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# Presence of steroid hormones in Lake Titicaca and drinking water, Puno (Peru)

## Presencia de hormonas esteroides en el lago Titicaca y agua potable, Puno (Perú)

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**Abstract**
The presence of steroid hormones in lake waters causes contamination of aquatic ecosystems, which may cause endocrine alterations in the organisms that inhabit them. Moreover, many of these waters are purified and distributed to populations located around the lake. Therefore, these effects could be repeated in humans consuming the water. This study reports the presence of steroid hormone residues in the waters of the inner bay of Lake Titicaca and drinking water in the city of Puno (Peru). The solid phase extraction method was used for sample preparation, and the analyses were developed in an HPLC-DAD system. Results show maximum concentrations of steroid hormones estrone (E1) 1.56, 17 β- estradiol (E2) 2.27, 17 α- ethinylestradiol (EE2) 13. 88 ng $L^{-1}$ respectively. These concentrations vary at the different monitoring points, and their presence could cause ecotoxicological effects to the endemic aquatic biota that inhabit this part of the lake. At the same time they also could affect the health of the human population that consumes this water.
**Keywords**: Liquid chromatography, environmental risk, emerging contaminant, Titicaca.

**Resumen**
La presencia de hormonas esteroides en las aguas de los lagos provoca la contaminación de los ecosistemas acuáticos, lo que puede provocar alteraciones endocrinas en los organismos que los habitan. Además, muchas de estas aguas se depuran y distribuyen a las poblaciones ubicadas alrededor del lago; por tanto, estos efectos podrían repetirse en los seres humanos que consumen estas aguas. Este estudio reporta la presencia de residuos de hormonas esteroides en las aguas del interior de la bahía del lago Titicaca y agua potable en la ciudad de Puno (Perú). Para la preparación de la muestra se utilizó el método de extracción en fase sólida y los análisis se desarrollaron en un sistema HPLC-DAD, laboratorio CENA- USP- Brasil. Los resultados muestran concentraciones máximas de hormonas esteroides estrona (E1) 1,56, 17 β-estradiol (E2) 2,27, 17 α-etinilestradiol (EE2) 13. 88 ng L-1 respectivamente. Estas concentraciones varían en los diferentes puntos de monitoreo, y su presencia podría ocasionar efectos ecotoxicológicos a la biota acuática endémica que habita esta parte de este lago; al mismo tiempo también podrían afectar la salud de la población humana que consume esta agua.
**Palabras clave**: Cromatografía líquida, riesgo ambiental, contaminante emergente, Titicaca.

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## Introduction

Hormones are increasingly used around the world (Torres *et al*., 2021), such as the hormone 17 α-ethinylestradiol (EE2), which is one of the compounds in birth control pills (González-Hernando *et al*., 2013) as well as livestock; therefore, they are excreted by humans and animals (Fent, 2015) and are capable of interfering with the endocrine system in vertebrates and invertebrates (Bovier *et al*., 2018). Hormones such as estrone (E1) and estradiol (E2) are endogenous estrogens catalyzed by cytochrome P450 (CYP) enzymes which have biological activity in the liver, tissues, extrahepatic target cells, and extrahepatic target cells (Zhu & Lee, 2005), producing metabolites such as estrogen hydroxylation with a similar structure but with different physiological effects after metabolism in vivo (Xu *et al*., 2021). All these compounds are eliminated through urine and human feces in different amounts, depending on age, health status, diet, pregnancy status of women and also through animal manure (González *et al*., 2020).

Hormones of natural and synthetic origin are also detected in the aquatic environment (Sacdal *et al*., 2020) as estrogens in inland water bodies, tributaries and wastewater treatment plant effluents (Deich *et al.,* 2021),

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because of the ineffectiveness of conventional treatments (Torres *et al.*, 2021). Runoff from farms and effluent from sewage treatment plants are affected by these pollutants (Sacdal *et al.,* 2020), and lakes collecting discharges from urban areas are affected these types of pollutants. Concentrations of 85.15 ng $L^{-1}$, 4.72 ng $L^{-1}$ ,and 64.24 ng $L^{-1}$ of E1, E2 and EE2, respectively, were found in the wastewater treatment plant of the city of Chascomús, Argentina (González *et al.*, 2020), and its presence causes endocrine disruption that can affect aquatic organisms such as inhibition of gonadal growth and suppression of spermatogenesis (S. Wang et al., 2018). as it can also have adverse effects on the human body as an endocrine disruptor (Xu et al., 2021), It is therefore important to determine and monitor these emerging pollutants in water bodies (Katrina & Espino, 2020).

There is concern about emerging pollution in many lakes, which are important sources of water, considering that lakes are sometimes watershed drains and reservoirs that are altered by changes in land use and environmental conditions (Sacdal et al., 2020). The presence of hormones in lakes such as Laguna Lake, Philippines was found to be 0.03 and 0.30 ng $L^{-1}$ of E1 and 0.36 ng $L^{-1}$ of EE2 (Katrina & Espino, 2020), in Buyukcekmece Lake, Istanbul Turkey from <0.5 ng $L^{-1}$ to 5.74 ng $L^{-1}$ of E1, <0.5 ng $L^{-1}$ to 10.2 ng $L^{-1}$ of E2 and <0.5 ng $L^{-1}$ to 11.7 ng $L^{-1}$ of EE2 (Aydin & Talinli, 2013), while in East Lake, Wuhan China, nd to 9.62 ng $L^{-1}$ E1, nd to 17.26 ng $L^{-1}$ E2, and nd to 6.85 ng $L^{-1}$ EE2 were found (J. Wang & Zhu, 2017), Also in Honghu Lake, China an average of 17.64 of E1, 7.26 ng $L^{-1}$ of E2, and 17.73 ng $L^{-1}$ respectively were found, as well as in Dongting Lake, China an average of 5.63 of E1, 10.32 of E2, 3.04 of EE2, 3.04 ng $L^{-1}$ of EE2 (Yang et al., 2015).Space therefore, this study reports the presence of steroid hormone residues in the waters of the inner bay of Lake Titicaca and drinking water in the city of Puno.

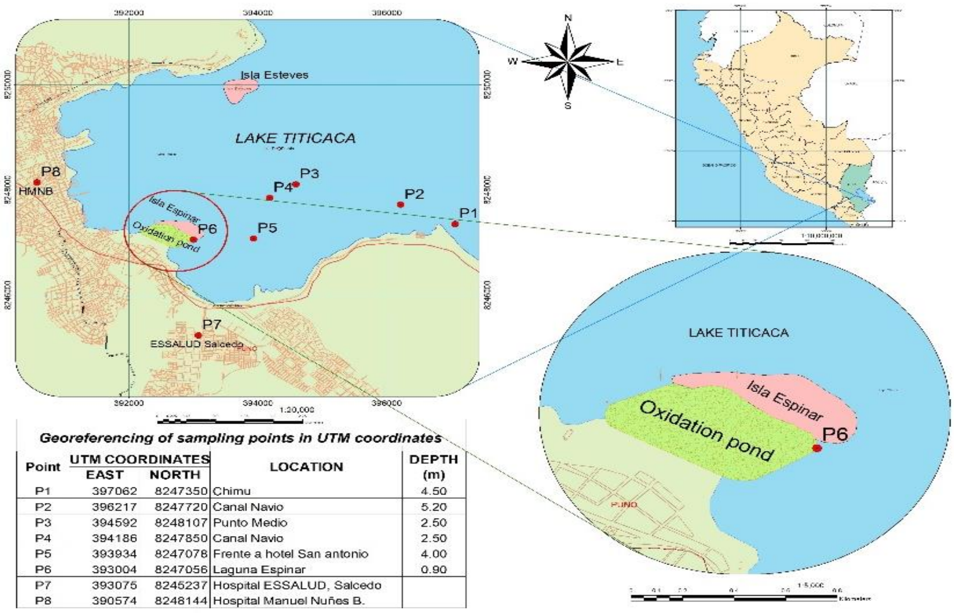
The research focuses on the integral ecological system and contributes to science, specifically, to conservation of biodiversity and environmental studies (Escobar-Mamani, F., Branca, D., y Haller, A., 2020). In a transdisciplinary perspective and sustainable development (Haller, A. y Branca, D., 2020), environmental management is not isolated from the socio-economic, cultural, or physical characteristics of the environment.

The objective of this work was to evaluate the presence of steroid hormones in the waters of the interior bay of Lake Titicaca and drinking water of Puno by High-Efficiency Liquid Chromatography

## Materials and methods

### *Sampling*

The present study considered eight sampling points, collected in March 2020, at six points located in the inner bay of Lake Titicaca (distance 4 km), and two points located in houses supplied with water from the inner bay of Lake Titicaca (distance 3.5.km), (Figure 1).




Georeferencing of sampling points in UTM coordinates

| Point | UTM COORDINATES EAST | UTM COORDINATES NORTH | LOCATION | DEPTH (m) |
|---|---|---|---|---|
| P1 | 397062 | 8247350 | Chimu | 4.50 |
| P2 | 396217 | 8247720 | Canal Navio | 5.20 |
| P3 | 394592 | 8248107 | Punto Medio | 2.50 |
| P4 | 394186 | 8247850 | Canal Navio | 2.50 |
| P5 | 393934 | 8247078 | Frente a hotel San antonio | 4.00 |
| P6 | 393004 | 8247056 | Laguna Espinar | 0.90 |
| P7 | 393075 | 8245237 | Hospital ESSALUD, Salcedo | |
| P8 | 390574 | 8248144 | Hospital Manuel Nuñes B. | |

**Figure 1.** *Location, 6 sampling points, Lake Titicaca inland bay and two points located in houses supplied with water from the lake – Puno (Perú).*

Sampling was performed using a Niskin Teflon sampler, which is suitable for lake water sampling (Golnick et al., 2016), Once the sample was taken, it was transferred to a 1L amber glass bottle with a screw cap, which was previously sterilized; the samples were kept at a temperature below 4 °C, to avoid degradation by photolysis. They were then were transported in thermal boxes to a laboratory to proceed with the extraction process.

### *Sample preparation*

HPLC grade water (Pura-Q. Brazil), HPLC grade methanol, Sigma-Aldrich sodium formate, and analytical standards for the hormones presented in Table 1 were used. Once in the laboratory, the samples were vacuum filtered with a 2.5 µm pore size paper filter to remove particulate matter from the sample. The hormone extraction process was carried out in the laboratory of the Universidad Nacional del Altiplano (Peru), by preconditioning the STRATA Polymeric Reversed Phase 500 mg/6 mL, Tubes, (SPE) solid-phase extraction cartridges were preconditioned with 5 mL of ultrapure water, followed by 5 mL of HPLC grade methanol, for each filtered sample (1L) and then dried, placed in cold chain and transported to the laboratories of CENA - USP -Brazil, where the analyses were carried out. Cartridges were rinsed with 5 mL of HPLC grade water at a flow rate of 1. 5 mL min$^{-1}$, the elution process was developed using 10 mL of HPLC grade methanol and placed in tip test tubes; subsequent to this, the samples were dried in a turbovap at a temperature of 40 °C and with a constant flow of constant nitrogen at 12 PSI. Once the sample was almost dry, it was reconstituted with 1 mL of a 50 % HPLC grade methanol and 50 % ultrapure water solution, filtered and transferred to a vial for subsequent analysis by HPLC-DAD; this extraction method was previously validated by Hortense Torres, (2014) in the laboratory where the analyses of the present study were carried out.

**Table 1.** Characteristics of hormones used for standards.

| Hormone | Purity % | Supply | Chemical Origin | Molecular Formula | Molecular Weight (g mol-1) |
|---|---|---|---|---|---|
| E1 | 99.0 | Dr, Ehrenstorfer GmbH, Augsburg, Germany | Natural hormone | $C_{18}H_{24}O_3$ | 270.37 |
| 17 alfa-EE2 | 98.5 | Dr, Ehrenstorfer GmbH, Augsburg, Germany | Synthetic hormone | $C_{20}H_{24}O_2$ | 296.40 |
| 17 beta- E2 | 99.0 | Dr, Ehrenstorfer GmbH, Augsburg, Germany | Natural hormone | $C_{12}H_{24}O_2$ | 272.38 |

### *Chromatographic analysis*

Chromatographic analyses were performed on a liquid phase HPLC-DAD chromatograph (Agilent, model 1200), with an Agilent Zorbax Eclipse Plus C18 column (100 x 3 mm, 3.5 µm); the chromatographic conditions used are shown in Table 2. For the quantification of the hormones present in the samples, standard solutions of the hormone standards Estrone (E1), 17α ethinylestradiol (EE2) and 17 β-estradiol (E2) were prepared in triplicate in concentrations of 0.5, 1, 3, 6 and 10 ug L$^{-1}$ respectively, these solutions were prepared in HPLC-grade methanol, the mobile phase consisted of 20:80 (V/V) (A/B) (mobile phase A ultrapure water with 5 mmol sodium formate and mobile phase B was HPLC grade methanol) used in isocratic mode at a flow rate of 1 mL-1, with an injection volume of 10 µL for all samples and standards, method used which has been developed and validated by (Hortense Torres, 2014).

**Table 2.** Chromatographic conditions for the identification and quantification of hormones.

| Parameter | Conditions |
|---|---|
| Flow | 1 mL min$^{-1}$ |
| Wavelength | 282 nm |
| Analytical run time | 15 minutos |
| Injection volume | 10 µL |
| Column temperature | 30°C |
| DAD Detector | 282 nm |

## Results

Hormone residues were found at the monitoring points considered in this study; the results are shown in Table 3.

**Table 3.** Steroid hormone concentration expressed in ng $L^{-1}$ at the sampling points.

| Hormones | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 |
|---|---|---|---|---|---|---|---|---|
| 17 β-estradiol E2 | 1.61 | 2.27 | 0.90 | 1.12 | 1.50 | 1.12 | 1.53 | 1.21 |
| 17 α-etinilestradiol EE2 | 1.38 | 1.62 | 5.76 | 5.80 | 8.97 | 13.88 | 1.03 | 0.81 |
| Estrone E1 | 0.76 | 0.74 | 0.83 | 0.72 | 0.75 | 1.56 | 0.80 | 0.77 |

This presence could be attributed to the fact that near the inner bay of this lake there is an oxidation lagoon, which is inefficient in the degradation and/or removal of hormone residues and therefore, the poorly-treated water is being discharged into the lake (Torres et al., 2021). Thus at point 6, the main point of outflow (oxidation lagoon) presents concentrations of 17 α-ethinylestradiol (EE2) 13.88 ng $L^{-1}$ and estrone (E1) 1.56 ng $L^{-1}$, as well as 17 β-estradiol (E2) 1. 12 ng $L^{-1}$, and as it travels until it reaches point P1- Chimú- (water intake for purification) these concentrations decrease. Point 1. concentrations of 17 α-ethinylestradiol (EE2) 1.38 ng L-1 and estrone (E1) 0.76 ng L-1, as well as 17 β-estradiol (E2) 1.61 ng L-. drinking water point 7 with 1.03 ngL-1 of EE2, 1.03 ngL-1 of E2, 1.56 ngL-1 E1 and point 8 with 0.81 ngL-1 of EE2, 1.21 ngL-1 of E2 and 0.77 ngL-1 of E1. It is shown in Figure 2.

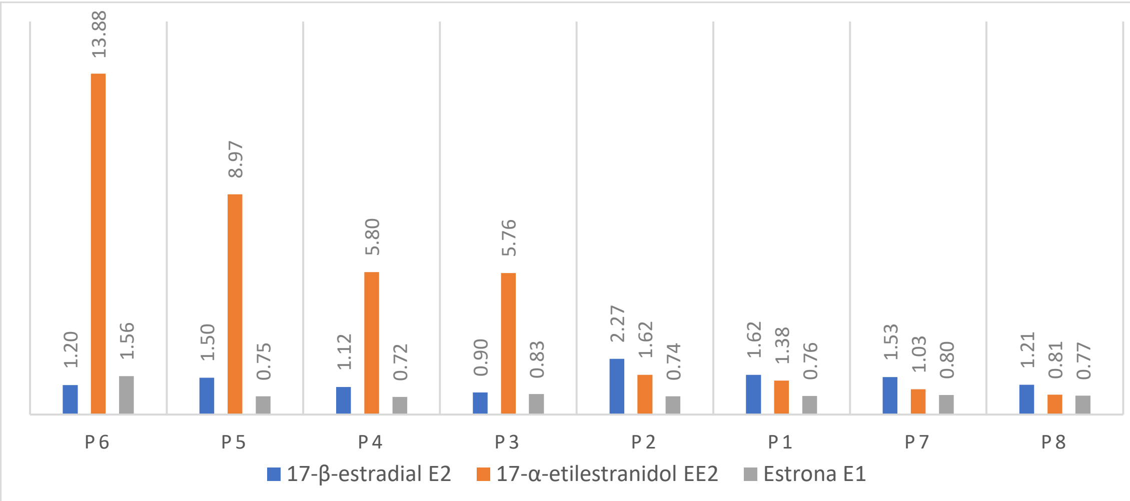



**Figure 2.** Graphic representation of the concentration of hormones Vs Study Points P6 a P1 Lake water, P7 a P8 drinking water –Puno.

## Discussion

This is due to their dispersion in water and depth, considering that these chemical compounds have lipophilic properties (Langenbach, 2013), low vapor pressure between 2.3 x 10-10 and 6.7 x 10-15 mm Hg, and also present log Kow values of the steroids of 2.81 for estriol and 3.94 for 17β-estradiol, while the steroid 7α-ethinylestradiol has a Log Kow value of 4.15. a characteristic of these compounds that allows them not to be soluble in water so it is expected to sediment and is a cause for reduced concentrations in the aqueous phase (Adeel et al., 2017), as well as the effect of sunlight exposure that produces a phototransformation of E2 and EE2 with a yield of 0.06 mol einstein-1 at 254 nm irradiance. (Mazellier et al., 2008).

The concentration of 17 β-estradiol E2 at point 1 was 1.61 ng $L^{-1}$, this is lower than that found in Lake Luoma, China which ranged from 2.52 ng $L^{-1}$ to 21.82 ng $L^{-1}$, mean concentration of 9.41 ng $L^{-1}$ (Dan Liu et al., 2017), and similar to that found in Buyukcekmece lake, Istanbul (Turkey) which ranged from <0.5-10.2 ng $L^{-1}$ (Aydin & Talinli, 2013) these values are related to population density. The presence of these compounds in the ecosystem of the lake's inner bay could have toxicological effects on the endemic species that inhabit this ecosystem. 17 β-estradiol (E2) is capable of producing the oxidation of macromolecules, generating the formation of the superoxide radical anion (Cavalieri et al., 2000), which causes loss of physicochemical properties that may result in cell death upon prolonged exposure, thus at 2 μg $L^{-1}$ of 17 β-estradiol affects several physiological processes, including the spliceosome, phototransduction, amino sugar and nuclear sugar metabolism, hypotaurine metabolism and the renin-angiotensin system. It was evidenced that exposure of *O. curvinotus* juveniles to increasing concentrations of 17 β-estradiol (2 ng L-1, 20 ng $L^{-1}$, 200 ng $L^{-1}$, and 2 µg $L^{-1}$) leads to a significant increase in the mRNA expression of vitellogenins (VTGS) and choriogenins (CHGS) (Dong et al., 2020).

The concentration 17 α-ethinylestradiol EE2 at point 1 was 1.38 ng $L^{-1}$, which is lower than that found in Lake Luoma, China (4.25 ng L-1 to 12.85 ng $L^{-1}$; mean concentration of 7.97 ng $L^{-1}$) (Dan Liu et al., 2017). However, at point 6 (outflow) it was 13.88 ng $L^{-1}$, lower than that found in wastewater and revert to the Chascomús lagoon, Argentina of 64.24 ng $L^{-1}$ and 47.55 ng $L^{-1}$ (at 200 m). (González et al., 2020), This difference could be attributed to the population density that generates domestic wastewater, sewage effluents, aquaculture, runoff from livestock farming (Zhou et al., 2016), industrial waters (Jia et al., 2019). Likewise, 17-α-ethinyl-estradiol is a non-translocating and non-competitive inhibitor of proton-coupled amino acids (Nielsen et al., 2021) being capable of inducing alterations in the endocrine system in living organisms at concentrations in the water below 1 ng $L^{-1}$ (Hansen et al., 1998; Purdom et al., 1994).

The presence of Estrone E1 at point 1 was 0.76 and is higher than that reported in surface waters of Laguna Lake, Philippines (0.03 and 0.30 ng $L^{-1}$) which is used for drinking water production (Katrina & Espino, 2020), and the Langat River is also used as a source of drinking water in Malaysia (0.09-0.18 ng $L^{-1}$; mean of 0.12 ng $L^{-1}$ ± 0.04) (Wee et al., 2019). At point 6 it was 1.12 ng $L^{-1}$, lower than reported in wastewater and discharged to the Chascomús lagoon, Argentina of 85.15 ng $L^{-1}$, 56.11 ng $L^{-1}$, and 1.54 (at 300 m upstream) (González et al., 2020). Estrone is converted to estriol by natural processes in surface waters (Aydin & Talinli, 2013) while high concentrations of E1 (>10 mg $L^{-1}$) significantly decrease the productivity of extracellular polymeric substances and metabolic activity (Zhang et al., 2021).

Several studies have shown abnormal development of fish in water with hormone residues, such as the feminization of male fish, affecting reproduction and inducing vitellogenin production (Torres et al., 2021), growth of certain aquatic organisms (Sacdal et al., 2020), Therefore, it is important to consider toxicity concentrations in the life of the fish, taking into account the lipophilic nature of the hormones that collaborate with bioaccumulation; in Carassius auratus fish after 72 hours of dietary exposure containing 100 ng $g^{-1}$ of EE2, the concentration was 1.7 ng $g^{-1}$ ±0.29 (Al-Ansari et al., 2011).

However, the presence of hormone residues at points 7 and 8 (drinking water), raises concerns about potential human exposure to endocrine disrupting compounds through dietary intake of food and especially the drinking water supply. (Wee et al., 2019). Hormone residues were within the range found in treated waters in the northern, central and southern regions of Taiwan E1 ND - 2.3 ng $L^{-1}$, E2 ND - 4.8ng $L^{-1}$, and EE2 ND - 3.8 ng $L^{-1}$ (Pai et al., 2020); estrogens 17-β-estradiol (E2) and 17-α-ethinylestradiol (EE2) are considered to be highly endocrine disrupting agents and have been included in the EU monitoring list on emerging aquatic pollutants due to their presence in animal tissues and fluids (Barreiros et al., 2016).

## Conclusions

The waters of the inner bay of Lake Titicaca and drinking water whose main source comes from this bay, show concentrations of steroid hormones: estrone (E1) 1.56, 17 β-estradiol (E2) 2.27, 17 α- ethinylestradiol (EE2) 13. 88 ng $L^{-1}$ respectively,

These concentrations vary in the different monitoring points as they move away from the main emission point (oxidation lagoon); the presence of these contaminants could cause ecotoxicological effects to the endemic aquatic biota that inhabit this lake, and at the same time it would be affecting the health of the population around the lake since they consume water from this lake.

The research focuses on the integral ecological system and contributes to science, specifically. Conservation of biodiversity, and environmental studies. In a transdisciplinary perspective and sustainable development, not isolated from the socioeconomic, cultural or physical characteristics of the environment

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### References

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

Al-Ansari, A. M., Saleem, A., Kimpe, L. E., Trudeau, V. L. y Blais, J. M. (2011). The development of an optimized sample preparation for trace level detection of 17α-ethinylestradiol and estrone in whole fish tissue. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *879*(30), 3649–3652. DOI: 10.1016/j.jchromb.2011.09.033

Aydin, E. y Talinli, I. (2013). Analysis, occurrence and fate of commonly used pharmaceuticals and hormones in the Buyukcekmece Watershed, Turkey. *Chemosphere*. DOI: 10.1016/j.chemosphere.2012.10.074

Barreiros, L., Queiroz, J. F., Magalhães, L. M., Silva, A. M. T. y Segundo, M. A. (2016). Analysis of 17-β-estradiol and 17-α-ethinylestradiol in biological and environmental matrices - A review. *Microchemical Journal*, *126*, 243–262. DOI: 10.1016/j.microc.2015.12.003

Bovier, T. F., Rossi, S., Mita, D. G., & Digilio, F. A. (2018). Effects of the synthetic estrogen 17-α-ethinylestradiol on Drosophila melanogaster: Dose and gender dependence. *Ecotoxicology and Environmental Safety*, *162*(June), 625–632. DOI: 10.1016/j.ecoenv.2018.07.020

Cavalieri, E., Frenkel, K., Liehr, J. G., Rogan, E. y Roy, D. (2000). Estrogens as Endogenous Genotoxic Agents — DNA Adducts and Mutations. *J. Natl. Cancer Inst.*, 27, 75–93. http://10.1093/oxfordjournals.jncimonographs.a024247

Dan Liu, Wu, S., Xu, H., Zhang, Q., Zhang, S., Shi, L., Yao, C., Liu, Y., & Cheng, J. (2017). Distribution and bioaccumulation of endocrine disrupting chemicals in water, sediment and fishes in a shallow Chinese freshwater lake: Implications for ecological and human health risks. *Ecotoxicology and Environmental Safety*. DOI: 10.1016/j.ecoenv.2017.02.045

Deich, C., Frazão, H. C., Appelt, J. S., Li, W., Pohlmann, T., & Waniek, J. J. (2021). Occurrence and distribution of estrogenic substances in the northern South China Sea. *Science of the Total Environment*, *770*, 145239. DOI: 10.1016/j.scitotenv.2021.145239

Dong, Z., Li, X., Huang, S., Zhang, N., Guo, Y., & Wang, Z. (2020). Vitellogenins and choriogenins are biomarkers for monitoring Oryzias curvinotus juveniles exposed to 17 β - estradiol. *Comparative Biochemistry and Physiology Part - C: Toxicology and Pharmacology*, *236*(April), 108800. DOI: 10.1016/j.cbpc.2020.108800

Fent, K. (2015). Progestins as endocrine disrupters in aquatic ecosystems: Concentrations, effects and risk assessment. In *Environment International*. DOI: 10.1016/j.envint.2015.06.012

Golnick, P. C., Chaffin, J. D., Bridgeman, T. B., Zellner, B. C., & Simons, V. E. (2016). A comparison of water sampling and analytical methods in western Lake Erie. *Journal of Great Lakes Research*, *42*(5), 965–971. DOI: 10.1016/j.jglr.2016.07.031

González-Hernando, C., Souza-de Almeida, M., Martín-Villamor, P., Cao-Torija, M. J., & Castro-Alija, M. J. (2013). La píldora anticonceptiva a debate. *Enfermería Universitaria*, *10*(3), 98–104. DOI: 10.1016/s1665-7063(13)72635-6

González, A., Kroll, K. J., Silva-Sanchez, C., Carriquiriborde, P., Fernandino, J. I., Denslow, N. D., & Somoza, G. M. (2020). Steroid hormones and estrogenic activity in the wastewater outfall and receiving waters of the Chascomús chained shallow lakes system (Argentina). *Science of the Total Environment*, *743*, 140401. DOI: 10.1016/j.scitotenv.2020.140401

Hansen, P. D., Dizer, H., Hock, B., Marx, A., Sherry, J., McMaster, M., & Blaise, C. (1998). Vitellogenin - A biomarker for endocrine disruptors. *TrAC - Trends in Analytical Chemistry*. DOI: 10.1016/S0165-9936(98)00020-X

Hortense Torres, N. (2014). *Determinação de hormônios e antimicrobianos no Rio Piracicaba e testes de toxicidade aguda com Daphnia magna* [Universidade de Sao Paulo]. https://teses.usp.br/teses/disponiveis/64/64135/tde-16042014-155332/es.php

Jia, Y., Hammers-Wirtz, M., Crawford, S. E., Chen, Q., Seiler, T. B., Schäffer, A., & Hollert, H. (2019). Effect-based and chemical analyses of agonistic and antagonistic endocrine disruptors in multiple matrices of eutrophic freshwaters. *Science of the Total Environment*, *651*, 1096–1104. DOI: 10.1016/j.scitotenv.2018.09.199

Katrina, K. M., & Espino, M. P. (2020). Occurrence and distribution of hormones and bisphenol A in Laguna Lake, Philippines. *Chemosphere*, *256*, 127122. DOI: 10.1016/j.chemosphere.2020.127122

Langenbach, T. (2013). Persistence and Bioaccumulation of Persistent Organic Pollutants (POPs). *Applied Bioremediation - Active and Passive Approaches*. 10.5772/56418

Mazellier, P., Méité, L., & De Laat, J. (2008). Photodegradation of the steroid hormones 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) in dilute aqueous solution. *Chemosphere*. DOI: 10.1016/j.chemosphere.2008.07.046

Nielsen, C. U., Pedersen, M., Müller, S., Kæstel, T., Bjerg, M., Ulaganathan, N., Nielsen, S., Carlsen, K. L., Nøhr, M. K., & Holm, R. (2021). Inhibitory Effects of 17-α-Ethinyl-Estradiol and 17-β-Estradiol on Transport Via the Intestinal Proton-Coupled Amino

Acid Transporter (PAT1) Investigated In Vitro and In Vivo. *Journal of Pharmaceutical Sciences*, *110*(1), 354–364. DOI: 10.1016/j.xphs.2020.08.010

Pai, C. W., Leong, D., Chen, C. Y., & Wang, G. S. (2020). Occurrences of pharmaceuticals and personal care products in the drinking water of Taiwan and their removal in conventional water treatment processes. *Chemosphere*, *256*, 127002. DOI: 10.1016/j.chemosphere.2020.127002

Purdom, C. E., Hardiman, P. A., Bye, V. J., Eno, N. C., Tyler, C. R., & Sumpter, J. P. (1994). Estrogenic Effects of Effluents from Sewage Treatment Works. *Chemistry and Ecology*. DOI: 10.1080/02757549408038554

Sacdal, R., Madriaga, J., & Espino, M. P. (2020). Overview of the analysis, occurrence and ecological effects of hormones in lake waters in Asia. *Environmental Research*, *182*(November 2019), 109091. DOI: 10.1016/j.envres.2019.109091

Torres, N. H., Santos, G. de O. S., Romanholo Ferreira, L. F., Américo-Pinheiro, J. H. P., Eguiluz, K. I. B., & Salazar-Banda, G. R. (2021). Environmental aspects of hormones estriol, 17β-estradiol and 17α-ethinylestradiol: Electrochemical processes as next-generation technologies for their removal in water matrices. *Chemosphere*, *267*, 128888. DOI: 10.1016/j.chemosphere.2020.128888

Wang, J., & Zhu, Y. (2017). Occurrence and risk assessment of estrogenic compounds in the East Lake, China. *Environmental Toxicology and Pharmacology*. DOI: 10.1016/j.etap.2017.03.018

Wang, S., Zhu, Z., He, J., Yue, X., Pan, J., & Wang, Z. (2018). Steroidal and phenolic endocrine disrupting chemicals (EDCs) in surface water of Bahe River, China: Distribution, bioaccumulation, risk assessment and estrogenic effect on Hemiculter leucisculus. *Environmental Pollution*, *243*, 103–114. DOI: 10.1016/j.envpol.2018.08.063

Wee, S. Y., Aris, A. Z., Yusoff, F. M., & Praveena, S. M. (2019). Occurrence and risk assessment of multiclass endocrine disrupting compounds in an urban tropical river and a proposed risk management and monitoring framework. *Science of the Total Environment*, *671*, 431–442. DOI: 10.1016/j.scitotenv.2019.03.243

Xu, S., Sun, J., Zhang, Y., Ji, J., & Sun, X. (2021). Opposite estrogen effects of estrone and 2-hydroxyestrone on MCF-7 sensitivity to the cytotoxic action of cell growth, oxidative stress and inflammation activity. *Ecotoxicology and Environmental Safety*, *209*, 111754. DOI: 10.1016/j.ecoenv.2020.111754

Yang, Y., Cao, X., Zhang, M., & Wang, J. (2015). Occurrence and distribution of endocrine-disrupting compounds in the Honghu Lake and East Dongting Lake along the Central Yangtze River, China. *Environmental Science and Pollution Research*, *22*(22), 17644–17652. DOI: 10.1007/s11356-015-4980-y

Zhang, F., Yu, Y., Pan, C., Saleem, M., & Wu, Y. (2021). Response of periphytic biofilm in water to estrone exposure: Phenomenon and mechanism. *Ecotoxicology and Environmental Safety*, *207*, 111513. DOI: 10.1016/j.ecoenv.2020.111513

Zhou, L. J., Zhang, B. B., Zhao, Y. G., & Wu, Q. L. (2016). Occurrence, spatiotemporal distribution, and ecological risks of steroids in a large shallow Chinese lake, Lake Taihu. *Science of the Total Environment*, *557–558*, 68–79. DOI: 10.1016/j.scitotenv.2016.03.059

Zhu, B. T., & Lee, A. J. (2005). NADPH-dependent metabolism of 17β-estradiol and estrone to polar and nonpolar metabolites by human tissues and cytochrome P450 isoforms. *Steroids*, *70*(4), 225–244. DOI: 10.1016/j.steroids.2005.01.002