the health of both marine living organisms and humans might be threatened. The danger of estradiol encouraged EPA to frequently monitor these hormones to the most accurate levels (EPA, 2012).

The estrogenic effects of E2 appear to be the most compelling mechanism of endocrine disruption and affect fish in both fresh water and marine environments (Matthiessen et al., 2002). It leads to improper expression of egg proteins; vitellogenin, and zonaradiata proteins (Knoebl et al., 2004). Furthermore, semantic modulation of secondary sexual structures may be noticed (Kirby et al., 2003). Several effects were found on aquatic creatures, including reproductive effects on Roach (*Rutilus rutilus*) (Jobling et al., 2002), (*Pomatoschistus minus*) (Kirby et al., 2003; Robinson et al., 2004), and sand goby (Kirby et al., 2003). Moreover, disruption of growth hormone and prolactin mRNA expression in the rainbow trout (Elango et al., 2006) and changing vitellogenin level in adult male zebra fish (Andersen et al., 2006) were also reported.

In fact, vitellogenin and cyp19b gene expression in zebra fish, were found to be affected by E2 during early embryogenesis and organogenesis (Wang et al., 2011). Mussels were also tested *in vivo* by being injected E2 in the presence of Chlorpyrifos (a common pesticide). They were found to be subjective by an internal interaction between both of them in the digestive gland (Canesi et al., 2011).

A laboratory study and field surveys were carried out in UK by Allen et al. (1999) on euryhaline flounder (*Platichthys flesus*) to evaluate the presence of biological responses in fish to the amount of estrogens and their alternatives in water, and to discover whether the effects are likely to be harmful to populations. They used yolk protein vitellogenin as an indicator of exposure to estrogens in male fish. They revealed that synthetic estrogens had a decreased effect on the vitellogenin response in *Platichthys flesus* than the freshwater species rainbow trout.

In fact, E2 as well as other estrogens are also able to change immune parameters of fish with functional consequences on their ability to cope with pathogens as reported by (Wenger et al., 2011) on his study on rainbow trout (*Oncorhynchus mykiss*).

Actually, (Denslow & Sepúlveda, 2007) reported that exposure of fish to endocrine disrupting chemicals can affect fish in different ways. Sexually differentiated exposure of fish to endocrine disrupting chemicals causes a clear subsidence in the bioavailability of sex hormones and gonadotropins; this consequently leads to impaired gonadal development, altered reproductive behaviors, and decreased fecundity and fertility.

Also in females, reduction of production of E2 leads to modifications in vitellogenesis. This ends up by detrimental effects on oogenesis and egg quality, ultimately leading to developmental abnormalities, increased embryo and sac fry mortality, and even spawning inhibition. These reproductive changes are usually reversible (activational) with animals. So animals are capable of returning back to normal after the exposure finishes. But exposure during the period of sex differentiation can result in irreversible structural (organizational) changes leading to modified reproductive produce and permanent (irreversible) masculinization or feminization of fish. This effect is expected to be magnified with recycling contaminated water in fish farms. The recycling appears to be appealing owing to its economic efficiency, but unfortunately, (Elnwisy, 2008). It may also lead to higher concentrations of E2 into second rounds of water recycling in farming.

In 1999, a study on Mersey estuary fish revealed that approximately 20% of male fish contained oocytes in their testes, but it was not seen elsewhere in UK (Allen et al., 1999). Actually, in Denmark, investigations on intersex showed that male fish in some areas were affected by contaminated wastewater discharges had intersex percentages between 5 and 11% (Jobling et al., 2002; Bjerregaard et al., 2006).

Therefore, this intersex feature and Vitellogenin changes in fish have been used as biomarkers for estrogenic exposure in the environment (Bjerregaard, 2012).

Also, (Brown et al., 2004) conducted a study, in which they found that E2 disrupts growth hormone and plasma insulin-like growth factor (IGF-I) concentrations in salmon. These effects are ecologically significant for survival of wild salmon even if unveiled to a little concentration. However, regardless of how small the amount of E2 is, aquatic organism would be able to survive though their internal function is not fully normal (Elnwisy et al., 2007). Thus, it was also suggested that it reduces hepatic sensitivity to growth hormone, peripheral production of insulin-like growth factor (Norbeck & Sheridan, 2011) in rainbow fish and vitellogenin induction, and a physiological response consistent with exposure to estrogenic compounds (Barber et al., 2011).

On the other hand, very few reports indicate that treatment of fish with E2 did not cause any change in growth or survival of sword tail fish *Xiphophorus hellerii* (Saeed et al., 2012a) or Green Tiger Barb Fish *Puntius tetrazona* (Saeed et al., 2012b).

Even algae were found to be a host environment for these compounds in higher trophic levels (Mason et al., 1996; Pflugmacher et al., 1999) via bio-concentration and bio-magnification, due to their substantial biomass, extensive range of habitat.

### 5. Impact of E2 and Estrogens on Humans

Estradiol can act as natural estrogens in human bodies by binding to estrogen receptors in the endocrine system (Ra et al., 2011). Some of these chemicals are suspected to cause human infertility or influence the development of children, or harm the reproductive processes (Guillette & Gunderson, 2001). In addition, some of these compounds can accumulate in sediment from where they can exert negative impacts on aquatic food webs (MPCA, 2008).

It is believed that the accumulation of estrogens in a human body has a role in breast carcinogenesis (Yaghjian & Colditz, 2011) and prostate cancer (Giese, 2003; Eliassen & Hankinson, 2008). Estradiol and other estrogens were also reported to increase proliferation of breast epithelium and stroma. Consequently, the chances of mutation increase in rapidly proliferating epithelium (Russo et al., 2002; Russo et al., 2006; Pattarozzi et al., 2008).

High amount of E2 is produced in female bodies via secretion result in production of enormous follicles in women and sudden decline in estrogen level because E2 is secreted from cumulus oophorus, resulting in poor-quality oocytes and embryos, lower fertilization, and higher miscarriage rates (Colakoglu et al., 2011).

It seems that the levels of E2, progesterone, cortisol and DHEA were found to be significantly high in patient of burning mouth syndrome (Kim et al., 2012).

### 6. Degradation and Removal of E2 and Estrogens

The natural estrogens including 17 $\beta$ -estradiol are not a persistent compound and could be degraded by sewage bacteria in aerobic and anaerobic conditions (Lee & Liu, 2002). Therefore, attention was directed towards developing possible methodologies for purification of water via phytoremediation (Imai et al., 2007) and biodegradation (Joss et al., 2004; Takeshi et al., 2004; Scherr et al., 2009) to eliminate these compounds from the water bodies.

Bacteria were the most recommended bioremediation method. In 2002, Fujii et al. (2002) isolated a new *Novosphingobium* species as a 17 $\beta$ -estradiol degrading bacterium from activated sludge in a sewage treatment plant in Japan, and proved that no toxic products were reproduced and accumulated in the medium.

Aerobic and anaerobic experiments made by (Lee & Liu, 2002) on the persistency of 17 $\beta$ -estradiol and its metabolites were intriguing. They used bacteria from activated sludge from a local sewage treatment plant in Canada. Their study depicted that E2 and the metabolites were not enduring and could be rapidly degraded by the used sewage bacteria. No other stable degradation products were noticed as shown in Figure 2.

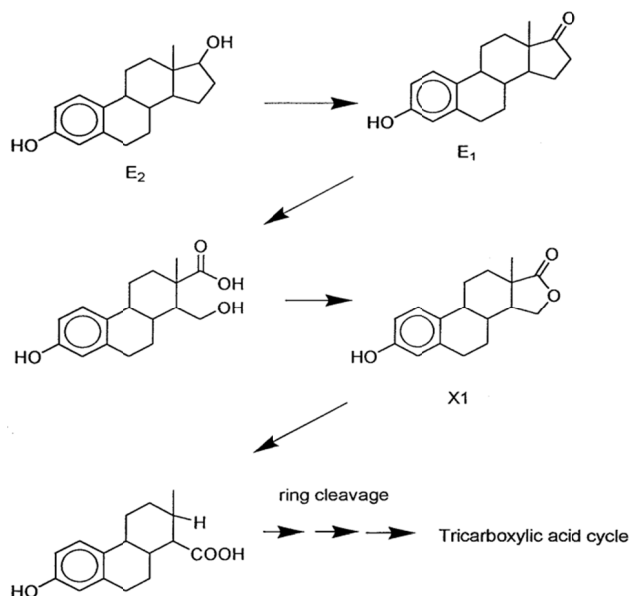


Figure 2. Proposed degradation pathway of estrone (Lee & Liu, 2002)

Different bacteria were discovered to be able to degrade 17 $\beta$ -estradiol successfully. For instance, *Enterobacter* sp. and *Bacillus* sp. were found successful estradiol degrading bacteria, by 57% and 37%, respectively, within 12 hours (Elnwshy et al., 2012). *Rhodococcus zopfii*, could completely degrade 100 mg/L of E<sub>2</sub> plus estrone, estriol, and ethinyl estradiol (Yoshimoto et al., 2004). Actually, *Rhodococcus equi*, and *Rhodococcus zopfii* were both suggested as E<sub>2</sub> degrading capable strains which degrade estradiol within 24 hours (Yoshimoto et al., 2004). Another bacterium (not named) was reported by (Lee & Liu, 2002) to be able to completely degrade 20 mg of E<sub>2</sub> within 18 hours. Many other species were also found efficient such as *Aminobacter*, *Brevundimonas*, *Escherichia*, *Flavobacterium*, *Microbacterium*, *Nocardioidea*, *Rhodococcus*, and *Sphingomonas* (Yu et al., 2007), *Novosphingobium* species (Fujii et al., 2002), ARI-1 and KC8 strains, as indicated by (Roh & Chu, 2010) *Sphingomonas* sp. and *Rhodococcus* (Kurusu et al., 2010). In fact, some bacteria are capable of degrading E<sub>2</sub> as well as estrone, estriol, 16 $\alpha$ -hydroxyestrone, 2-methoxy-estrone, and 2-methoxyestradiol (Lee & Liu, 2002). *Bacillus* spp., isolated from activated sludge, was also found effective in degrading E<sub>2</sub> (Jiang et al., 2010).

In 2004, it was possible to remove estrogens, estrone, 17 $\beta$ -estradiol, and estriol, from the water by nitrifying activated sludge and ammonia-oxidizing bacterium *Nitrosomonas europaea*. In fact, there was a suggestion referring that although E<sub>2</sub> can be degraded to estrone in the presence of nitrifying activated sludge and ammonia-oxidizing bacterium, but this might have been because of other heterotrophic bacteria, not by ammonia-oxidizing bacteria. This suggestion is due to absence of estrone during the degradation intervals (Shi et al., 2004). Later, the same compounds were successfully removed from water when they were treated with continuous flow algae and duckweed ponds (Shi et al., 2010).

Generally, it is not yet clear how E<sub>2</sub> is degraded. Yet, oxidation of E<sub>2</sub> to estrone is accepted to be the first step of the degradation pathway (Christoph & Juliane, 2009). This oxidation was suggested to be initiated in the biodegradation process at the hydroxyl group at C-17 (ring D) of E<sub>2</sub>, leading to the formation of the major metabolite (Lee & Liu, 2002).

It is repeatedly mentioned in studies that oxidation of E<sub>2</sub> to estrone occurs both in complex culture systems, such as activated sludge (Ternes et al., 1999) and also can occur in purified bacterial cultures (Chang-Ping et al., 2007). Nevertheless, the pathways of bacteria-mediated degradation are not yet fully well understood.

It was reported earlier that during the very early stage (1-5 hr.) of E<sub>2</sub> degradation by the culture, unknown metabolite was observed. The frequency of its occurrence was not really as detectable as that of estrone (Lee & Liu, 2002). Later, (Kurusu et al., 2010) conducted a study to identify E<sub>2</sub> metabolites; they incubated resting ED8 cells with E<sub>2</sub> and then put the meta-cleavage inhibitor "3-chlorocatechol" to inhibit benzene ring meta-cleavage, resulting in the accumulation of intermediate degradation products. In 3 hours, they detected the trimethylsilyl derivatives of E<sub>2</sub> and five other metabolites.



In the natural environment, different factors may be involved in the degradation process by bacteria, such as: degradation in the anaerobic river sediments which is more rapid than in the anaerobic marine sediments (Lopez et al., 2002; Tyler et al., 2005; Czajka & Londry, 2006; Christoph & Juliane, 2009), salinity factor that makes E2 more resistant to degradation, or failing of marine microorganism to involve in biodegradation of E2 are all possible factors.

Anaerobic biodegradation is less energy efficient than aerobic biodegradation, so the aerobic degradation of E2 is much faster (Lee & Liu, 2002). After a 22 hours contact of E2 with a culture containing sewage treatment plants, two-thirds of the spiked E2 ( $200 \mu\text{g L}^{-1}$ ) was oxidized to estrone. The spiked  $17\beta$ -estradiol was completely removed within 18 hours.

Activated sludge from wastewater treatment plants were used to isolate two strains of *Rhodococcus* as estrogens degraders (Yoshimoto et al., 2004). Strain *Rhodococcus zopfii* completely and rapidly degraded 100 mg of  $17\beta$ -estradiol, estrone, estriol, and ethinylestradiol/liter, the other was *Rhodococcus equi*, showed degradation activities comparable with *Rhodococcus zopfii*. *Rhodococcus zopfii* showed the highest activity, because it selectively degraded 100 mg/L of E2, even when glucose was used as a readily utilizable carbon source in the culture medium after 24 h.

Later, genera *Aminobacter*, *Brevundimonas*, *Escherichia*, *Flavobacterium*, *Microbacterium*, *Nocardioides*, *Rhodococcus*, and *Sphingomonas* were isolated from activated sludge of a wastewater treatment plant as well (Yu et al., 2007). These bacteria converted E2 to estrone, but only *Brevundimonas* and *Sphingomonas* showed the ability to degrade estrone.

Actually *Sphingomonas* seems to be an effective E2 degrader, thus it was examined and even tested for its genome sequence (Anyi et al., 2011). This step can lead to identifying the gene responsible for E2 degrading or resistance in the future. Also, *Novosphingobium* sp., (ARI-1) and KC8 were able to remove testosterone,  $17\beta$ -estradiol and estrone at the same time from wastewater rapidly when it is grown on complex nutrients containing  $17\beta$ -estradiol (Roh & Chu, 2010).

However, *Novosphingobium* sp. is a common estrogen-degrader which has no ability to degrade estrone after it is grown on a nutrient-estrogen-free medium for 7 days, while strain KC8 would still be able to degrade both  $17\beta$ -estradiol and estrone after growing on the same medium for 15 days.

They also detected concentrations of strain KC8 2-3 times higher than those of strain *Novosphingobium* sp. in the waste water treatment plants, which led them to suggest that strain KC8 is an ever-present strain in waste water treatment plants and might be very significant in estrogen removal.

## 7. Conclusion

The emerging water pollution with  $17\beta$ -estradiol—endocrine disrupting chemical—is present in most of organism in the environment leading to higher concentrations in human bodies. The impact of this pollutant on the environmental ecosystems and human welfare are equally dangerous. Though efforts on removal  $17\beta$ -estradiol have been carried out, further investigations on environmentally friendly alternative component should be considered.

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