



A Review on the Importance of Hormones Monitoring and Their Removal in Conventional Wastewater Treatment Systems

Mohammad Taghi Ghaneian¹, Roya Peirovi^{2,1*}, Ali Asghar Ebrahimi¹

¹ Environmental Science and Technology Research Center, Department of Environmental Health Engineering, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

² Faculty Member of Environmental Health Engineering Department, Gonabad University of Medical Sciences, Gonabad, Iran.

ARTICLE INFO

REVIEW ARTICLE

Article History:

Received: 10 January 2017

Accepted: 22 April 2017

***Corresponding Author:**

Roya Peirovi

Email:

rpeirovi@yahoo.com

Tel:

+989155683814

Keywords:

EDCs,
Gonadal Steroid Hormones,
Hormon Removal,
Wastewater Treatment.

ABSTRACT

Introduction: Micro pollutants have become one of the most important environmental concerns around the world. These natural and synthetic compounds have been called Endocrine Disrupting Chemicals (EDCs) due to their interfere with the normal function of the endocrine system in humans and animals. They include natural and synthetic hormones and their metabolites, surfactant, insecticide, as well as some pharmaceuticals and health care products. Because of these compounds effects, importance of their monitoring in Iran is perceived like other countries.

Materials and Methods: This paper was carried out on the basis of studies accomplished from 2000 to 2017 and also the ones published in databases such as Google Scholar, Elsevier, Scopus, Science direct, Magiran, and SID using hormone removal, micro pollutant removal, removal hormones from wastewater, sex hormones removal, steroidal hormones, hormone removal from wastewater, and removal efficiency of micro pollutants as the keywords. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement was used for selecting articles.

Results: Sexual reproduction of fish exposed to estrogenic compounds was changed. Phytoestrogens exist on a certain strain of clover which caused severe infertility in sheep grazing on them. Some studies have reported that a decrease in men's sperm and breast cancer in women are caused by exposure to estrogenic compounds.

Conclusion: According to the current study, further studies are needed to determine the entry routes of steroid hormones into aquatic environment, the detection techniques and measurements, as well as the best removal method in Iran.

Citation: Ghaneian MT, Peirovi R, Ebrahimi AA. **A Review on the Importance of Hormones Monitoring and their Removal in Conventional Wastewater Treatment Systems.** J Environ Health Sustain Dev. 2017; 2(2): 312-20.

Introduction

For decades, micro-pollutants have become one of the most important environmental concerns all over the world. Micro-pollutants can commonly be found in various concentrations ranging from nano-grams to micro-grams per liter in aquatic

environment¹⁻². Although low concentration of micro-pollutants may be no significant, they can create some problems with water and wastewater treatment processes³⁻⁴. Additionally, these pollutants can enter drinking water resources through different ways, thus dealing with them is

of primary importance due to health issues⁵⁻⁷. These natural and synthetic compounds have attracted special attention due to their interference in normal function of endocrine system in human and animals. All these disrupting compounds are called Endocrine Disrupting Compounds (EDCs), which are referred to emerging pollutants in some literatures⁸⁻⁹. When their concentration is higher than threshold in the environment, they will have adverse effects on humans and other creatures. EDCs mainly include natural hormones, synthetic hormones and their metabolites, non-steroidal hormones, and synthetic compounds which are used as plasticizer, fire retardant, surfactants, insecticides, and some pharmaceuticals^{8,10-11}.

Interference mechanisms in compounds include: imitation of hormones effect, fighting against the effects of hormones, and disruption in hormones synthesis, hormones metabolism and building special hormone receptors. Most of these compounds reacting with hormone receptors are estrogenic compounds and few of them have androgenic or anti-androgenic potentiality. Among these disrupting compounds, hormones resulting from human and animal wastes are often known as steroidal hormones and are distinguished from others by their high estrogenic potential¹²⁻¹⁴.

Endocrine Disrupting Compounds properties

Some of natural and synthetic hormones resulting from human and animal waste act as destructors of endocrine glands. Furthermore, some of these estrogens imitate the compounds of plants. With regard to their origin, these compounds are classified as Natural estrogenic/androgenic hormones: E1, E2, E3, testosterone and others¹⁵, Synthetic hormones: EE2, Diethylstilbestrol, Norethindrone and others, Phytomyco estrogens: Diadzein, Genesis, Zalenon and others¹⁶. Studies showed that EE2 and E2 are estrogenic components with high potentials, followed by E1 and E3^{7, 17-18}. These natural and synthetic hormones have been of great concern, since with their low concentration (in nano-gram per liter range have potential of adverse effects on aquatic beings¹⁹⁻²¹. Natural estrogens have the

same tetra cyclic network including phenol ring, two cyclohexane rings and a ring of cyclopentane. Physical and chemical characteristics of these compounds play a significant role in predicting their fate in natural and engineered environment. Estrogens are not good soluble in water. Octanol - water partition coefficient (Kow) is defined as a proportion of dissolving a compound in octanol and water at a specific temperature under equilibrium conditions. Since dispersion of material between water and other natural environments is always relative, the amount of Kow is appropriate to predict the absorption or dissolution of a compound. The log of Kow estrogenic compounds is in the range of 2.5-4. As a result, their hydrophilicity is moderate and they tend to be broadly distributed in solid environments. A large number of total estrogens produced by body are in the form of conjugated estrogen with their urine. This polar conjugates are biologically inactive and their solubility in water is higher in comparison with compounds that are not conjugate. Despite most excreted hormones by humans are conjugated, studies have shown that most of estrogenic hormones present in the wastewater and effluent are in the form of free estrogens and sulfur estrogens. This indicates that the de-conjugation has happened at this time. De-conjugation can be done by *Escherichia coli* present in human intestinal flora and Beta-glucosidase enzyme²²⁻²⁴.

Hormones' sources and pathways in environment

Hormones can enter the environment through point sources (such as effluents of Water and Wastewater Treatment Plants (WWTPs)) and non-point sources (like agriculture runoff). As mentioned earlier, human and animal wastes are the main source of steroid hormones in aquatic environments. Natural and synthetic hormones and their metabolites finally enter the wastewater treatment plant. Both treated solid and liquid wastes are known as potential pathways for these compounds to enter the environment²⁵⁻²⁷. Three types of natural estrogenic compounds (E1, E2,

and E3) are produced in human body and are all driven from cholesterol. E2 is the first metabolite in pregnant women and has the highest potential and E3 is the metabolite of E1 and E2. An average estrogenic hormone in women feces is two times higher than that of men which can reach to more than 6 mg/day during pregnancy²⁸. In addition to the natural estrogenic compounds, synthetic estrogens used in medicine and are digested, eventually reach to sewage treatment plant through human waste. Synthetic estrogens in EE2 tablets act as the largest distributor of estrogen in wastewater treatment plants^{2, 29}.

Rules

In Europe, efforts to classify and regulate EDCs have started since 1999. In European Union, social strategy has been considered for endocrine destructors to take action against them. Under this strategy, a list consisting of 575 materials has been identified as substances suspected of EDC. In 2006, the law related to their registration, evaluation, guidance, and limitations was accepted. Several hormones are part of EPA Unregulated

Contaminant Monitoring program that include estriol, estrone, estradiol, ethylene estradiol, equilin, androstenedione, and testosterone³⁰.

Materials and Methods

This paper was carried out on the basis of studies accomplished from 2000 to 2017 and also the ones published in databases such as Google Scholar, Elsevier, Scopus, Science direct, Magiran, and SID using hormone removal, micro pollutant removal, removal hormones from wastewater, steroidal hormones, hormone removal from wastewater, and removal efficiency of micro pollutants as the keywords. Selecting articles process is shown in Figure 1. As it can be seen in Figure 1, with removal of unrelated found cases and additional search, 87 cases including reviews: 7, research:71, technical notes: 2, thesis: 1, rapid communication: 1, application note: 1, discussion: 1, rules: 1, final Report: 1, method: 1 were investigated. The above mentioned sources were then classified according to journal's name (Table 1), subjects (Chart 1), and keywords (Chart 2).

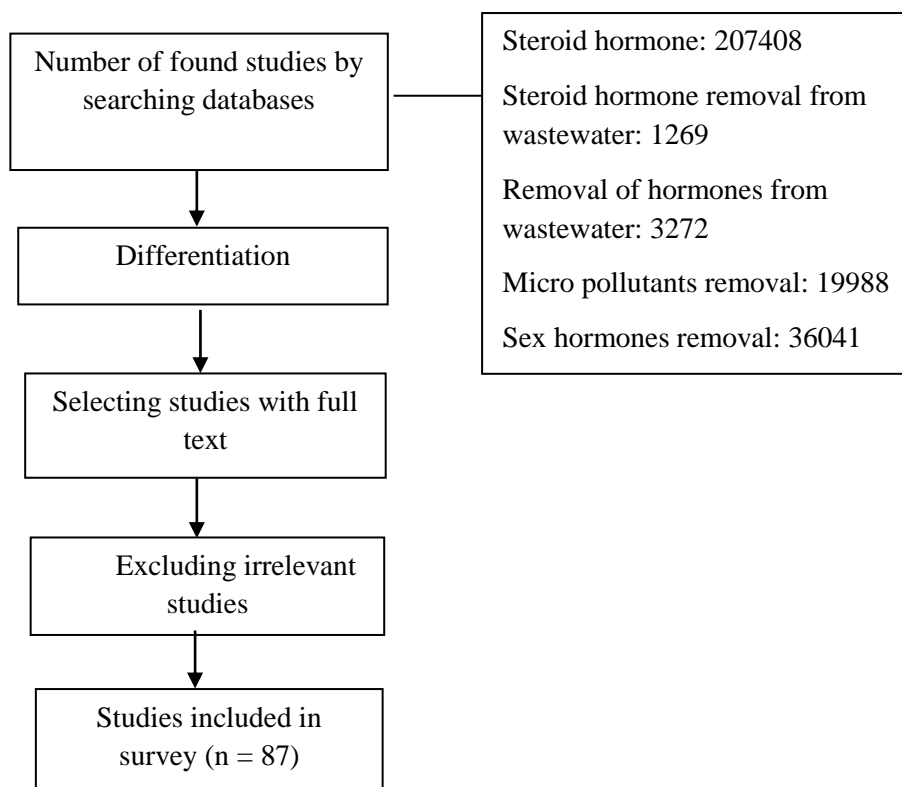


Figure 1: The process of selecting articles for this study

Table 1: Number of articles based on journals' names

Journals' names	NO	Journals' names	NO
Applied Surface Science	1	Journal of Food And Drug Analysis	1
Journal of Chromatography A	9	Journal of Environmental Health Science And Engineering	1
Chemosphere	12	Journal of Environmental Management	1
Science of The Total Environment	20	Aquatic Toxicology	1
Water Research	9	Environmental Research	1
Environmental Pollution	3	Iranian South Medical Journal	1
Journal of Bioscience and Bioengineering	1	International Biodeterioration & Biodegradation	1
Analytical Chemical Acta	2	Desalination	1
Talanta	4	Journal of Pharmaceutical And Biomedical Analysis	1
Trends In Analytical Chemistry	3	Journal of Chromatography B	1
Aquatic Toxicology	2	Journal of Membrane Science	1
Ultrasonics Sonochemistry	1	Journal of Hazardous Materials	2
Chemical Engineering	1	Bioresource Technology	2

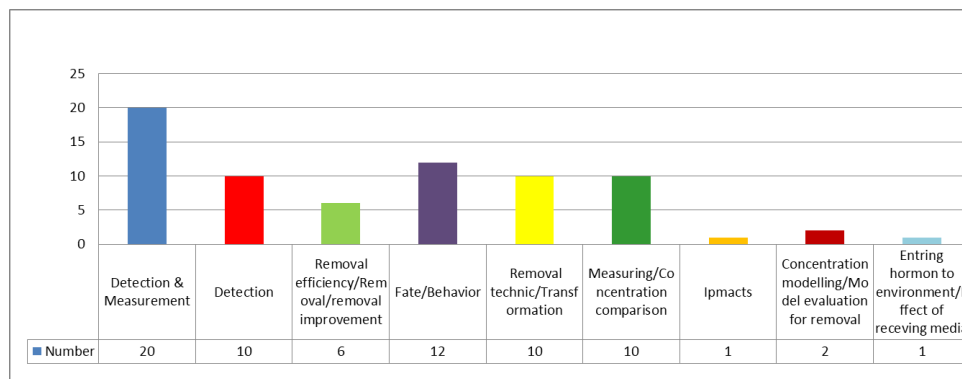


Chart 1: Classification of studies based on their subjects

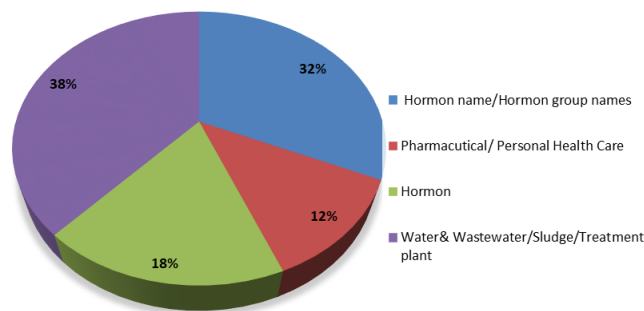


Chart 2: Frequency of keywords in studies

Results

Health and environmental impacts of estrogenic compounds

Health impacts surveys have shown that estrogenic compounds changed the sexual reproduction of fish exposed to them³²⁻³³. Most sexual interferences were observed among males.

High concentrations of EE2 in a lake showed a change of gender from male to female among species exposed to these pollutants. Exposure to EE2 with a concentration of about 9.86 mg/L resulted in reduction of mating among Zebra fish and reduction in female reproduction³². There are Phytoestrogens on a particular species of clover plants that cause infertility disorders in sheep

feeding on them³⁴. In a field study on the increased level of vitellogenin in female painted turtle it was reported that E2 exposure may cause reproductive disorders and change the energy level for survival³⁵. Some studies have shown low sperm count in men, loss of sexual health and breast cancer in women owing to exposure to estrogenic compounds. While other studies rejected this and reported other factors like geographical and cultural differences factors as the most important factors in reducing sperm. Systemic study of health impacts on humans involves many challenges

because the long time between exposures and exacerbate clinical symptom depends on the age and duration of exposure³⁶⁻³⁹.

Estrogenic compounds removal methods

Possible removal methods of human hormones from treatment units include volatility, biological and non-biological degradation, and adsorption^{3, 40-41}. Table 2 shows the effect of each reported method in various studies. Investigating the efficiency of advanced oxidation processes was not the aim of this study.

Table 2: Efficiency comparison of hormones removal methods

Process	Efficiency
Coagulation & flocculation	Non- effective for most of micro pollutants
Adsorption (Activated Carbon)	More effective than coagulation & flocculation
Membranes	
UF	Very low removal (8%) for estradiol, but hydrophobic membranes (80%)
MF,UF	Removal mechanism is adsorption to the polymer membrane surface not by size.
Flat plate NF modules	Up to 60% for some compounds
Combination of RO &MF	Efficiency enhanced 56 to 90% except for Nonylphenol and ibuprofen compounds
MBR	Effective
Attached growth process	Different based on process and compound, generally effective
Volatility	Non- effective
Sorption	Effective

Estrogenic compounds' fate in engineered processes

Although the existing treatment plants are designed based on nitrogen, carbon, and phosphorus removal, partial removal of EDCs has been observed in many cases⁴². However, large differences in removal efficiency have been viewed by many studies. More than 98% removal of natural estrogens (E1 and E2) in Germany⁴³ and more than 61% removal of E1 in similar treatment plants in Italy⁴⁴⁻⁴⁵ were reported by researchers. Different efficiencies emphasize on the importance of location specific geographical parameters in removal of these compounds. Progress of technical

analysis in two decades, along with the development in industry have enabled researchers to study the occurrence and fate of normal and synthetic hormones in treatment plant even in mg/L level^{18, 46}. Removal efficiency of treatment plants regarding three major hormones are listed in Table 3. The conventional treatment plants consist of preliminary, primary, and secondary, they also include tertiary treatment in the case of effluent discharges to ground or surface waters. Although each unit helps the overall performance of the plant, secondary also called biological treatment has an important role in the removal of estrogenic hormones⁴⁷.

Table 3: Comparison of removal efficiencies in activated sludge (AS) system

Author	Process	Hormone	R (%)
Baronti	AS(Italy)	E1	61
		E2	87
		E3	95
		EE2	85
Ternes	AS (Brazil)	E1	83
		E2	99.9
		EE2	78
Similar studies		E1	90
		E3	99

The main reasons for low E1 removal in comparison to other natural estrogens, are E2 to E1 oxidation by microorganisms under aerobic conditions and stability of estrogen conjugations especially sulfate conjugation. In addition to E1, EE2 compounds despite having less concentration than natural estrogen compounds are resistant in wastewater due to their hard degradability nature.

Discussion

Among the biological conventional processes, activated sludge relative to other methods is of greater efficiency in the removal. However, two-stage trickling filter with the final two steps settling can have efficiency equal to activated sludge, while single-stage trickling filter can not^{14, 25, 47}.

Andersen et al. (2003) reported that the synthetic hormone EE2 was detected in the waste activated sludge at a level of around 11 ng/g. Another study was performed in a full-scale WWTP announced that all three estrogens measured (E1, E2, EE2) were present in the activated and excess sludge despite their absence in the final effluent. Furthermore, the concentration of hormones in the activated sludge did not change across the plant despite the extensive treatment (two-step denitrification followed by nitrification with a combined SRT of 12 days)⁴⁸. On the other hand, some researcher declare Conventional activated sludge system with nutrient removal process has shown high capability of estrogen removal. Anderson et al. described a municipal treatment plant with activated sludge systems for nitrification and de-nitrification of natural estrogens up to 98% and synthetic estrogens up to 90%. Natural estrogens decompose in nitrification and de-

nitrification while EE2 only decomposes in nitrification conditions⁴³.

Conclusion

While wastewater effluents is expected to be the major source of estrogen in aquatic environments, surface runoff and livestock sewage may be other sources of these compounds. Removal efficiency varies according to circumstances and type of process. Accuracy of measurement methods is also different. There is a gap in investigating estrogen's fate during the sludge stabilizing, which is mainly due to the problems associated with quantification of very low amounts of these micro-pollutants in sludge. Effect of operational parameters such as temperature was not identified well. Moreover, further research in this field is needed to determine the effects of thermal, mechanical, and chemical pre-treatments applied before aerobic and anaerobic digestion on fate and removal of estrogenic compounds that lead to increase decomposition capability, pathogen removal, and dehydrating of sludge. With regard to this study and its most important results, it can be concluded that further research can be conducted on determining steroidal hormones' entry paths to water resources, their detection and measurement techniques, as well as the best and most economical removal methods in Iran.

Acknowledgements

The authors would like to acknowledge all reviewers.

Funding

The work was not funded.

Conflict of interest

Authors have no competing interests.

This is an Open Access article distributed in accordance with the terms of the Creative Commons Attribution (CC BY 4.0) license, which permits others to distribute, remix, adapt and build upon this work, for commercial use.

References

- Ingrand V, Herry G, Beausse J, et al. Analysis of steroid hormones in effluents of wastewater treatment plants by liquid chromatography–tandem mass spectrometry. *J Chromatogr A*. 2003; 1020(1): 99-104.
- Vymazal J, Březinová T, Koželuh M. Occurrence and removal of estrogens, progesterone and testosterone in three constructed wetlands treating municipal sewage in the Czech Republic. *Sci Total Environ*. 2015; 536: 625-31.
- Luo Y, Guo W, Ngo HH, et al. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Sci Total Environ*. 2014; 473: 619-41.
- Nguyen LN, Hai FI, Yang S, et al. Removal of pharmaceuticals, steroid hormones, phytoestrogens, UV-filters, industrial chemicals and pesticides by *Trametes versicolor*: Role of biosorption and biodegradation. *Int Biodeterior Biodegradation*. 2014; 88: 169-75.
- Alidadi H, Peiravi R, Dehghan AA, et al. Survey of heavy metals concentration in Mashhad drinking water in 2011. *Razi Journal of Medical Sciences*. 2014; 20(116): 27-34.
- Peiravi R, Alidadi H, Dehghan AA, et al. Heavy Metals Concentrations in Mashhad Drinking Water Network. *Zahedan Journal of Research in Medical Sciences*. 2013; 15(9): 74-6.
- Matić I, Grujić S, Jauković Z, et al. Trace analysis of selected hormones and sterols in river sediments by liquid chromatography–atmospheric pressure chemical ionization–tandem mass spectrometry. *J Chromatogr A*. 2014; 1364: 117-27.
- Jasinska EJ, Goss GG, Gillis PL, et al. Assessment of biomarkers for contaminants of emerging concern on aquatic organisms downstream of a municipal wastewater discharge. *Sci Total Environ*. 2015; 530: 140-53.
- Rezaee A, Ghanizadeh G, Yazdanbakhsh A, et al. Removal of endotoxin in water using ozonation process. *Aust J Basic Appl Sci*. 2008; 2: 495-9.
- Peirovi R, Alidadi H, Jamali F, et al. conventional and advanced water treatment processes: Marandiz; 2016; 72,164.
- Virkutyte J, Varma RS, Jegatheesan V. Treatment of Micropollutants in Water and wastewater: IWA; 2010; 165.
- Silva CP, Otero M, Esteves V. Processes for the elimination of estrogenic steroid hormones from water: A review. *Environ Pollut*. 2012; 165: 38-58.
- Shargil D, Gerstl Z, Fine P, et al. Impact of biosolids and wastewater effluent application to agricultural land on steroidal hormone content in lettuce plants. *Sci Total Environ*. 2015; 505: 357-66.
- Hamid H, Eskicioglu C. Fate of estrogenic hormones in wastewater and sludge treatment: A review of properties and analytical detection techniques in sludge matrix. *Water Res*. 2012; 46(18): 5813-33.
- Tan Y, Jing L, Ding Y, et al. A novel double-layer molecularly imprinted polymer film based surface plasmon resonance for determination of testosterone in aqueous media. *Appl Surf Sci*. 2015; 342: 84-91.
- Albero B, Sánchez-Brunete C, Miguel E, et al. Analysis of natural-occurring and synthetic sexual hormones in sludge-amended soils by matrix solid-phase dispersion and isotope dilution gas chromatography–tandem mass spectrometry. *J Chromatogr A*. 2013; 1283: 39-45.
- Folmar LC, Hemmer MJ, Denslow ND, et al. A comparison of the estrogenic potencies of estradiol, ethynylestradiol, diethylstilbestrol, nonylphenol and methoxychlor in vivo and in vitro. *Aquat Toxicol*. 2002; 60(1): 101-10.

18. Azzouz A, Ballesteros E. Combined microwave-assisted extraction and continuous solid-phase extraction prior to gas chromatography–mass spectrometry determination of pharmaceuticals, personal care products and hormones in soils, sediments and sludge. *Sci Total Environ.* 2012; 419: 208-15.
19. Irwin LK, Gray S, Oberdörster E. Vitellogenin induction in painted turtle, *Chrysemys picta*, as a biomarker of exposure to environmental levels of estradiol. *Aquat Toxicol.* 2001; 55(1): 49-60.
20. Andrási N, Molnár B, Dobos B, et al. Determination of steroids in the dissolved and in the suspended phases of wastewater and Danube River samples by gas chromatography, tandem mass spectrometry. *Talanta.* 2013; 115: 367-73.
21. Aufartová J, Mahugo-Santana C, Sosa-Ferrera Z, et al. Determination of steroid hormones in biological and environmental samples using green microextraction techniques: An overview. *Analytica Chimica Acta.* 2011; 704(1–2): 33-46.
22. Liu Z-h, Kanjo Y, Mizutani S. Removal mechanisms for endocrine disrupting compounds (EDCs) in wastewater treatment—physical means, biodegradation, and chemical advanced oxidation: a review. *Sci Total Environ.* 2009; 407(2): 731-48.
23. Jones-Lepp T, Stevens R. Pharmaceuticals and personal care products in biosolids/sewage sludge: the interface between analytical chemistry and regulation. *Anal Bioanal Chem.* 2007; 387(4): 1173-83.
24. Zuo Y, Lin Y. “Solvent effects on the silylation-gas chromatography–mass spectrometric determination of natural and synthetic estrogenic steroid hormones” Comment on “Formation of chlorinated estrones via hypochlorous disinfection of wastewater effluent containing estrone” by Hideyuki Nakamura, Ryoko Kuruto-Niwa, Mitsuo Uchida and Yoshiyasu Terao [*Chemosphere* 66 (2007) 1441–1448]. *Chemosphere.* 2007; 69(7): 1175-6.
25. Carballa M, Omil F, Lema JM. Comparison of predicted and measured concentrations of selected pharmaceuticals, fragrances and hormones in Spanish sewage. *Chemosphere.* 2008; 72(8): 1118-23.
26. Carballa M, Omil F, Lema JM, et al. Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. *Water Res.* 2004; 38(12): 2918-26.
27. Nagarnaik PM, Mills MA, Boulanger B. Concentrations and mass loadings of hormones, alkylphenols, and alkylphenol ethoxylates in healthcare facility wastewaters. *Chemosphere.* 2010; 78(8): 1056-62.
28. Rodriguez-Navas C, Björklund E, Halling-Sørensen B, et al. Biogas final digestive byproduct applied to croplands as fertilizer contains high levels of steroid hormones. *Environ Pollut.* 2013; 180: 368-71.
29. Manickum T, John W. Occurrence, fate and environmental risk assessment of endocrine disrupting compounds at the wastewater treatment works in Pietermaritzburg (South Africa). *Sci Total Environ.* 2014; 468–469: 584-97.
30. Fisher C, Lopez L. Automated Extraction and Determination of Human Hormones in Drinking Water Using Solid-Phase Extraction and HPLC with UV Detection.
31. Phillips P, Schubert C, Argue D, et al. Concentrations of hormones, pharmaceuticals and other micropollutants in groundwater affected by septic systems in New England and New York. *Sci Total Environ.* 2015; 512: 43-54.
32. Leet JK, Sassman S, Amberg JJ, et al. Environmental hormones and their impacts on sex differentiation in fathead minnows. *Aquat Toxicol.* 2015; 158: 98-107.
33. Pollock MS, Dubé MG, Schryer R. Investigating the link between pulp mill effluent and endocrine disruption: attempts to explain the presence of intersex fish in the Wabigoon River, Ontario, Canada. *Environ Toxicol Chem.* 2010; 29(4): 952-65.
34. Adams N. Clover phyto-oestrogens in sheep in Western Australia. *Pure Appl Chem.* 1998; 70(9): 1855-62.
35. Panter G, Thompson R, Sumpter J. Adverse reproductive effects in male fathead minnows (*Pimephales promelas*) exposed to

- environmentally relevant concentrations of the natural oestrogens, oestradiol and oestrone. *Aquat Toxicol.* 1998; 42(4): 243-53.
36. Lopez de Alda MaJ, Díaz-Cruz S, Petrovic M, et al. Liquid chromatography–(tandem) mass spectrometry of selected emerging pollutants (steroid sex hormones, drugs and alkylphenolic surfactants) in the aquatic environment. *J Chromatogr A.* 2003; 1000(1–2): 503-26.
37. Lopez J. Endocrine-disrupting chemical pollution: why the EPA should regulate these chemicals under the clean water act. *Sustainable Development Law & Policy.* 2010; 10(3): 7.
38. López-Fernández R, Martínez L, Villaverde S. Membrane bioreactor for the treatment of pharmaceutical wastewater containing corticosteroids. *Desalination.* 2012; 300: 19-23.
39. Han J, Meng S, Dong Y, et al. Capturing hormones and bisphenol A from water via sustained hydrogen bond driven sorption in polyamide microfiltration membranes. *Water Res.* 2013; 47(1): 197-208.
40. Blair B, Nikolaus A, Hedman C, et al. Evaluating the degradation, sorption, and negative mass balances of pharmaceuticals and personal care products during wastewater treatment. *Chemosphere.* 2015; 134: 395-401.
41. Wojnarowicz P, Yang W, Zhou H, et al. Changes in hormone and stress-inducing activities of municipal wastewater in a conventional activated sludge wastewater treatment plant. *Water Res.* 2014; 66: 265-72.
42. Andersen H, Siegrist H, Halling-Sørensen B, et al. Fate of estrogens in a municipal sewage treatment plant. *Environ Sci Technol.* 2003; 37(18): 4021-6.
43. Baronti C, Curini R, D'Ascenzo G, et al. Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water. *Environ Sci Technol.* 2000; 34(24): 5059-66.
44. D'ascenzo G, Di Corcia A, Gentili A, et al. Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities. *Sci Total Environ.* 2003; 302(1): 199-209.
45. Azzouz A, Souhail B, Ballesteros E. Continuous solid-phase extraction and gas chromatography–mass spectrometry determination of pharmaceuticals and hormones in water samples. *J Chromatogr A.* 2010; 1217(17): 2956-63.
46. Plósz BG, Leknes H, Liltved H, et al. Diurnal variations in the occurrence and the fate of hormones and antibiotics in activated sludge wastewater treatment in Oslo, Norway. *Sci Total Environ.* 2010; 408(8): 1915-24.
47. Hamid H, Eskicioglu C. Effect of microwave hydrolysis on transformation of steroidal hormones during anaerobic digestion of municipal sludge cake. *Water Res.* 2013; 47(14): 4966-77.
48. Esperanza M, Suidan MT, Marfil-Vega R, Gonzalez C, Sorial GA, McCauley P, et al. Fate of sex hormones in two pilot-scale municipal wastewater treatment plants: Conventional treatment. *Chemosphere.* 2007; 66(8): 1535-44.