Regulation of the synthetic estrogen 17α-ethinylestradiol in water bodies in Europe, the United States, and Brazil

Regulamentação do estrogênio sintético 17α-etinilestradiol em matrizes aquáticas na Europa, Estados Unidos e Brasil

Regulación del estrógeno sintético 17α-etinilestradiol en matrices acuáticas en Europa, Estados Unidos y Brasil

Danieli Lima da Cunha [1,2]
Samuel Muylaert Camargo da Silva [2]
Daniele Maia Bila [3]
Jaime Lopes da Mota Oliveira [1]
Paula de Novaes Sarcinelli [1]
Ariane Leites Larentis [1]

Abstract

The synthetic estrogen 17α-ethinylestradiol, the principal component of oral contraceptives, has been identified as one of the main compounds accounting for adverse effects on the endocrine system in various species. This study aimed to analyze the state-of-the-art in legislation and guidelines for the control of this synthetic estrogen in water bodies in Europe and the United States and to draw a parallel with the Brazilian reality. Countries have generally attempted to expand the regulation and monitoring of certain emerging micropollutants not previously covered by legislation. Europe is more advanced in terms of water quality, while in the United States this estrogen is only regulated in water for human consumption. Brazil still lacks legal provisions or standards for this estrogen, which can be explained by the relatively limited maturity of the country's system for controlling water pollutants.

Endocrine Disruptors; Ethinyl Estradiol; Water Quality Criteria

[1] *Fundação Oswaldo Cruz, Rio de Janeiro, Brasil.*
[2] *Universidade Federal Fluminense, Niterói, Brasil.*
[3] *Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brasil.*

Correspondence
D. L. Cunha
Rua General Andrade Neves 2, apto. 202, Niterói, RJ 24210-000, Brasil.
danielicunha@hotmail.com

Introduction

A key environmental issue in recent years is emerging micropollutants, or organic and inorganic compounds that pose high potential risk to human health and the environment, even at low concentrations (between $\mu g.L^{-1}$ and $ng.L^{-1}$). Such pollutants include a wide range of natural and synthetic chemical compounds, including pharmaceuticals, personal care products, hormones, surfactants, flame retardants, pesticides, and nanoparticles [1,2,3]. These micropollutants feature a group known as endocrine disruptors, including exogenous substances with the capacity to alter the endocrine system's functions, thereby provoking adverse effects for human and animal health [3,4,5,6].

Of the various micropollutants classified as endocrine disruptors, the synthetic estrogen 17α-ethinylestradiol, the principal estrogenic component used in formulation of oral contraceptives and one of the most extensively consumed medicines in the world, has raised great concern in the academic community, since it has been identified as the main compound responsible for endocrine alterations in aquatic organisms.

This destabilization of the endocrine system leads to observable biochemical and histopathological alterations (of the liver, gonads, and kidneys), modifications in the reproductive process and development, and behavioral changes, among others. Table 1 lists studies from the last 15 years on the effects of exposure to 17α-ethinylestradiol in various species in different life phases. The most alarming adverse effect is feminization – the development of female sex characteristics, including female reproductive anatomy [7]. This process compromises the affected population's reproductive cycle and can trigger an imbalance in its ecosystem.

Fish species, due to their inherent ecological and economic importance, are among the most extensively studied organisms, acting as important indicators of the potential effects of endocrine disruption, especially those involved in reproductive physiology, since their reproductive system is regulated by estrogens that are similar to those of mammals [8,9,10].

The effects on fish, amphibians, and birds have also been assessed by the presence of biomarkers like vitellogenin (VTG) and zona radiata protein (ZRP), both normally found only in females [11,12,13,14]. A key function of estrogens in oviparous organisms is to produce these markers during ovogenesis [15,16,17], and estrogenic micropollutants can increase the expression of these sexual proteins.

There are still no studies that prove the effects of 17α-ethinylestradiol on human health based on environmental exposures [18,19], which may be partially explained by the serious difficulty in demonstrating causality when analyzing endocrine disruption [20]. Given an endocrine disruptor with the capacity to provoke profound physiological changes with only a "trigger" dose, one can formulate the hypothesis that once present in the water ingested by humans, even at low concentrations, this hormone can trigger adverse effects in some individuals.

Basically, 17α-ethinylestradiol is introduced into water by two main routes: excretion and disposal. Once consumed, the estrogen is excreted in urine and feces in its conjugated forms (sulfates and glucuronides) [21,22]. These are dumped in sewage, which later reaches aquatic environments in natura or in the form of effluents treated in sewage treatment stations [23,24,25]. Importantly, conventional sewage treatment technologies have limited ability to remove this estrogen [26,27,28,29,30,31]. The situation is aggravated by the fact that 17α-ethinylestradiol is the most persistent of the estrogens, with a half-life in water of approximately 17 days and a low photodegradation rate [32,33].

Since water pollution threatens aquatic environments, with various negative effects on the species that inhabit water bodies or ingest the water from them (including humans), some countries have created a series of legal provisions and standards to limit the concentrations of these pollutants in water bodies.

To discuss the legislation on 17α-ethinylestradiol, two types of provisions apply to water: water quality for the protection of aquatic life and protection of drinking water for human health. The current study thus analyzes the state-of-the-art of legal provisions and standards on the control of the synthetic estrogen 17α-ethinylestradiol in water in Europe and the United States and to draw a parallel with the Brazilian reality.

Legal frameworks for regulation of 17α-ethinylestradiol in water

European Union

Due to the geopolitical structure of the European Union, various member countries share rivers and lakes. The member states have thus pursued an integrated solution for the environmental recovery of their water bodies. This joint effort spearheaded by the European Parliament and Council of the European Union resulted in

Table 1

Adverse effects of 17α-ethinylestradiol in different species.

Species/Life phase of the organism at the beginning of the test	Observed effects
Fishes	
Danio rerio	
Embryos	Change in mating behavior [57]; Induction of vitellogenin synthesis in males; Anomalies and mortality in embryos [58]
Adult males and females	Histopathological changes in females [59]
Adult males	Induction of vitellogenin synthesis and behavior changes [60]
Different stages	Indução da síntese de viteloginina; Atraso e redução da desova; Redução das taxas de fertilização [61]
Oryzias latipes	
Embryos	Significant reduction in fertilization rate [62]
Adult males	Induction of vitellogenin synthesis and feminization [63]
One day after birth	Significant change in sex ratio (more females) and hermaphroditism [64]
Oryzias melastigma	
Adult males and females	Changes in mating behavior and inhibition of spawning [65]
Pimephales promelas	
Embryos	Decrease in body size and induction of vitellogenin synthesis [8]; Decrease in fertilization of eggs and feminization [66]; Induction of vitellogenin synthesis; feminization; Significant inhibition of spermatogenesis [67]
Adult males	Changes in reproductive behavior, reduction in hormone levels, and secondary sexual characteristics [68]
Betta splendens	
Young and adult males and females	Changes in mating behavior [69]
Pomatoschistus minutus	
Adult males	Induction of vitellogenin synthesis and zona radiata protein [11]
Gasterosteus aculeatus	
Adult males	Changes in mating behavior [63]
Gobiocypris rarus	
Adult males and females	Induction of vitellogenin synthesis in both sexes, alterations in liver and kidneys, feminization [70]
Amphibians	
Xenopus laevis	
Adult males	Induction of vitellogenin synthesis and hepatic alterations [71]; Changes in mating behavior [72]; Significant behavior changes [73]
Hyalella azteca	
4 to 6 weeks after gametogenesis	Growth reduction in males [61]
Gammarus pulex	
Different stages	Significant increase in mean population size; change in adult sex ratio (2:1 in favor of females) [74]
Crustaceans	
Daphnia magna	
Larvae	Decrease in number of progeny per female [75]
Ceriodaphnia reticulata	
Larvae	Increased mortality in newborns [76]
Sida crystallina	
Larvae	Shorter juvenile phase [76]

(continues)

Table 1 (continued)

Species/Life phase of the organism at the beginning of the test	Observed effects
Mollusks	
Lymnea stagnalis	
Ova	Significant hatching delay; Deformations in developing snails; Growth reduction in progeny [61]
Adult males and females	Reproductive changes [77]
Porifera	
Hydra vulgaris	
Adult males and females	Significant reductions in number of oocytes (females) and sperm activity (males) [61]
Echinoderms	
Hemicentrotus pulcherrimus/Strongylocentrotus nudus	
Embryos and larvae	Altered morphogenesis [78]

Directives to set binding water quality standards for the member states.

Among the aspects comprising the "good state" of European rivers and lakes, in addition to hydrological and ecological characteristics, various physical and chemical parameters are monitored. Standards have been developed for monitoring water quality in order to maintain these parameters within the established ranges [34,35].

Directive 2000/60/EC, known as the Water Framework Directive (WFD) [36], was passed in December 2000 to protect and recover the quality of water in Europe and to ensure its sustainable long-term use. The directive establishes various measures to protect aquatic ecosystems, in addition to gradual timeframes for reaching the goals [34,35,36].

As an initial step in the strategy laid out in the WFD, in November 2001, *Decision 2455/2001/EC* established the first list of priority substances (total of 33) [37]. This first decision did not set any maximum concentrations. It was not until 2008 that *Directive 2008/105/EC* (Environmental Quality Standards – EQS) set these limits for such substances in surface waters [38].

Since the Directives provide for periodical revision and updating of the standards, in January 2012 the European Commission published the document *COM(2011)876*, proposing the inclusion of a second list of 15 new priority substances and their respective EQS [39], targeted for monitoring in the implementation round of the European Union water policy from 2015 to 2021 [40] (Table 2). The new substances include pesticides, dioxins, industrial chemicals, and pharmaceuticals.

Selection of substances was based on a prioritization procedure obtained from scientific data, drawing on a list of 2,000 substances initially considered as potentially hazardous [41]. A more detailed look at 17α-ethinylestradiol shows that before its inclusion on the list of priority substances, this synthetic estrogen was the target of some discussion, and a limit of $0.035 ng.L^{-1}$ was finally proposed for its concentration in water bodies. This proposed limit considered a series of studies on its ecotoxicological effects [7,42]. Figure 1 shows the potential adverse effects in fish associated with different concentrations of 17α-ethinylestradiol in surface waters.

After a series of discussions, in August 2013 the European Union issued *Directive 2013/39/EU 65*, approving the inclusion of 12 of the 15 substances proposed in *COM(2011)876*. For the pharmaceuticals whose inclusion was not approved (17α-ethinylestradiol, 17β-estradiol, and diclofenac), no maximum tolerable concentrations were determined. The three pharmaceuticals were included in a Surveillance List in order to collect more monitoring data and back the definition of appropriate limits in relation to the risk posed by these substances [43].

One explanation for not setting EQS for these three pharmaceuticals was the high inherent cost of meeting such standards, requiring major changes in sewage treatment stations. England and Wales would have to spend some 41 billion dollars to install new systems, plus 15 billion more over the course of the following ten years. Even in the face of such high costs, some authors believe that the retrofitting would be positive, bringing additional benefits: using the technology, wastewater treatment would remove many other pollutants that have caused concern [7,42].

As for the quality of water for human consumption in EU member countries, the main goal of *Directive 98/83/ANDC* of November 1998 is to protect human health from the harmful

Table 2

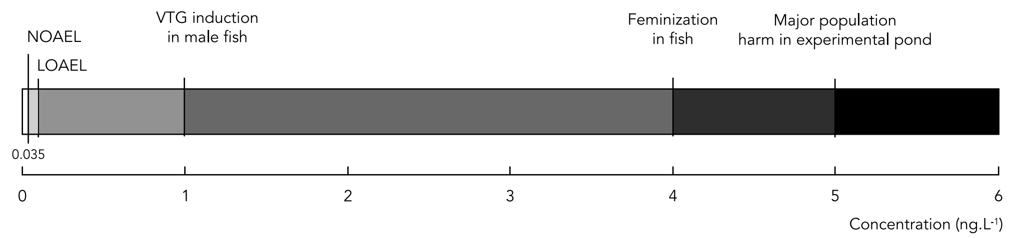
List of the 15 priority substances classified by *Directive 2013/39/UE* [43].

Substance	Type/Use	Effect
17α-ethinylestradiol	Pharmaceutical/synthetic estrogen used mainly in oral contraceptives	Endocrine disruptor, mainly in fish
17β-estradiol	Estrogen excreted naturally (approximately 90%) in human and animal urine. Also used in formulations of pharmaceuticals for female hormone replacement therapy	Endocrine disruptor, mainly in fish
Aclonifen	Herbicide used in a variety of field crops	Toxic to various aquatic organisms
Bifenox	Herbicide used to kill broadleaf weeds in grain crops and pastures	Toxic to various aquatic organisms
Irgarol	Algicide used as antifouling agent in coatings of boat and ship hulls	Toxic, degrades very slowly; persistent in sediments
Cypermethrin	Pyrethroid insecticide and biocide used in field crops, salmon farming, and timber and wood preservation	Toxic to various aquatic organisms; accumulates in sediments
Dichlorvos	Organophosphate insecticide and biocide used to protect grain crops	Particularly toxic to various aquatic organisms and possible carcinogenic to humans
Diclofenac	Pharmaceuticals/NSAID	Toxic, both directly (e.g., chronic studies show effects in fishes) and by secondary poisoning (for example, vultures in India affected by veterinary use in bovines)
Dicofol	Organochloride acaricide authorized for use on fruits and vegetables	Toxic, similar to DDT, recommended for designation as POP; possibly carcinogenic to humans and possible endocrine disruptor
Dioxins	Dioxins, byproducts of thermal combustion of chlorinated compounds; PCBs, chlorinated organic compounds previously used in manufacturing electrical equipment; some are also produced by combustion	PBT and POP, some forms probably carcinogenic in humans; other possible effects include endocrine disruption and immune and nervous system impairment. Limits have already been set for its presence in human foodstuffs and livestock feed
HBCDD	Industrial chemical used as flame retardant, especially in polystyrene, including insulation panels	PBT and POP, possibly toxic to human reproduction
Heptachlor/Heptachlor epoxide	Organochlorine insecticide restricted in many countries by the Stockholm Convention, but possible as secondary emissions	POP, highly toxic to aquatic organisms, possibly/probably carcinogenic in humans and possible endocrine disruptor
PFOS	Industrial chemical, used in aircraft hydraulic fluids, photography, and electroplating; Present in many existing products, especially textiles	PBT and POP, toxic to animals, especially mammals; possible carcinogen in humans and possible effects on thyroid function
Quinoxyfen	Fungicide used mainly in grain crops and vineyards	PBT and vPvB properties, accumulates particularly in sediments
Terbutryn	Herbicide used especially in building façades as a preservative between coats of paint	Toxic especially to algae and aquatic plants

DDT: dichlorodiphenyltrichloroethane; HBCDD: Hexabromocyclododecane; NSAID: non-steroidal anti-inflammatory pharmaceuticals; PBT: persistent, bioaccumulative, and toxic; PCBs: polychlorinated biphenyls; POP: persistent organic pollutant; PFOS: perfluorooctane sulfonate; vPvB: very persistent and very bioaccumulative.

Figure 1

Adverse effects in fish associated with different concentrations of 17α-ethinylestradiol in surface waters [7].



LOAEL: lowest observed adverse effect level; NOAEL: no observable adverse effect level; VTG: vitellogenin.

effects of contamination of any drinking water, ensuring its quality and safety. The directive requires regular monitoring and testing of a total of 48 parameters, including microbiological and chemical indicators and contaminants. These chemical compounds include 26 substances, mainly metals, pesticides, and polycyclic aromatic hydrocarbons (PAH's), some also included on the list of priority substances regulated by *Directive 2008/105/EC*, but do not include 17α-ethinylestradiol [44].

Importantly, member states are not allowed to set lower standards for the protection of human health when compared to the European Union guidelines. The law further provides that member states may include additional requirements such as regulating additional substances that are relevant in their respective territories, or establish more stringent standards [44].

United States

In the United States, the Environmental Protection Agency (EPA) is responsible for regulating the quality of water resources and that of water for human consumption. The *Clean Water Act* (CWA), passed in 1948 and amended several times over the years, is the legal reference that establishes the basic structure for regulating the disposal of pollutants in waters and quality standards for U.S. surface waters. As for pollution control, the national standards for quality of surface water include 126 substances, but no estrogens, either natural or synthetic. The CWA provides that the states hold triennial public hearings to review their water quality standards, in order ensure compliance with current scientific evidence and water uses by humans and aquatic life [45].

As for the quality of water for human consumption, the EPA also acts as a regulatory agency. The *Safe Drinking Water Act* (SDWA) passed by Congress in 1974 and amended in 1986 and 1996 is the prevailing legislation for all as water actually or potentially used for human consumption, applying to both surface and table waters. The law authorizes the EPA to set standards for the public water supply and requires that the system's operators comply with the regulations for protection of human health [46].

The SDWA adopts two categories for drinking water standards, both with mandatory compliance: the *National Primary Drinking Water Regulation* (NPDWR), which sets the primary standards for chemical and microbiological contaminants with potential adverse effects on human health; and the *National Secondary Drinking Water Regulation* (NSDWR), pertaining to secondary standards for substances with potential aesthetic and/or organoleptic effects [47]. Currently, *EPA 816-F-09-0004*, issued in May 2009, sets primary standards for a list of 88 contaminants, including microorganisms, disinfectants, byproducts of disinfection, organic and inorganic compounds, and radioactive nucleotides [48]. However, the list does not include 17α-ethinylestradiol or any other estrogen.

The drinking water standard is defined by an assessment of the risk to human health, using methodologies developed and validated by the EPA itself. Such methodologies include the following steps to assess the risk associated with exposure to a given contaminant: identification of the hazard, assessment of the exposure, dose-response assessment, and risk characterization [46].

As part of the system of risk assessment for human health, the EPA has a drinking water monitoring program for pollutants that have still not been regulated, known as Unregulated Contaminant Monitoring (UCM). This monitoring aims to collect data on pollutants suspected of being present in the water. Through this program, every five years is published a list of pollutants, the *Contaminant Candidate List* (CCL), which are still not subject to regulation, but which pose potential risks to health and which are known to occur in the water supply [49].

The first CCL (CCL1) was published in March 1998 and had 60 contaminants (10 microbiological and 50 chemical). CCL2, published in February 2005, reduced the list to 51 contaminants (9 microbiological and 42 chemical), and CCL3 was published in late 2009, expanding the list to 116 contaminants (12 microbiological and 104 chemical). CCL3 includes pesticides, byproducts of disinfection, commercial chemical products, pathogenic agents, biological toxins, and pharmaceutical products, including 17α-ethinylestradiol and other endocrine disruptors. CCL3 drew on an assessment of 7,500 chemical and microbiological contaminants. CCL4 was proposed recently, in April 2015, and is still under review. The new proposed list includes 112 contaminants (12 microbiological and 100 chemical), among which 17α-ethinylestradiol.

Once included on the CCL, contaminants are further assessed to determine whether there are sufficient data to meet the criteria for Regulatory Determination. The EPA characterizes each contaminant included on the CCL based on three aspects: effects on health, occurrence in the aquatic environment, and analytical methods. If the data are sufficient to raise the grade of a given contaminant, a Regulatory Determination can be issued [49].

Brazil

The principal legal provisions that impact the environmental quality of water in Brazil are the responsibility of the Ministry of the Environment, through the National Council on the Environment (CONAMA). This agency establishes a series of resolutions with mandatory nationwide compliance for maintaining the quality of water resources in the country. These resolutions are normally based on the environmental standards set by the United States EPA. An important provision is CONAMA *Resolution n. 357* of 2005, appended in 2011 by CONAMA *Resolution n. 430*. These resolutions rule on the classification of bodies of surface water and the respective environmental standards, as well as establishing the conditions and standards for disposing of effluents [50,51]. CONAMA *Resolution n. 396* of 2008 [52] further defines the quality of table water, including for direct or indirect human use.

To guarantee minimum quality standards consistent with the preponderant uses of the country's waters, these resolutions are important for inducing improvements in water bodies. However, nearly ten years after CONAMA 357 was issued, little progress has been made with the compliance of water bodies, with extremely few rivers meeting the standards and water pollution control still lacking a series of crucial improvements.

These provisions generally define various physical and chemical parameters, organic and inorganic chemicals, algae, and microorganisms in monitoring water quality. The concentrations for each substance vary according to the classification of the respective body of water. As for persistent organic pollutants, Brazil passed *Legislative Order n. 204* of 2004 [53], adopting as its basis the standards set by the Stockholm Convention on Persistent Organic Pollutants. These resolutions set limits on a series of substances with the potential to disrupt the endocrine system, such as aldrin, DDT, heptachlor, and polychlorinated biphenyls (PCBs), but not 17α-ethinylestradiol. CONAMA 357 further provides that government can set water quality standards for substances that can compromise the use of water for intended purposes, providing a technical basis.

The Brazilian Ministry of Health issues rulings with water quality standards for human consumption. Monitoring water quality for human consumption is also the responsibility of the Ministry of Health through its Division of Environmental Health Surveillance. *Ruling n. 2.914* of 2011 [54] is the prevailing legislation that sets water quality standards for human consumption. This ruling was based on recommendations by the World Health Organization (WHO) and various international standards.

This Ruling sets standards for microorganisms, cyanotoxins, radioactive nucleotides, and various organic and inorganic chemical compounds such as pesticides, disinfectants, and byproducts of disinfection, but does not include 17α-ethinylestradiol or other potential endocrine disruptors [54].

On this issue, the São Paulo State Chapter of the Brazilian Association of Health and Environmental Engineering (ABES/SP) published a document in 2012 entitled *Guidelines on Drinking Water Quality and Chemical Substances*, with a list of 291 priority substances present in water and still unregulated. These compounds were prioritized according to their use, the amounts produced, persistence, and effects, based on the scientific literature. Then, based on established criteria for the exclusion or maintenance, the general list was refined on a list of priority substances. 17α-ethinylestradiol was included on the general list, but after the combination of these criteria it was excluded from the principal list, which contains 72 chemical substances. This same study calls attention to the effects of endocrine disruptors on aquatic organisms and potential effects on human health, and suggests that endocrine disruptors tend to be increasingly regulated by law [55].

Brazil also has a huge environmental health liability, since it has not solved the challenge of basic sanitation. The country fails to provide safe running water for the entire population (coverage now stands at 82.7%). More alarmingly, fewer than half of Brazilian households (48.3%) are connected to the sewage system and only 38.7% of all sewage is treated before dumping into the water bodies [56]. It is thus scarcely realistic to expect that Brazil will deal immediately with emerging micropollutants like 17α-ethinylestradiol when the country has failed to solve more basic problems.

Final remarks

When analyzing current regulation of water pollutants and focusing more specifically on the synthetic estrogen 17α-ethinylestradiol, with scientific progress and increasing knowledge on the effects of such pollution, countries have generally endeavored to expand the regulation and monitoring of some emerging micropollutants not previously covered by legislation.

The European Union has made significant progress in water pollution control through measures such a list of 15 priority substances, amongst which 17α-ethinylestradiol. As for water for human consumption, despite Europe's mature legislation, this synthetic estrogen is still not regulated. The opposite is true in the United States, where 17α-ethinylestradiol is regulated in water for human consumption but is not included on the list of quality control standards for water bodies.

In Brazil, 17α-ethinylestradiol is not covered by any water legislation, either for human consumption or for quality of water bodies. This can be partially explained by a comparison with the situation in Europe and North America, since Brazil lags significantly behind in water pollution control in general. The Brazilian situation with water pollution involves more than the legal provisions and standards, since important provisions in the country's existing legislation have still not been enforced satisfactorily.

The control of emerging micropollutants, including 17α-ethinylestradiol, calls for urgent progress. As a relatively new issue, progress at the national level requires monitoring the current conditions of water bodies and the elaboration of studies as the basis for feasible and effective measures.

Contributors

D. L. Cunha and S. M. C. Silva contributed to the elaboration of the article. D. M. Bila, J. L. M. Oliveira, P. N. Sarcinelli, and A. L. Larentis contributed to the critical revision.

Acknowledgments

The authors wish to thank the Capes agency for granting a Master's scholarship.

References

1. Conselho Nacional de Recursos Hídricos. Moção MMA nº 61, de 10 de julho de 2012. Recomenda promoção de ações de ciência e tecnologia para melhoria de técnicas de monitoramento e de tratamento de água de abastecimento e de efluentes, visando à remoção de micropoluentes emergentes e eliminação de microrganismos patogênicos emergentes. ftp://ftp.saude.sp.gov.br/ftpsessp/bibliote/informe_eletronico/2012/iels.ago.12/Iels153/U_MO-MMA-CNRH-61_100712.pdf (accessed on 24/Jun/2015).
2. Barceló D, Petrovic M. Emerging contaminants from industrial and municipal waste: occurrence, analysis and effects. Berlin: Springer; 2008.
3. Bila DM, Dezotti M. Desreguladores endócrinos no meio ambiente: efeitos e conseqüências. Quím Nova 2007; 30:651-66.
4. Bergman A, Heindel JJ, Jobling S, Kidd KA, Zoeller TR. State of the science of endocrine disrupting chemicals. Geneva: United Nations Environment Programme/World Health Organization; 2012.
5. Diamanti-Kandarakis E, Bourguignon J, Giudice LC, Hauser R, Prins GS, Soto AM, et al. Endocrine-disrupting chemicals: an endocrine society scientific statement. Endocr Rev 2009; 30:293-342.
6. Sumpter JP, Johnson AC. Lessons from endocrine disruption and their application to other issues concerning trace organics in the aquatic environment. Environ Sci Technol 2005; 39:4321-32.
7. Gilbert N. Drug-pollution law all washed up. Nature 2012; 491:503-4.
8. Johns SM, Denslow ND, Kane MD, Watanabe KH, Orlando EF, Sepúlveda MS. Effects of estrogens and antiestrogens on gene expression of fathead minnow (*Pimephales promelas*) early life stages. Environ Toxicol 2011; 26:195-206.
9. Maruska KP, Gelsleichter J. Hormones and reproduction in chondrichthyan fishes. In: Norris DO, Lopez KH, editors. Hormones and reproduction of vertebrates. v. 1. Oxford: Elsevier; 2011. p. 209-37.
10. Moraes NV, Grando MD, Valerio DAR, Oliveira DP. Exposição ambiental a desreguladores endócrinos: alterações na homeostase dos hormônios esteroidais e tireoideanos. Rev Bras Toxicol 2008; 21:1-8.
11. Humble JL, Hands E, Saaristo M, Lindström K, Lehtonen KK, Diaz de Cerio O, et al. Characterization of genes transcriptionally upregulated in the liver of sand goby (*Pomatoschistus minutus*) by 17α-ethinyloestradiol: identification of distinct vitellogenin and zona radiata protein transcripts. Chemosphere 2013; 90:2722-9.
12. Chandra K, Bosker T, Hogan N, Lister A, MacLatchy D, Currie S. Sustained high temperature increases the vitellogenin response to 17α-ethynylestradiol in mummichog (*Fundulus heteroclitus*). Aquat Toxicol 2012; 118-119:130-40.
13. Woods M, Kumar A. Vitellogenin induction by 17α-estradiol and 17β-ethynylestradiol in male Murray rainbow fish (*Melanotaenia fluviatilis*). Environ Toxicol Chem 2011; 30:2620-7.
14. Folmar LC, Hemmer M, Hemmer R, Bowman C, Kroll K, Denslow ND. Comparative estrogenicity of estradiol, ethynyl estradiol and diethylstilbestrol in an in vivo, male sheepshead minnow (*Cyprinodon variegatus*), vitellogenin bioassay. Aquat Toxicol 2000; 49:77-88.
15. Damstra T, Barlow S, Bergman A, Kavlock R, Kraak GVD. Global assessment of the: state of the science of endocrine disrupors. Geneva: World Health Organization; 2002. (WHO Publication, WHO/PCS/EDC/02.2).
16. Hiramatsu N, Cheek AO, Sullivan CV, Matsubara T, Hara A. Vitellogenesis and endocrine disruption. Biochemistry and Molecular Biology of Fishes 2005; 6:431-71.
17. Arukwe A, Celius T, Walther BT, Goksøyr A. Effects of xenoestrogen treatment on zona radiata protein and vitellogenin expression in Atlantic salmon (*Salmo salar*). Aquat Toxicol 2000; 49:159-70.
18. Cao Q, Yu Q, Connell DW. Fate simulation and risk assessment of endocrine disrupting chemicals in a reservoir receiving recycled wastewater. Sci Total Environ 2010; 408:6243-50.
19. Caldwell DJ, Mastrocco F, Nowak E, Johnston J, Yekel H, Pfeiffer D, et al. An assessment of potential exposure and risk from estrogens in drinking water. Environ Health Perspect 2010; 118:338-44.
20. Parlamento Europeu. Proposta de resolução do Parlamento Europeu sobre a proteção da saúde pública contra os desreguladores endócrinos. http://www.europarl.europa.eu/sides/getDoc.do?pubRef=-//EP//TEXT+REPORT+A7-2013-0027+0+DOC+XML+V0//PT (accessed on 24/Jun/2015).
21. Heberer T. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicol Lett 2002; 131:5-17.
22. Cano A, Roura AC, Cortit LI. Farmacología de los anticonceptivos hormonales orales. In: Buil C, editor. Manual de anticoncepcíon hormonal oral. Zaragoza: Sociedad Española de Contracepción; 1997. p. 75-99.
23. Wu QY, Shao YR, Wang C, Sun HY, Hu H. Health risk induced by estrogens during unplanned indirect potable reuse of reclaimed water from domestic wastewater. Huan Jing Ke Xue 2014; 35:1041-50.
24. Esteban S, Gorga M, Petrovic M, González-Alonso S, Barceló D, Valcárcel Y. Analysis and occurrence of endocrine-disrupting compounds and estrogenic activity in the surface waters of Central Spain. Sci Total Environ 2014; 466-467:939-51.
25. Jin S, Yang F, Xu Y, Dai H, Liu W. Risk assessment of xenoestrogens in a typical domestic sewage-holding lake in China. Chemosphere 2013; 93:892-8.
26. Mills RM, Salazar KA, Baynes A, Shen LQ, Churchley J, Beresford N, et al. Removal of ecotoxicity of 17α-ethinylestradiol using TAML/peroxide water treatment. Sci Rep 2015; 5:10511.
27. Cong VH, Iwaya S, Sakakibara Y. Removal of estrogens by electrochemical oxidation process. J Environ Sci (China) 2014; 26:1355-60.

28. Brandt EMF, Queiroz FB, Afonso RJCF, Aquino SF, Chernicharo CAL. Behaviour of pharmaceuticals and endocrine disrupting chemicals in simplified sewage treatment systems. J Environ Manage 2013; 128:718-26.
29. Fent K, Weston AA, Caminada D. Ecotoxicology of human pharmaceuticals. Aquat Toxicol 2006; 76:122-59.
30. Ternes TA, Kreckel P, Mueller J. Behaviour and occurrence of estrogens in municipal sewage treatment plants-II. Aerobic batch experiments with activated sludge. Sci Total Environ 1999; 225:91-9.
31. Jobling S, Nolan M, Tyler CR, Brighty G, Sumpter JP. Widespread sexual disruption in wild fish. Environ Sci Technol 1998; 32:2498-506.
32. Atkinson SK, Marlatt VL, Kimpe LE, Lean DRS, Trudeau VL, Blais JM. Environmental factors affecting ultraviolet photodegradation rates and estrogenicity of estrone and ethinylestradiol in natural waters. Arch Environ Contam Toxicol 2011; 60:1-7.
33. Jurgens MD, Holthaus KIE, Johnson AC, Smith JJL, Hetheridge M, Williams RJ. The potential for estradiol and ethinylestradiol degradation in English rivers. Environ Toxicol Chem 2002; 21:480-8.
34. European Commission. The EU Water Framework Directive - integrated river basin management for Europe. http://ec.europa.eu/environment/water/water-framework/index_en.html (accessed on 19/Jun/2015).
35. European Commission. WISE - Water Information System Europe. Water Notes on the Implementation of the Water Framework Directive. http://ec.europa.eu/environment/water/participation/pdf/waternotes/WATER%20INFO%20NOTES%201%20-%20PT.pdf.
36. Directiva 2000/60/CE do Parlamento Europeu e do Conselho, de 23 de outubro de 2000, que estabelece um quadro de acção comunitária no domínio da política da água. Jornal Oficial das Comunidades Europeias. http://eur-lex.europa.eu/resource.html?uri=cellar:5c835afb-2ec6-4577-bdf8-756d3d694eeb.0009.02/DOC_1&format=PDF (accessed on 29/Jun/2015).
37. Decisão nº 2455/2001/CE do Parlamento Europeu e do Conselho, de 20 de novembro de 2001, que estabelece a lista das substâncias prioritárias no domínio da política da água e altera a Directiva 2000/60/CE. Jornal Oficial das Comunidades Europeias. http://eur-lex.europa.eu/legal-content/PT/TXT/PDF/?uri=CELEX:32001D2455&from=EN (accessed on 29/Jun/2015).
38. Directiva 2008/105/CE do Parlamento Europeu e do Conselho, de 16 de dezembro de 2008, relativa a normas de qualidade ambiental no domínio da política da água, que altera e subsequentemente revoga as Directivas 82/176/CEE, 83/513/CEE, 84/156/CEE, 84/491/CEE e 86/280/CEE do Conselho, e que altera a Directiva 2000/60/CE. Jornal Oficial das Comunidades Europeias. http://eur lex.europa.eu/legal-content/PT/TXT/PDF/?uri=CELEX:32008L0105&from=EN (accessed on 30/Jun/2015).
39. Proposta de Diretiva do Parlamento Europeu e do Conselho, que altera as Diretivas 2000/60/CE e 2008/105/CE no que respeita às substâncias prioritárias no domínio da política da água. http://eur lex.europa.eu/legal-content/PT/TXT/PDF/?uri=CELEX:52011PC0876&from=EN (accessed on 30/Jun/2015).
40. Proposal for a revised directive of the European Parliament and of the Council on Priority Substances in the field of water quality. http://europa.eu/rapid/press-release_MEMO-12-59_en.htm (accessed on 19/Jun/2015).
41. EurActiv. Commission to add pharma pollutants to water law. http://www.euractiv.com/sustainability/commission-add-new-chemicals-water-regulation-news-510511 (accessed on 19/Jun/2015).
42. Owen R, Jobling S. The hidden cost of flexible fertility. Nature 2012; 485:441.
43. Diretiva 2013/39/UE do Parlamento Europeu e do Conselho, de 12 de agosto de 2013, que altera as Diretivas 2000/60/CE e 2008/105/CE no que respeita às substâncias prioritárias no domínio da política da água. Jornal Oficial da União Europeia. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:226:0001:0017:PT:PDF (accessed on 19/Jun/2015).
44. Directiva 98/83/CE del Consejo, de 3 de noviembre de 1998, relativa a la calidad de las aguas destinadas al consumo humano. Diario Oficial de las Comunidades Europeas. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1998:330:0032:0054:ES:PDF (accessed on 19/Jun/2015).
45. U. S. Environmental Protection Agency. Summary of the Clean Water Act. http://www2.epa.gov/laws-regulations/summary-clean-water-act (accessed on 15/Jun/2015).
46. U. S. Environmental Protection Agency. Safe Drinking Water Act (SDWA). http://water.epa.gov/lawsregs/rulesregs/sdwa/index.cfm (accessed on 15/Jun/2015).
47. U. S. Environmental Protection Agency. Drinking water contaminants. http://water.epa.gov/drink/contaminants/ (accessed on 15/Jun/2015).
48. U. S. Environmental Protection Agency. EPA 816-F-09-004 – Referent national primary drinking water regulations. http://www.epa.gov/ogwdw/consumer/pdf/mcl.pdf (accessed on 01/Jul/2015).
49. U. S. Environmental Protection Agency. Drinking water contaminant Candidate List (CCL) and regulatory determination. http://www2.epa.gov/ccl (accessed on 15/Jun/2015).
50. Conselho Nacional do Meio Ambiente. Resolução CONAMA nº 357, de 17 de março de 2005. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. Diário Oficial da União 2005; 18 mar.
51. Conselho Nacional do Meio Ambiente. Resolução CONAMA nº 430, de 13 de maio de 2011. Dispõe sobre as condições e padrões de lançamento de efluentes, complementa e altera a Resolução no 357, de 17 de março de 2005, do Conselho Nacional do Meio Ambiente-CONAMA. Diário Oficial da União 2011; 16 mai.

52. Conselho Nacional do Meio Ambiente. Resolução CONAMA nº 396, de 3 de abril de 2008. Dispõe sobre a classificação e diretrizes ambientais para o enquadramento das águas subterrâneas e dá outras providências. Diário Oficial da União 2008; 7 abr.
53. Senado Federal. Decreto Legislativo nº 204. Aprova o texto da Convenção de Estocolmo sobre Poluentes Orgânicos Persistentes, adotada, naquela cidade, em 22 de maio de 2001. Diário Oficial da União 2004; 10 mai.
54. Ministério da Saúde. Portaria MS nº 2.914, de 12 de dezembro de 2011. Dispõe sobre os procedimentos de controle e de vigilância da qualidade da água para consumo humano e seu padrão de potabilidade. Diário Oficial da União 2011; 14 dez.
55. Umbuzeiro GA. Guia de potabilidade para substâncias químicas. São Paulo: Limiar; 2012.
56. Instituto Trata Brasil. Saneamento no Brasil. http://www.tratabrasil.org.br/saneamento-no-brasil (accessed on 22/Jun/2015).
57. Volkova K, Reyhanian NC, Porseryd T, Hallgren S, Dinnétz P, Porsch-Hällström I. Developmental exposure of zebrafish (*Danio rerio*) to 17-ethinylestradiol affects non-reproductive behavior and fertility as adults, and increases anxiety in unexposed progeny. Horm Behav 2015; 73:30-8.
58. Soares J, Coimbra AM, Reis-Henriques MA, Monteiro NM, Vieira MN, Oliveira JM, et al. Disruption of zebrafish (*Danio rerio*) embryonic development after full life-cycle parental exposure to low levels of ethinylestradiol. Aquat Toxicol 2009; 95:330-8.
59. Silva P, Rocha MJ, Cruzeiro C, Malhão F, Reis B, Urbatzka R, et al. Testing the effects of ethinylestradiol and of an environmentally relevant mixture of xenoestrogens as found in the Douro River (Portugal) on the maturation of fish gonads: a stereological study using the zebrafish (*Danio rerio*) as model. Aquat Toxicol 2012; 124-125:1-10.
60. Reyhanian N, Volkova K, Hallgren S, Bollner T, Olsson PE, Olsén H, et al. 17α-ethinyl estradiol affects anxiety and shoaling behavior in adult male zebra fish (*Danio rerio*). Aquat Toxicol 2011; 105:41-8.
61. Segner H, Caroll K, Fenske M, Janssen CR, Maack G, Pascoe D, et al. Identification of endocrine-disrupting effects in aquatic vertebrates and invertebrates: report from the European IDEA project. Ecotoxicol Environ Saf 2003; 54:302-14.
62. Bhandari RK, Vom Saal FS, Tillitt DE. Transgenerational effects from early developmental exposures to bisphenol A or 17α-ethinylestradiol in medaka, Oryzias latipes. Sci Rep 2015; 5:9303.
63. Hirakawa I, Miyagawa S, Katsu Y, Kagami Y, Tatarazako N, Kobayashi T, et al. Gene expression profiles in the test is associated with testis-ova in adult Japanese medaka (*Oryzias latipes*) exposed to 17α-ethinylestradiol. Chemosphere 2012; 87:668-74.
64. Metcalfe CD, Metcalfe TL, Kiparissis Y, Koenig BG, Khan C, Hughes RJ, et al. Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by in vivo assays with Japanese medaka (*Oryzias latipes*). Environ Toxicol Chem 2001; 20:297-308.
65. Lee PY, Lin CY, Chen TH. Environmentally relevant exposure of 17α-ethinylestradiol impairs spawning and reproductive behavior in the brackish medaka *Oryzias melastigma*. Mar Pollut Bull 2014; 85:338-43.
66. Parrott JL, Blunt BR. Life-cycle exposure of fathead minnows (*Pimephales promelas*) to an ethinylestradiol concentration below 1 ng/L reduces egg fertilization success and demasculinizes males. Environ Toxicol 2005; 20:131-41.
67. van Aerle R, Pounds N, Hutchinson TH, Maddix S, Tyler CR. Window of sensitivity for the estrogenic effects of ethinylestradiol in early life-stages of fathead minnow, *Pimephales promelas*. Ecotoxicology 2002; 11:423-34.
68. Salierno JD, Kane AS. 17alpha-ethinylestradiol alters reproductive behaviors, circulating hormones, and sexual morphology in male fathead minnows (*Pimephales promelas*). Environ Toxicol Chem 2009; 28:953-61.
69. Dzieweczynski TL, Campbell BA, Marks JM, Logan B. Acute exposure to 17α-ethinylestradiol alters boldness behavioral syndrome in female Siamese fighting fish. Horm Behav 2013; 66:577-84.
70. Zha J, Wang Z, Wang N, Ingersoll C. Histological alternation and vitellogenin induction in adult rare minnow (*Gobiocypris rarus*) after exposure to ethynylestradiol and nonylphenol. Chemosphere 2007; 66:488-95.
71. Garmshausen J, Kloas W, Hoffmann F. 17α-ethinylestradiol can disrupt hemoglobin catabolism in amphibians. Comp Biochem Physiol C Toxicol Pharmacol 2015; 171:34-40.
72. Hoffmann F, Kloas W. Estrogens can disrupt amphibian mating behavior. PLoS One 2012; 7:e32097.
73. Hoffmann F, Kloas W. The antiestrogens tamoxifen and fulvestrant abolish estrogenic impacts of 17α-ethinylestradiol on male calling behavior of Xenopus laevis. PLoS One 2012; 7:e44715.
74. Watts MM, Pascoe D, Carroll K. Population responses of the freshwater amphipod *Gammarus pulex* (L.) to an environmental estrogen, 17alpha-ethinylestradiol. Environ Toxicol Chem 2002; 21:445-50.
75. Luna TO, Plautz SC, Salice CJ. Chronic effects of 17α-ethinylestradiol, fluoxetine, and the mixture on individual and population-level end points in *Daphnia magna*. Arch Environ Contam Toxicol 2015; 68:603-11.
76. Jaser W, Severin GF, Jütting U, Jüttner I, Schramm KW, Kettrup A. Effects of 17alpha-ethinylestradiol on the reproduction of the cladoceran species *Ceriodaphnia reticulata* and *Sida crystallina*. Environ Int 2003; 28:633-8.
77. Giusti A, Lagadic L, Barsi A, Thomé JP, Joaquim-Justo C, Ducrot V. Investigating apical adverse effects of four endocrine active substances in the freshwater gastropod *Lymnaea stagnalis*. Sci Total Environ 2014; 493:147-55.
78. Kiyomoto M, Kikuchi A, Morinaga S, Unuma T, Yokota Y. Exogastrulation and interference with the expression of major yolk protein by estrogens administered to sea urchins. Cell Biol Toxicol 2008; 24:611-20.

Resumo

O estrogênio sintético 17α-etinilestradiol, principal componente utilizado em formulações de contraceptivos orais, tem sido apontado como um dos principais compostos responsáveis por provocar efeitos adversos no sistema endócrino de várias espécies. O objetivo deste estudo foi analisar o estado da arte dos dispositivos legais e normativos referentes ao controle desse estrogênio sintético nas águas da Europa e dos Estados Unidos, e traçar um paralelo com a realidade brasileira. No geral, os países têm buscado ampliar a regulamentação e monitoramento de alguns micropoluentes emergentes que antes não eram objeto de atenção por parte dos dispositivos legais. A Europa está mais avançada no que tange à qualidade dos corpos hídricos, enquanto que nos Estados Unidos esta substância é alvo de regulamentação apenas para a água destinada ao consumo humano. No Brasil, ainda não há nenhum dispositivo legal ou normativo que aborde esse estrogênio, o que pode ser associado a uma baixa maturidade do sistema brasileiro quanto ao controle de poluentes hídricos.

Disruptores Endócrinos; Etinilestradiol;
Critérios de Qualidade da Água

Resumen

El estrógeno sintético 17α-etinilestradiol, principal componente utilizado en fórmulas de contraceptivos orales, ha sido apuntado como uno de los principales compuestos responsables por provocar efectos adversos en el sistema endócrino de varias especies. El objetivo de este estudio fue analizar el estado de la cuestión de los dispositivos legales y normativos referentes al control de este estrógeno sintético en las aguas de Europa y de los Estados Unidos, y trazar un paralelo con la realidad brasileña. En general, los países han buscado ampliar la regulación y el monitoreo de algunos micro-contaminantes emergentes que antes no eran objeto de atención por parte de los dispositivos legales. Europa está más avanzada en lo que se refiere a la calidad de los cuerpos hídricos, mientras que en los Estados Unidos esta substancia es objeto de regulación solamente para el agua destinada al consumo humano. En Brasil todavía no existe ningún dispositivo legal o normativo que aborde este estrógeno, lo que puede ser asociado a una inmadurez del sistema brasileño respecto al control de contaminantes hídricos.

Disruptores Endocrinos; Etinilestradiol;
Criterios de Calidad del Agua

Submitted on 10/Apr/2015
Final version resubmitted on 03/Sep/2015
Approved on 21/Sep/2015