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Removal of endocrine-disrupting chemical mixtures in water using chlorination and photolysis

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ABSTRACT

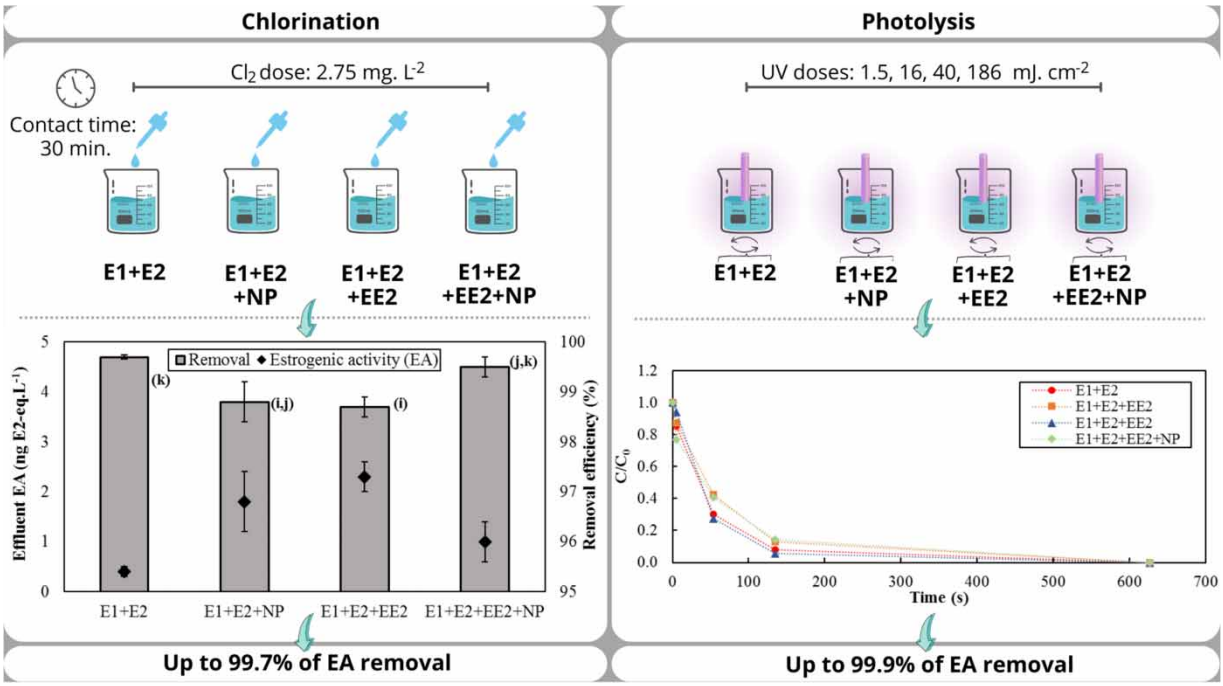
Micropollutants have been continuously detected in freshwater. In parallel, the potential adverse effects of human exposure to endocrine-disrupting chemicals (EDCs) through drinking water have been gaining the attention of researchers and health authorities. Given this fact, this study aimed to evaluate the effectiveness of chlorination and photolysis to remove the estrogenic activity caused by mixtures of EDCs in water: estrone (E1: 100 ng L^{-1}), 17β-estradiol (E2: 100 ng L^{-1}), ethinylestradiol (EE2: 50 ng L^{-1}), and nonylphenol (NP: 1,000 ng L^{-1}) under operating conditions applicable for water treatment plants. The tests were performed using freshwater spiked with the following mixtures: E1 + E2, E1 + E2 + EE2, E1 + E2 + NP, and E1 + E2 + EE2 + NP). Removal efficiencies of up to 99.7% were achieved at a chlorine dose of 2.75 mg L^{-1} and 30 min of contact time. In photolysis, estrogenic activity removal was higher than 99.9% at a UV dose of 186 mJ cm^{-2}. Results indicated that both chlorination and photolysis can be efficient to remove the estrogenic activity caused by the tested EDC mixtures in water. Furthermore, experiments suggested that EDC mixtures can be efficiently removed at feasible water disinfection operating conditions.

Key words: disinfection, hormones, micropollutants, oxidation, surfactants, water treatment

HIGHLIGHTS

- Removal of E1, E2, EE2, and nonylphenol from water was investigated.
- Tests were carried out at conditions applicable for water disinfection.
- Chlorination removed up to 99.7% of the estrogenic activity.
- Efficiencies of up to 99.9% were achieved using photolysis.

GRAPHICAL ABSTRACT



INTRODUCTION

Concerns about water quality emerged at the end of the 19th century when the physician John Snow evidenced cholera as a waterborne disease. Thereafter, the major objective of water treatment has been to minimize the risks caused by pathogens in water (Libânio 2010; Jain *et al.* 2014). Until now, drinking water treatment plants are still designed to remove microorganisms and improve the organoleptic properties (e.g., color and turbidity) of water, whereas chemical substances that may pose risks to human health usually are not the focus of drinking water treatment (Jain *et al.* 2014; Pai *et al.* 2020).

Within this scenario, endocrine-disrupting chemicals (EDCs) are commonly not included in drinking water legislations (Aquino *et al.* 2021). However, these substances are capable to affect the endocrine system of humans and animals (Pereira *et al.* 2013). The risks associated with EDCs exposure are still not fully understood (Wee & Aris 2019). Despite this, several studies reported associations between exposure to EDCs and alterations in the reproductive, endocrine, and digestive systems (Colborn *et al.* 1993; Safe 2000; AWWA 2007; Gallo *et al.* 2016; Sheikh *et al.* 2016, 2017; Sweeney *et al.* 2016; USEPA 2016; Vilela *et al.* 2018).

Currently, diverse chemical substances are classified as EDCs, such as some natural and synthetic hormones, pesticides, surfactants, plasticizers, pharmaceuticals, and personal care products (Bila & Dezzotti 2007; Aquino *et al.* 2013). Due to their estrogenic activity potency and their growing use, some of these substances have been gaining special attention from the scientific community and public health agencies. Among these substances, estrone (E1), 17β-estradiol (E2), ethinylestradiol (EE2), and nonylphenol (NP) can be cited. E1 and E2 are natural hormones produced and excreted by humans and animals, which can also be used to prevent and control disorders in the female reproductive system (NIH 2022). EE2 is a synthetic estrogen that is widely used in contraceptives and hormone replacement therapies (NIH 2022). In addition, NP is known as the major degradation product of NP ethoxylates, which are nonionic surfactants (Kim 2014).

These EDCs can be released into water sources through raw and treated wastewaters discharge or even through runoff from animal-feeding operations (Ternes *et al.* 1999; Aquino *et al.* 2013; Gomes *et al.* 2022). As a consequence of their massive use, E1, E2, EE2, and NP have been frequently found in water sources and drinking waters (Ding & Tzing 1998; Snyder *et al.* 1999; Kolpin *et al.* 2002; Johnson & Jürgens 2003; Moreira *et al.* 2009, 2011; Resende *et al.* 2017; Aquino *et al.* 2021).

Given the increasing concern about EDCs, many analytical methods to detect and quantify these compounds in environmental matrices have been developed in the last decades (Routledge & Sumpter 1996; Alda & Barceló 2001; Magi *et al.* 2010;

Gunatilake *et al.* 2014; Lopardo *et al.* 2019; Martin-Yken 2020). In this context, chromatographic methods and *in vitro* and *in vivo* assays have been widely used to analyze EDCs in waters (Robitaille *et al.* 2022). *In vitro* assays can evaluate the estrogenic activity of a sample based on mechanisms of action of EDCs, such as the case of cell proliferation assays (Shanle & Xu 2011; Serra *et al.* 2020). A noticeable example is the *in vitro* yeast estrogen screen (YES), developed by Routledge & Sumpter (1996). The YES assay is capable to quantify a wide range of estrogenic activity concentrations resulting from the synergic effect of the different EDCs present in a sample, as occurs in the environment (Routledge & Sumpter 1996). Its additional advantages include low cost, operational simplicity, and relatively low detection limits (Martin-Yken 2020).

One factor that contributes to the ubiquitous presence of EDCs in the environment is the inefficiency of some treatment processes allied to their continued environmental release. For instance, water clarification is ineffective for EDCs removal (Chen *et al.* 2007; Azevedo *et al.* 2020; Pai *et al.* 2020). However, depending on the process and the operational conditions used, these compounds can be removed during water disinfection (WHO 2011). In this context, chlorination and photolysis have been increasingly studied for this purpose. Previous researches evidence that both chlorination and photolysis may lead to the removal of EDCs (Alum *et al.* 2004; Coleman *et al.* 2004; Westerhoff *et al.* 2005; Fonseca *et al.* 2011; Pereira *et al.* 2011, 2013; Schenck *et al.* 2012; Sarkar *et al.* 2014; Li *et al.* 2017; Huang *et al.* 2022). However, there is a lack of studies that evaluate the removal of the estrogenic activity caused by mixtures of EDCs during disinfection under technically and economically viable conditions.

Thus, the objective of this work was to evaluate the efficiency of chlorination and photolysis for the estrogenic activity removal resulting from mixtures of E1, E2, EE2, and NP in water.

METHOD

Reagents

E1 (≥99%), E2 (≥98%), EE2 (≥98%), NP (analytical standard), and sodium hypochlorite solution (10–15%) were purchased from Sigma-Aldrich. Acetone, hexane, hydrochloric acid, methanol, nitric acid, absolute ethanol, hydrogen peroxide, and the reagents used in the YES assay were purchased from Synth.

Water sampling and characterization

The water used in the experiments was sampled in a preserved area located in Torreões (Minas Gerais, Brazil). The sample was analyzed for pH, electrical conductivity (EC), apparent color, turbidity, total hardness, total carbon (TC), total organic carbon (TOC), and free and total chlorine. These analyses were conducted in triplicate (except for total hardness, TC, and TOC) and were performed according to the Standard Methods for the Examination of Water and Wastewater (APHA *et al.* 2005). Additionally, the sample was also tested to certify the absence of estrogenic activity, using the *in vitro* YES assay (Routledge & Sumpter 1996) (as described in item 2.2.2). The basic physicochemical properties of the water used in the experiments were as follows: apparent color = 12.8 ± 4.0 uH; turbidity = 0.1 ± 0.0 NTU; pH = 7.9 ± 0.5; EC = 18.6 ± 2.8 μS cm^{-1}; total hardness = 17 mg$CaCO_3$ L^{-1}; TC = 3.0 mg L^{-1}; and TOC = 0.18 mg L^{-1}.

Solid phase extraction

Before the YES assays, samples were extracted with BondElut C18 (500 mg, 6 mL) cartridges (Agilent). First, 600 mL of water samples were acidified to pH 3.0 with hydrochloric acid. The acidified samples were passed through the extraction cartridges, which were previously conditioned with 6.0 mL of hexane, 2.0 mL of acetone, 6.0 mL of methanol, and 10 mL of acidified deionized water (pH = 3.0). Subsequently, analytes were eluted with 4.0 mL of acetone and the eluates were evaporated under a gentle nitrogen flow. The SPE procedure followed the method described by Pereira (2011). Before the YES assays, analytes were resuspended in 1.0 mL absolute ethanol (Bila *et al.* 2007).

In vitro YES assay

The *in vitro* YES assay was used to quantify the estrogenic activity in water samples. In summary, the *in vitro* YES assay uses a recombinant yeast strain (*Saccharomyces cerevisiae*) developed by the Genetics Department, Glaxo. The yeast strain contains the human estrogen receptor (hER) and expression plasmids carrying the reporter gene lac-Z, which is capable to encode the enzyme β-galactosidase in the presence of EDCs. Subsequently, the β-galactosidase degrades the substrate chlorophenol red-b-D-galactopyranoside (CPRG) added in the medium into chlorophenol red, promoting a color change from yellow to red (Routledge & Sumpter 1996). Therefore, the estrogenic activity (in E2 equivalents, E2-eq) can be estimated by comparing

the absorbance of the medium to the standard curve generated from the absorbances of the assay using known concentrations of E2 (26.6 ng L^{-1} to 54.48 μg L^{-1}).

Sample preparation

The efficiencies of chlorination and photolysis in estrogenicity removal were tested using freshwater spiked with four different mixtures of EDCs: E1 + E2, E1 + E2 + NP, E1 + E2 + EE2, and E1 + E2 + EE2 + NP. In all conditions tested, initial concentrations of E1 and E2 were 100 ng L^{-1}, whereas concentrations of EE2 and NP were 50 and 1,000 ng L^{-1}, respectively. These concentrations were chosen to represent real concentrations found in surface waters, based on previous values reported in the literature (Ding & Tzing 1998; Snyder *et al.* 1999; Kolpin *et al.* 2002; Johnson & Jürgens 2003; Moreira *et al.* 2009, 2011; Resende *et al.* 2017; Aquino *et al.* 2021).

Experimental procedure

Experiments were carried out in the Environmental Quality Laboratory (LAQUA) of the Federal University of Juiz de Fora (UFJF). Each condition described below (i.e., different EDC mixtures and UV doses) was tested in triplicate.

Chlorination

Batch experiments were carried out in a beaker of 1 L containing 700 mL of EDCs solution. A chlorine dose of 2.75 mg L^{-1}, which is comparable to those usually used in drinking water treatment plants (0.2–5 mg L^{-1} (Brazil, 2021; WHO 2017)), was assessed in this work. The chlorine dose was obtained from a sodium hypochlorite stock solution with 1,000 mg L^{-1} Cl_2, which was preserved in an amber bottle at 4 °C. The contact time of chlorination was fixed at 30 min. Considering that the chlorine dose can vary according to the working temperature, the chlorine dose applied in the experiments was calibrated before the experiments. The removal of residual chlorine after the tests was carried out by the addition of 0.1-mL sodium metabisulphite 3% (m/V) for each 100-mL sample.

Photolysis

Photolysis experiments were performed in an 800 mL bench-scale reactor equipped with a low-pressure mercury lamp of 80 W placed in a quartz tube and submerged in an axial position in the samples. During the experiments, the system was maintained by continuous stirring with a magnetic stirrer. To maintain steady state conditions during the tests, the lamp was kept on for 15 min before the experiments (Nicole *et al.* 1990; Huang *et al.* 2022).

The UV light intensity (i) was determined by hydrogen peroxide (H_2O_2) actinometry, as described by Nicole *et al.* (1990). The method consists of evaluating the decay of the absorbance of an H_2O_2 solution with a known concentration (0.05 M) at its maximum wavelength (221 nm) over time. The absorbance was measured at 0, 3, 4, 5, 6, 7.5, 10, 12.5, 15, 20, 25, and 30 min. Then, the contact times (*t*–*s*) used in this work were estimated by Equation (1).

$$D = i \cdot t \tag{1}$$

where i is the UV light intensity ($mW \cdot cm^{-2}$) and D is the UV dose ($mJ \cdot cm^{-2}$).

The UV doses used in this work (1.5, 16, 40, and 186 $mJ \cdot cm^{-2}$) were chosen according to values previously established for disinfection processes (Brazil, 2021; LeChevallier & Au, 2004; NSF/ANSI 2021). The dose of 1.5 $mJ \cdot cm^{-2}$ is determined by the Brazilian drinking water standard as the minimum UV dose required for drinking water disinfection (Brazil, 2021). The UV dose of 16 $mJ \cdot cm^{-2}$ is established by the American National Standards Institute (ANSI) and it is recommended for supplemental treatment of waters that are already of good quality (Class B systems) (NSF/ANSI 2021). The dose of 40 $mJ \cdot cm^{-2}$ is recommended by the same institute (ANSI) for the removal of cryptosporidium, giardia, bacteria, and viruses from contaminated water (NSF/ANSI 2021). Finally, the dose of 186 $mJ \cdot cm^{-2}$ is recommended for the removal of 99.99% of bacteria and viruses from water according to a guide for water treatment and pathogen control by the World Health Organization (WHO) and The International Water Association (IWA) (LeChevallier & Au, 2004).

Removal kinetics

Previous studies indicate that EDCs removal usually follows pseudo first-order kinetics (Benotti *et al.*, 2009; Fernández *et al.* 2014; Ren *et al.* 2017). Thus, the removal rate (k) of estrogenic activity under different operating conditions was calculated based on the reaction time (t) and initial and final estrogenic activity concentrations (C_0 and C, respectively) (Equation (2)).

Subsequently, the estrogenic activity half-life ($t_{1/2}$) in each EDC mixture studied was estimated by Equation (3).

$$\ln\frac{C}{C_0} = -kt \tag{2}$$

$$t_{1/2} = \frac{\ln 2}{k} \tag{3}$$

Data analysis

Analysis of variance (ANOVA) and the Tukey test were used to compare estrogenicity removal efficiencies of chlorination and photolysis under different conditions at a 95% confidence level. For the case of chlorination, these analyses were used to compare estrogenic activity removal efficiencies from aqueous solutions with different mixtures of EDCs (E1 + E2, E1 + E2 + NP, E1 + E2 + EE2, and E1 + E2 + EE2 + NP). In the case of photolysis, ANOVA and Tukey test were carried out to assess statistically significant differences between the removal of estrogenic activity from both solutions with different mixtures of EDCs and during photolysis at different UV doses. These analyses were performed using SISVAR software.

RESULTS AND DISCUSSION

Chlorination

The physicochemical properties of the water before and after chlorination were summarized in Table S1 (Supplementary Material). As can be seen, in terms of the parameters analyzed, the water used in the experiments had good quality, and its general physicochemical properties were comparable to waters sampled after post-filtration in drinking water treatment plants (Brazil, 2021).

In all conditions tested, pH, apparent color, and turbidity remained practically unaltered after chlorination (Table S1, Supplementary Material). In contrast, EC at the end of the tests was about two-fold higher than in the freshwater, probably due to the addition of chlorine ions in the samples.

After chlorination, the concentration of free chlorine in the E1 + E2 solution was higher than in the other tests. This fact is expected, since a minor concentration of EDCs (100 ng · L^{-1} E1 and 100 ng · L^{-1} E2) were available to react with chlorine in this test. The solution prepared with a mixture of E1 + E2 + EE2 had the second highest concentration of free chlorine after chlorination, due to the less concentration of EDCs in the test (100 ng · L^{-1} E1, 100 ng · L^{-1} E2, and 50 ng · L^{-1} EE2) in comparison with E1 + E2 + NP (100 ng · L^{-1} E1, 100 ng · L^{-1} E2, and 50 ng · L^{-1} EE2) and E1 + E2 + EE2 + NP (100 ng · L^{-1} E1, 100 ng · L^{-1} E2, 50 ng · L^{-1} EE2, and 1,000 ng · L^{-1} NP). Accordingly, the concentration of free chlorine reached 1.2 mg · L^{-1} in the E1 + E2 + EE2 + NP test, as a result of the high reactivity of this solution in comparison with the other conditions tested. Furthermore, concentrations of total chlorine after chlorination varied from 2.2 to 2.5 mg · L^{-1}, whereas the combined chlorine varied from 0.1 to 0.9 mg · L^{-1}. This indicates a relatively low chlorine demand in the conditions studied (Smith *et al.* 2021).

Removal of estrogenic activity varied from 98.7 to 99.7%, with a maximum residual concentration of 2.3 ng E2-eq. L^{-1} (Figure 1). Statistically significant differences ($\alpha = 0.05$) between the removal efficiencies achieved during chlorination in the presence of different EDC mixtures were evidenced by ANOVA. As evidenced by the Tukey test, chlorination in the presence of E1 + E2 + NP and E1 + E2 + EE2 led to relatively low estrogenic activity removal efficiencies compared to the other experimental conditions, whereas median removal rates occurred for E1 + E2 + NP and E1 + E2 + EE2 + NP, and the highest removal efficiencies were observed for E1 + E2 and E1 + E2 + EE2 + NP. Despite this, the numerical difference between the lower (98.7%) and the higher (99.7%) removal efficiency of chlorination under the distinct experimental conditions was relatively small, resulting in a slight variation in the estrogenic activity effluent concentration (from 2.3 to 0.4 ng E2-eq. L^{-1}).

As can be seen, the highest removal efficiency (99.7%) occurred in the experiment with E1 + E2, which resulted in a residual estrogenic activity of 0.4 ng E2-eq. L^{-1}. Noticeably, the removal efficiency decreased to 98.8% when adding NP to this mixture (E1 + E2 + NP). As the relative estrogenic activity potency of NP is about 2.57×10^5 times lower than E2 (Beck *et al.* 2006), the addition of NP did not increase substantially the initial estrogenic activity of the solution. Nevertheless, the presence of NP available to react with chlorine resulted in a reduction in estrogenic activity removal efficiency and, consequently, in a higher effluent concentration (1.4 ng E2-eq. L^{-1}), which can indicate the formation of chlorinated by-products that can cause estrogenic activity in the final effluent. A comparable outcome occurred in the experiment with E1 + E2 + EE2, which had a removal efficiency of 98.7%. As EE2 has a relative estrogenic activity potency of about 0.75–2.46

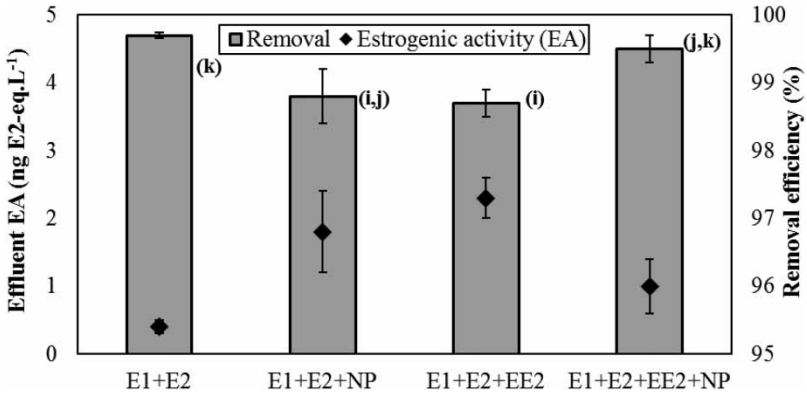


Figure 1 | Estrogenic activity (EA) effluent concentrations and removal efficiencies during chlorination of water spiked with different mixtures of endocrine disrupting compounds (EDCs). *Notes*: Different indexes *i*, *j*, and *k* represent statistically significant differences between the removal efficiencies at a 95% confidence level. E1 + E2: water spiked with 100 ng · L^{-1} of E1 and 100 ng · L^{-1} of E2. E1 + E2 + NP: water spiked with 100 ng · L^{-1} of E1, 100 ng · L^{-1} of E2, and 1,000 ng · L^{-1} of NP. E1 + E2 + EE2: water spiked with 100 ng · L^{-1} of E1, 100 ng · L^{-1} of E2, and 50 ng · L^{-1} of EE2. E1 + E2 + EE2 + NP: water spiked with 100 ng · L^{-1} of E1, 100 ng · L^{-1} of E2, 50 ng · L^{-1} of EE2, and 1,000 ng · L^{-1} of NP. Values were expressed as mean ± standard deviation of three replicates.

(Beck *et al.* 2006; Combalbert & Hernandez-Raquet, 2010), the initial estrogenic activity increased due to the addition of EE2, leading to an estrogenic activity of 2.3 ng E2-eq. L^{-1} after chlorination. Surprisingly, the addition of NP and EE2 (E1 + E2 + EE2 + NP) did not affect the average removal efficiency of chlorination which was maintained equal to 99.5%, with a residual estrogenic activity concentration of 1.0 ± 0.4 ng E2-eq. L^{-1}. This is interesting, as EDCs tend to occur simultaneously in the environment. Therefore, these results evidence that chlorination can be an effective process to remove the estrogenic activity from complex matrices such as freshwater.

The remaining estrogenic activity after chlorination obtained in this study agreed with values reported in the literature. Fan *et al.* (2013) assessed the presence of EDCs in finished waters from 62 drinking water treatment plants in China. In their work, E1 and E2 were detected in 53 plants, with maximum concentrations of 0.1 and 1.7 ng · L^{-1}, respectively, whereas NP was found in 55 plants with concentrations of up to 558 ng · L^{-1}. A similar study was conducted in Brazil by Gerolin (2008), in which E1, E2, EE2, and NP were identified at concentrations of 0.1, 1.48, 472, and 87 ng · L^{-1}, respectively. In Spain, Rodriguez-Mozaz *et al.* (2004) detected EE2 at a concentration of 2.5 ng · L^{-1}, which is lower than the value reported by Gerolin (2008) and more concordant with the concentrations obtained in the present study.

Despite the satisfactory results, the risks associated with human exposure to estrogens are not fully understood. Therefore, it is not possible to affirm that the remaining estrogenic activity determined in this work is not like to affect human health. Different values for the acceptable daily intake (ADI) of EDCs and their possible guideline values in drinking water have been proposed. ADIs from 13 to 50 ng · kg^{-1} body weight d^{-1} were established for E1 by previous toxicological studies, which evidenced estrogenic responses and effects on the endocrine system and liver of humans with no-observed-adverse-effect levels (NOAEL) varying from 0.004 to 0.005 ng · kg^{-1} body weight d^{-1} (AWWA 2007; USEPA 2016). For the case of E2, an ADI of 50 ng · kg^{-1} body weight d^{-1} was established based on the promotion of serum levels of follicle-stimulating hormone, angiotensinogen, sex hormone binding globulin, and corticosteroid-binding globulin in women (AWWA 2007; EPHC/NRMMC/AHMC, 2008; FAO/WHO, 2000). Additionally, dose–response studies evidenced the development of breast, uterus, and mesentery cancers in rats after exposure to E2, with a slope factor of 1/39,000 (USEPA 2016). More restrictive ADIs, from 0.1 to 5 ng · kg^{-1} body weight d^{-1}, were established for EE2 based on its effects on the endocrine system and liver of humans (AWWA 2007; USEPA 2016). In contrast, ADIs from 15,000 to 50,000 ng · kg^{-1} body weight d^{-1} were estimated for NP, based on effects on the reproductive system and decreases in body and organ weight of rats (EU, 2002; AWWA 2007; EMEA, 2015; USEPA 2016). Considering these ADIs, possible guideline values for maximum acceptable concentrations of E1, E2, EE2, and NP in drinking waters may vary from 78 to 300 ng · L^{-1} (E1), 8.0 to 300 ng · L^{-1} (E2), 3.0 to 150 ng · L^{-1} (EE2), and 90,000 to 300,000 ng · L^{-1} (NP) (Aquino *et al.* 2021).

Previous studies evidenced that the presence of chlorine can promote the removal of EDCs from water by aromatic halogenation and removal efficiencies of up to 98% had already been reported (Hu *et al.* 2003; Alum *et al.* 2004; Westerhoff *et al.* 2005; Choi *et al.* 2006; Pereira *et al.* 2011; Schenck *et al.* 2012; Li *et al.* 2017; Shao *et al.* 2018). However, the initial

concentrations of EDCs used in these studies were at least 10 times higher than those used in the present work. This fact suggests that chlorination can be effective to remove EDCs even at low concentrations which are more likely to be found in the environment. The Europe Union (EU) established a watch list of substances and compounds of concern for water intended for human consumption in the revised Drinking Water Directive (EU Directive 2020/2184) (EU, 2020). In this watch list E2 and NP have guidance values of 1 and 300 $ng \cdot L^{-1}$, respectively (EU, 2020). Values considering the worst case, for E2, that was achieved only for the E1 + E2 mixture.

It is worth mentioning that chlorination of EDCs may lead to the formation of different by-products and the toxicity of many of these products is still not well described in the literature (Bila *et al.* 2007; Pereira *et al.* 2011; He *et al.* 2016; Li *et al.* 2017; Leusch *et al.* 2019). According to Leusch *et al.* (2019), by-products formed during chlorination are unlikely to increase estrogenic activity. However, their findings suggest that these by-products may be more reactive and have increased non-specific toxicity (Leusch *et al.* 2019). It is also important to highlight that the potential by-products from the reaction between chlorine and EDCs may include trihalomethanes and halogenic acetic acids (He *et al.* 2016; Li *et al.* 2017).

Photolysis

In this work, the UV light intensity was estimated as 0.297 $mW \cdot cm^{-2}$, which is near to the lower value used by Zhang *et al.* (2010) (0.246 $mW \cdot cm^{-2}$) and lower than other light intensities reported in the literature (1.5–500 $mW \cdot cm^{-2}$) (Caupos *et al.* 2011; Chowdhury *et al.* 2011; Kovacic *et al.* 2018; Li *et al.* 2013; Liu & Liu 2004; Liu *et al.* 2017; Martínez-Zapata *et al.* 2013; Mboula *et al.* 2015; Zhang *et al.* 2010). After determining the light intensity, the times required to provide the tested UV doses were estimated as follows: 5 s for the dose of 1.5 $mJ \cdot cm^{-2}$, 54 s for the dose of 16 $mJ \cdot cm^{-2}$, 75 s for 40 $mJ \cdot cm^{-2}$, and 626 s for 186 $mJ \cdot cm^{-2}$. As expected, photolysis did not lead to major changes in the basic physicochemical properties of water in all combinations and doses tested (Table S2).

Previous researches evidence that pH may affect photolysis efficiency (Neamţu & Frimmel 2006). Some studies suggest that photolysis in alkaline media may result in slightly increased EDCs removal efficiencies (Liu & Liu 2004; Neamţu & Frimmel 2006), but this is not a consensus (Chowdhury *et al.*, 2011). Thus, as a preliminary analysis on the effectiveness of photolysis in the proposed scenario, no adjustments in pH before photolysis were performed in this work. Given this fact and considering possible costs related to the adjustment of pH prior to photolysis in a real scale operation, further analyses on the effects of changes in pH on photolysis at the proposed optimal conditions and the potential technical and economic aspects are recommended. Water temperature was higher for the dose of 186 mJ cm^{-2} than for the other doses, as a response of the heat generated during UV light use. In fact, the use of cooling systems can be required for large exposure times.

Photolysis efficiently removed estrogenic activity induced by all mixtures of EDCs tested particularly at the UV doses of 40 (85.5–94.4%) and 186 $mJ \cdot cm^{-2}$ (>99.9%) (Figure 2). Significantly increases ($\alpha = 0.05$) in removal efficiencies were observed with the increase of UV doses from 1.5 to 40 $mJ \cdot cm^{-2}$.

The lowest dose tested, which is recommended for disinfection by the Brazilian drinking water standard (Brazil, 2021) was capable to remove 6–23% of estrogenic activity, with effluent estrogenic activity of up to 138 ng E2-eq. $\cdot L^{-1}$. The UV doses established by the American National Standards Institute (NSF/ANSI 2021) (16–40 $mJ \cdot cm^{-2}$) led to removals from 57.8 to 94.4%. As aforementioned, the dose of 40 $mJ \cdot cm^{-2}$ is recommended to remove viruses and pathogenic bacteria from water. Regarding estrogenic activity, concentrations of up to 24 ng E2-eq. $\cdot L^{-1}$ remained after photolysis. This value is higher than possible health-based guideline values for E2 and EE2 (8 and 3 $ng \cdot L^{-1}$) (Aquino *et al.* 2021), which suggests that the refereed UV dose (40 $mJ \cdot cm^{-2}$) may not be sufficient to minimize potential risks of the exposure to EDCs through drinking water intake. On the other hand, the dose recommended by ANSI was sufficient to remove the estrogenic activity from the mixtures with a remaining concentration of less than the method detection limit (approximately 1 $ng \cdot L^{-1}$). For this, an exposure time of 10 min and 26 s was required. However, it is worth to mention that this time can be reduced by providing a high UV light intensity.

High removal efficiencies of the studied EDCs during photolysis had already been reported in the literature. However, EDC concentrations tested in earlier studies were considerably higher (10–1,950 $\mu g \cdot L^{-1}$ for estrogens and up to 10 $mg \cdot L^{-1}$ for NP) than those tested in the present work (in the 50–100 $ng \cdot L^{-1}$ for estrogens and 1,000 $ng \cdot L^{-1}$ for NP) (Zhang *et al.* 2010; Martínez-Zapata *et al.* 2013; Carlson *et al.* 2015 Huang *et al.* 2022). One of the major reasons for using relatively high initial concentrations during experiments to assess efficiencies of treatment processes can be associated with the dependency on analytical methods capable to detect EDCs at low concentrations. Within this context, in the case of EDCs with relatively high estrogen activity potency (e.g., E2 and EE2) or mixtures of EDCs, methods to detect/quantify estrogenic

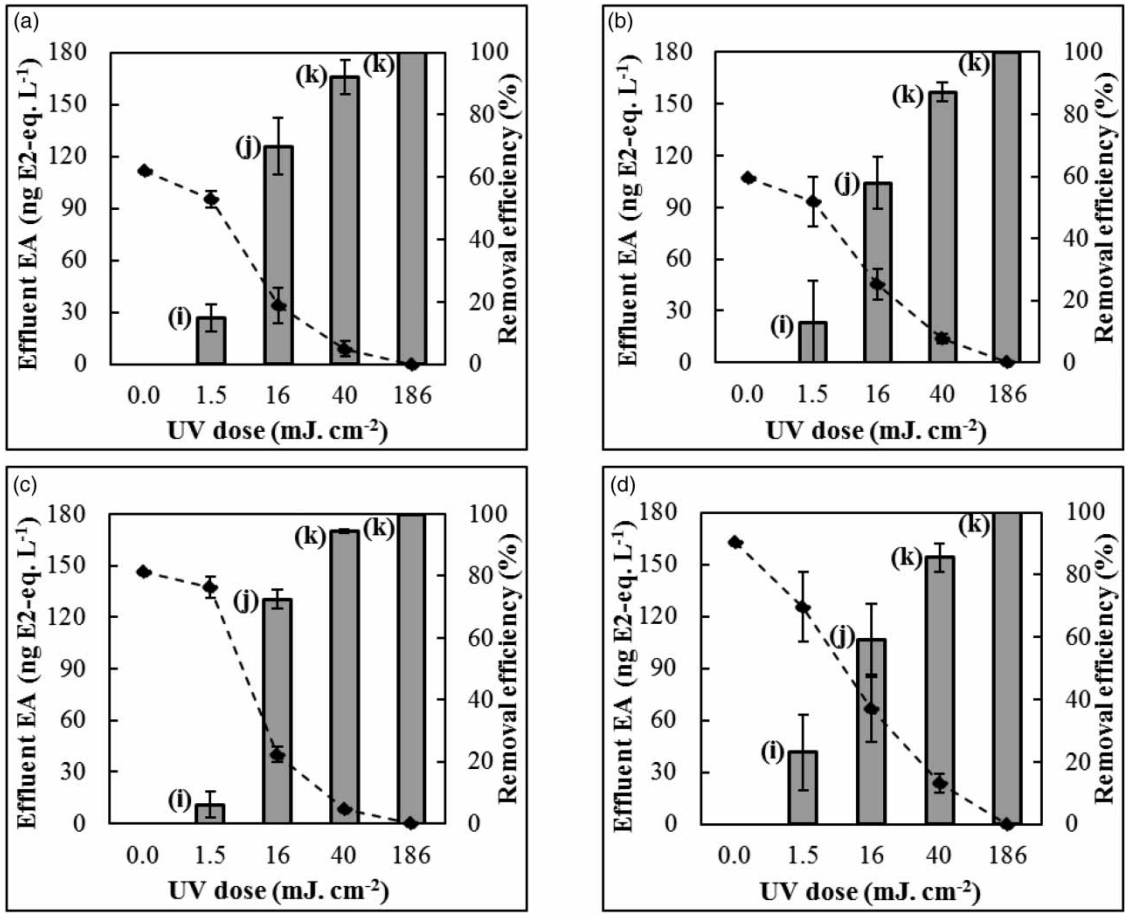


Figure 2 | Estrogenic activity (EA) effluent concentrations and removal efficiencies during photolysis of water spiked with different mixtures of endocrine disrupting compounds (EDCs): (a) E1 + E2; (b) E1 + E2 + NP; (c) E1 + E2 + EE2; (d) E1 + E2 + EE2 + NP. Notes: E1 + E2: water spiked with 100 ng · L^{-1} of E1 and 100 ng · L^{-1} of E2. E1 + E2 + NP: water spiked with 100 ng · L^{-1} of E1, 100 ng · L^{-1} of E2, and 1,000 ng · L^{-1} of NP. E1 + E2 + EE2: water spiked with 100 ng · L^{-1} of E1, 100 ng · L^{-1} of E2, and 50 ng · L^{-1} of EE2. E1 + E2 + EE2 + NP: water spiked with 100 ng · L^{-1} of E1, 100 ng · L^{-1} of E2, 50 ng · L^{-1} of EE2, and 1,000 ng · L^{-1} of NP. Values were expressed as mean ± standard deviation of three replicates.

activity (e.g., YES assay) can be useful tools for assessing processes efficiency at initial concentrations comparable to those which naturally occur in the environment.

Estrogenic activity removal followed a pseudo first-order kinetic in all combinations tested (Figure 3), as expected (Benotti *et al.*, 2009; Fernández *et al.*, 2014; Ren *et al.* 2017). The highest rate constant ($k = 1.296$ min^{-1}) was observed for the mixture of E1 + E2 + EE2, evidencing that the degradation of these compounds by photolysis undergo more rapidly (Table 1). The rate constants estimated in this work were higher than values reported in the literature. This fact may be related to the large discrepancy between the initial concentration used in this and in previous studies. Consequently, the half-life times (i.e., the time required to remove 50% of the initial concentration) of estrogenic activity observed in the conditions tested in this work were notoriously higher than those reported in earlier studies.

Carlson *et al.* (2015) reported pseudo first-order rate constants (k) in the order of 10^{-4} min^{-1} for the removal of E1, E2, EE2, and NP (initial concentrations: 20–1,500 μg · L^{-1}) from deionized water during photolysis. A rate constant in the order of 10^{-3} was observed for the removal NP (10 mg · L^{-1}) from ultrapure water in the study of Martínez-Zapata *et al.* (2013), whereas Huang *et al.* (2022) observed rate constants from 10^{-3} to 10^{-2} for E1, E2, E3, and EE2 (10 μg · L^{-1}). Similarly, Zhang *et al.* (2010) reported that the removal rate constant of EE2 (610–1,950 μg · L^{-1}) photolysis increased from 10^{-3} to 10^{-1} min^{-1} with increasing the UV light intensity from 0.2456 to 1.5 mW cm^{-2}.

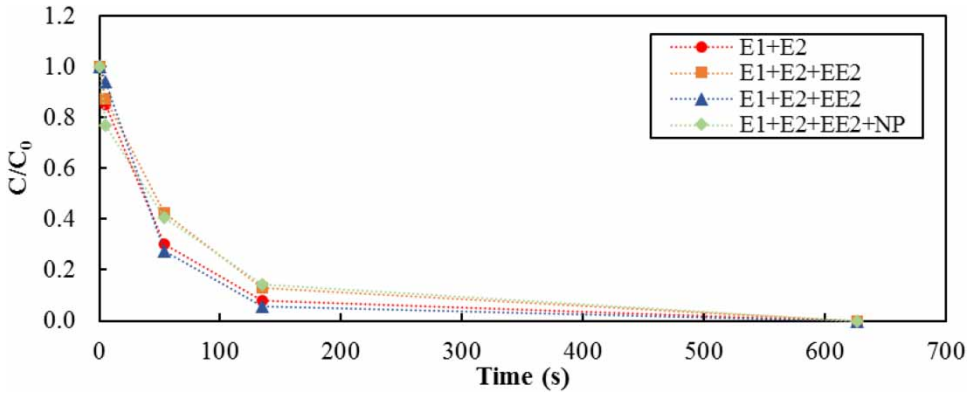


Figure 3 | Decay of the estrogenic activity (EA) during photolysis of water spiked with different mixtures of endocrine disrupting compounds. Notes: E1 + E2: water spiked with 100 ng · L^{-1} of E1 and 100 ng · L^{-1} of E2. E1 + E2 + NP: water spiked with 100 ng · L^{-1} of E1, 100 ng · L^{-1} of E2, and 1,000 ng · L^{-1} of NP. E1 + E2 + EE2: water spiked with 100 ng · L^{-1} of E1, 100 ng · L^{-1} of E2, and 50 ng · L^{-1} of EE2. E1 + E2 + EE2 + NP: water spiked with 100 ng · L^{-1} of E1, 100 ng·L^{-1} of E2, 50 ng · L^{-1} of EE2, and 1,000 ng · L^{-1} of NP. Values were expressed as mean of three replicates.

Table 1 | Pseudo first-order kinetic parameters obtained for the decay of estrogenic activity during photolysis

EDC mixtures	R^2	k (min^{-1})	$t_{1/2}$ (s)
E1 + E2[a]	0.995	1.122	37
E1 + E2 + NP[b]	0.999	0.900	46
E1 + E2 + EE2[c]	0.997	1.296	32
E1 + E2 + EE2 + NP[d]	0.990	0.822	51

[a]Water spiked with 100 ng · L^{-1} of E1 and 100 ng · L^{-1} of E2.
[b]Water spiked with 100 ng · L^{-1} of E1, 100 ng · L^{-1} of E2, and 50 ng · L^{-1} of NP.
[c]Water spiked with 100 ng · L^{-1} of E1, 100 ng · L^{-1} of E2, and 50 ng · L^{-1} of EE2.
[d]Water spiked with 100 ng · L^{-1} of E1, 100 ng · L^{-1} of E2, 50 ng · L^{-1} of EE2, and 50 ng · L^{-1} of NP.

This suggest therefore that EDCs degradation rate during photolysis may be strongly influenced by the initial concentration of EDCs in water. Interestingly, as concentrations of EDCs in freshwater usually remain in the order of ng L^{-1} (Ding & Tzing 1998; Snyder *et al.* 1999; Kolpin *et al.* 2002; Johnson & Jürgens 2003; Moreira *et al.* 2009, 2011; Resende *et al.* 2017; Aquino *et al.* 2021), experiments with low concentrations may reflect more closely the EDCs degradation in a real application scenario.

The results therefore evidence that photolysis can be efficient for the removal of estrogenic activity caused by the studied EDCs during drinking water treatment. This can be achieved by providing an UV dose capable to remove estrogenic activity from water. At the operating conditions of this work, this dose can be obtained with an exposure time of about 10 min, but this contact time can be decreased by using reactors that can provide high UV light intensities.

Some studies have been reported the formation of EDC by-products during photolysis, raising concerns about the toxicity of these products (Caupos *et al.* 2011; Li *et al.* 2013; Mboula *et al.* 2015; Souissi *et al.* 2012). However, little information concerning their toxicity is currently available. In this regard, studies have pointed out that the phenol ring of EDCs may persist after photolysis, suggesting the endocrine disrupting potential of by-products (Mboula *et al.* 2015; Souissi *et al.* 2012). Furthermore, as reported by Souissi *et al.* (2012) photolysis can lead to the formation of hydroxylated by-products and it is important to highlight that some hydroxylated products of EDCs (e.g., 2-hydroxyestradiol, 4-hydroxyestradiol, 16α-hydroxyestradiol, and 4-hydroxyestrone) can be carcinogenic.

CONCLUSIONS

At the conditions investigated, both chlorination and photolysis resulted in high removal efficiencies of the estrogenic activity induced by the mixtures of EDCs tested in this work. Chlorination resulted in a removal of up to 99.7% of the estrogenic

activity of samples at a chlorine dose of 2.75 mg · L^{-1} and contact time of 30 min. In all EDC solutions tested, photolysis led to removal efficiencies higher than 99.9% at a UV dose of 186 mJ · cm^{-2}. It is worth highlighting that the EDC concentrations used in this study were based on values observed in water sources, whereas the conditions tested were determined according to water disinfection operating conditions, which corroborates the applicability of the proposed techniques in drinking water treatment plants. Furthermore, these processes may lead to the formation of by-products and little information concerning the health risks associated with these products in water is currently available. In this context, a combination of *in vivo* and *in vitro* techniques can be valuable for the assessment of the occurrence and the potential risks related to these products in treated waters.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Material.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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