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Efficient Removal of Estrogenic Activity during Oxidative Treatment of Waters Containing Steroid Estrogens

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The discharge of synthetic and natural steroid estrogens from municipal wastewaters to the aquatic environment has received increased attention because of their potential reproductive effects on fish. Using 17α-ethinylestradiol (EE2) as a representative steroid estrogen, several oxidants applied in wastewater treatment (chlorine, bromine, ozone, hydroxyl radical, chlorine dioxide, and ferrate) were shown to selectively and rapidly transform EE2. For typically applied oxidant doses, these transformations occur in the time range of seconds to minutes. The resulting initial transformation products of EE2 exhibit a substantially lower in vitro estrogenic activity (<13% of EE2). For selected structural derivatives of EE2, a quantitative structure–activity relationship was established between substituents changed on the phenolic moiety and the relative in vitro estrogenic activity. In addition, the initial EE2 transformation products that still exhibit residual estrogenic activity are quickly further transformed by most of the tested oxidants. Therefore, oxidative wastewater treatment may serve as a powerful tool to remove estrogenic activity induced by steroid estrogens.

Introduction

In recent years, chemicals with estrogenic activity have received increased attention due to reproductive effects on aquatic organisms (*1*). In particular, steroid estrogens, such as the synthetic 17α-ethinylestradiol (EE2) used in the contraceptive pill, the natural 17β-estradiol (E2), and estrone (E1), have been found in the effluent of wastewater treatment plants (WWTPs) at concentrations in the ng L^{-1} range and are believed to be responsible for observed endocrine disrupting effects in wild fish (i.e., feminization) living downstream of effluents of wastewater treatment plants (Supporting Information, Text S1). Furthermore, a recent study has demonstrated that exposures to 5 ng L^{-1} of EE2 pose a serious threat to the sustainability of wild fish populations (*2*).

As one of the measures to minimize the risk of adverse effects caused by the estrogenic compounds discharged to surface waters, it has been suggested to optimize wastewater treatment to go beyond the removal of nutrients (C, N, and P) and to include a polishing step for removal of estrogenic compounds (*3*). Chemical oxidation has been widely applied for disinfection of drinking water and wastewaters and for the elimination of undesired micropollutants from drinking water (*4*). Ozone, chlorine, and chlorine dioxide are currently widely used oxidants in water treatment (*4*). OH radicals, whether generated in situ as secondary oxidants during ozonation or as a primary oxidant in advanced oxidation processes (AOPs) such as UV/H_2O_2 and O_3/H_2O_2, have been shown to be effective for the oxidation of many classes of compounds (*5*). In addition, bromine, which is typically an in situ generated secondary oxidant during chlorination of bromide-containing waters, may also be an important oxidant in drinking water and wastewater treatment (*6*). Finally, ferrate is an emerging water treatment oxidant that can be dosed directly to water (*7*).

There is an ongoing discussion as to whether such oxidation processes should be applied to municipal wastewater treatment to remove organic micropollutants, including estrogenic compounds. Recent studies showed that several oxidants (ozone, chlorine, chlorine dioxide, OH radical, and ferrate) react at moderate to very rapid rates with steroid estrogens (Figure 1). In addition, efficient removal of steroid estrogens during oxidative treatment of wastewater was demonstrated (*8*). Based on these investigations, oxidation processes are a promising option for controlling steroid estrogens during water treatment.

However, because full mineralization does not occur with common oxidant doses applied in water treatment, oxidation of these compounds yields a variety of transformation products. Therefore, it is important not only to identify the major initial transformation products but also to assess their

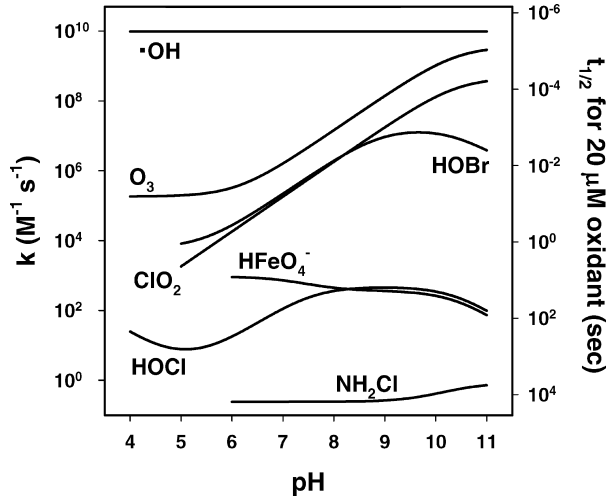

FIGURE 1. Apparent second-order rate constants and half-lives ($t_{1/2}$) for the reactions of 17α-ethinylestradiol (EE2) with different oxidants as a function of pH. The half-lives are calculated for an oxidant concentration of 20 μM. In the case of the OH radical (·OH), the half-lives for the reactions of EE2 are longer than ~1 s because concentrations of ·OH are generally below 10^{-10} M during oxidative water treatment with ·OH (advanced oxidation processes) (*5*). The rate constants for ·OH, ozone (O_3), chlorine dioxide (ClO_2), ferrate ($HFeO_4^-$), and chlorine (HOCl) were taken from refs *16, 17, 18, 7,* and *19*, respectively. The rate constants for bromine (HOBr) and monochloramine (NH_2Cl) were taken from ref *20*.

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estrogenic activity relative to the parent compounds. The term initial is used in a general sense in this paper, including transformation products between the first and the third stage of sequential reaction with the oxidant. Additionally, the kinetics of the formation and further transformation of the initial products is crucial for determining their persistence under given oxidation conditions.

In the present study, the oxidation of the oral contraceptive EE2 was investigated as a model steroid estrogen because of its significant contribution to the estrogenic activity in effluents of WWTPs (Supporting Information, Text S1). The oxidants ozone, chlorine, chlorine dioxide, OH radicals, bromine, and ferrate were examined. The first step was to compare second-order rate constants for the reaction of EE2 with various oxidants. In the next step, to test to what extent the initial transformation of EE2 diminishes the estrogenic activity, aqueous solutions of EE2 were treated with a range of oxidant doses and subsequently tested for the residual EE2 concentration and estrogenic activity. Using this approach, the total contribution of transformation products to the estrogenic activity can be deduced from the difference between the measured relative estrogenic activity and the measured EE2 concentration. Huber et al. (*9*) first applied this method and found that initial oxidation products of EE2 by ozone have approximately 200-times lower estrogenic activity than EE2. Several previous studies have also reported efficient removal of estrogenic activity during ozonation or chlorination of steroid estrogens (*10, 11*). Tabata et al. (*12*) reported removal of in vivo estrogenic activity during chlorination of steroid estrogens. Nevertheless, because these studies (*10–12*) did not examine quantitative changes of both the estrogenic activity and the concentration of steroid estrogens as a function of the degree of oxidation of the steroid estrogens, it is difficult to assess the estrogenic activity of initial transformation products. In the following step, initial transformation products and their reactivity toward further transformation during the reaction of EE2 with each oxidant were investigated. Furthermore, the estrogenic activity was measured for a series of structural derivatives of EE2, including the identified initial oxidative transformation products of EE2. The measured estrogenic activities of individual estrogens were used to establish a quantative structure–activity relationship (QSAR) between substituents change on the phenolic moiety and the relative estrogenic activity of estrogens. For some of the halogenated products of EE2, the measured estrogenic activities were compared with those reported in previous studies (*13*).

Experimental Section

Chemical Reagents. All reagents were of 95% purity or higher. Descriptions of chemical sources for estrogens, synthesis of chlorinated products of EE2, and preparation of oxidants are provided in the Supporting Information, Text S2.

Yeast Estrogen Screen. The recombinant yeast estrogen screen (YES) was performed as described by Routledge and Sumpter (*14*) with minor changes and data evaluation as reported by Rutishauser et al. (*15*). All estrogenic activity measurements in this study are performed by this in vitro YES assay. The details are described in the Supporting Information, Text S3.

Estrogenic Activity of Transformation Mixtures of EE2: EE2 vs ethinylestradiol equivalents (EEEQ). Transformation mixtures of EE2 were obtained by reacting 10 μM EE2 with oxidants at pH 8 (5 mM phosphate buffer). After completion of the reaction, remaining EE2 concentrations were determined by a HPLC/UV system. The reaction solutions were also subjected to solid-phase extraction (SPE). SPE was performed to transfer EE2 and transformation products of EE2 from water to the ethanol phase for subsequent estrogenic activity measurements by the YES. Details on the reaction of EE2 with oxidants, determination of EE2, and SPE procedures are described in the Supporting Information, Text S4.

Evaluation of the Concentration−Effect Curves. The concentration−effect curve of each sample containing transformation mixtures of EE2 was fitted to a symmetric logistic function (eq 1) by optimizing relative concentration factors (RCF) causing a 50% effect (EC_{50}). The relative concentration factor (RCF) is calculated by multiplying the enrichment factor in the SPE with the dilution factor in the YES. The estrogenic activity of samples, which is expressed as ethinylestradiol equivalent concentration (EEEQ) was then calculated as the ratio of the EC_{50} for EE2 to the EC_{50} for samples ($EEEQ = EC_{50}(EE2)/EC_{50}(samples)$). The details are described in the Supporting Information, Text S5.

$$\text{Effect}(\%) = \frac{100\%}{1 + 10^{(\log EC_{50} - \log RCF)m}} \quad (1)$$

Evolution of Products during Chlorination and Bromination of EE2. Various concentrations of chlorine or bromine (0−28 μM) were added to 10 μM EE2 at pH 8 (5 mM phosphate buffer). After completion of the reactions, the solutions were analyzed by a HPLC/UV system. For identification and quantification of chlorinated or brominated EE2, HPLC/UV chromatograms were compared between treated samples and standards of each chloro- and bromo-EE2s.

Relative Estrogenic Potencies of Structural-derivatives of EE2. Relative estrogenic potencies (RPs) of several representative initial transformation products of EE2 were measured (see Figure S7 for the concentration-effect curves and Figure S8 for the structures of the initial transformation products). Known amounts of each compound were dissolved in ethanol and series of dilutions were made and tested with the YES. The EC_{50} values of each compound were determined by fitting the concentration−effect curve (eq 1) while optimizing the actual concentration of the compound in the final YES medium (instead of RCF) causing a 50% effect (EC_{50}). Relative potencies were then calculated as the ratio of the EC_{50} for EE2 to the EC_{50} for each structural derivative of EE2.

Results and Discussion

Kinetics for Oxidative Transformation of EE2. Figure 1 summarizes the second-order rate constants and half-lives for the transformation of EE2 by several water treatment oxidants. All oxidants except monochloramine (NH_2Cl) are reactive enough to transform EE2 significantly at common oxidant concentrations applied in water treatment (a few mg L^{-1}). Oxidation of EE2 will be poor during chloramination (NH_2Cl) or chlorination of water containing high concentrations of ammonia (e.g., chlorine-to-ammonia molar ratio less than 1) due to the rapid conversion of chlorine to NH_2Cl (*4*).

Estrogenic Activity of Transformation Mixtures of EE2 (EE2 vs EEEQ). Figure 2 shows the decrease of the relative EE2 concentration and the relative estrogenic activity as a function of the oxidant dose for the six selected oxidants. The EE2 concentrations and EEEQs in Figure 2 were normalized to the values of the untreated solution. With all six investigated oxidants, the reduction of the EEEQ was linearly proportional to the decrease of the EE2 concentration with a direct one-to-one correlation (see slopes of "1" in insets of Figure 2 for the relative EEEQ vs the relative EE2 concentration). This indicates that the estrogenic activity of the products resulting from the initial transformation step is insignificant relative to EE2. Alternatively, there is also the possibility that the initial transformation products still have considerable estrogenic activity, but they are rapidly further oxidized to products exhibiting negligible estrogenic activity.

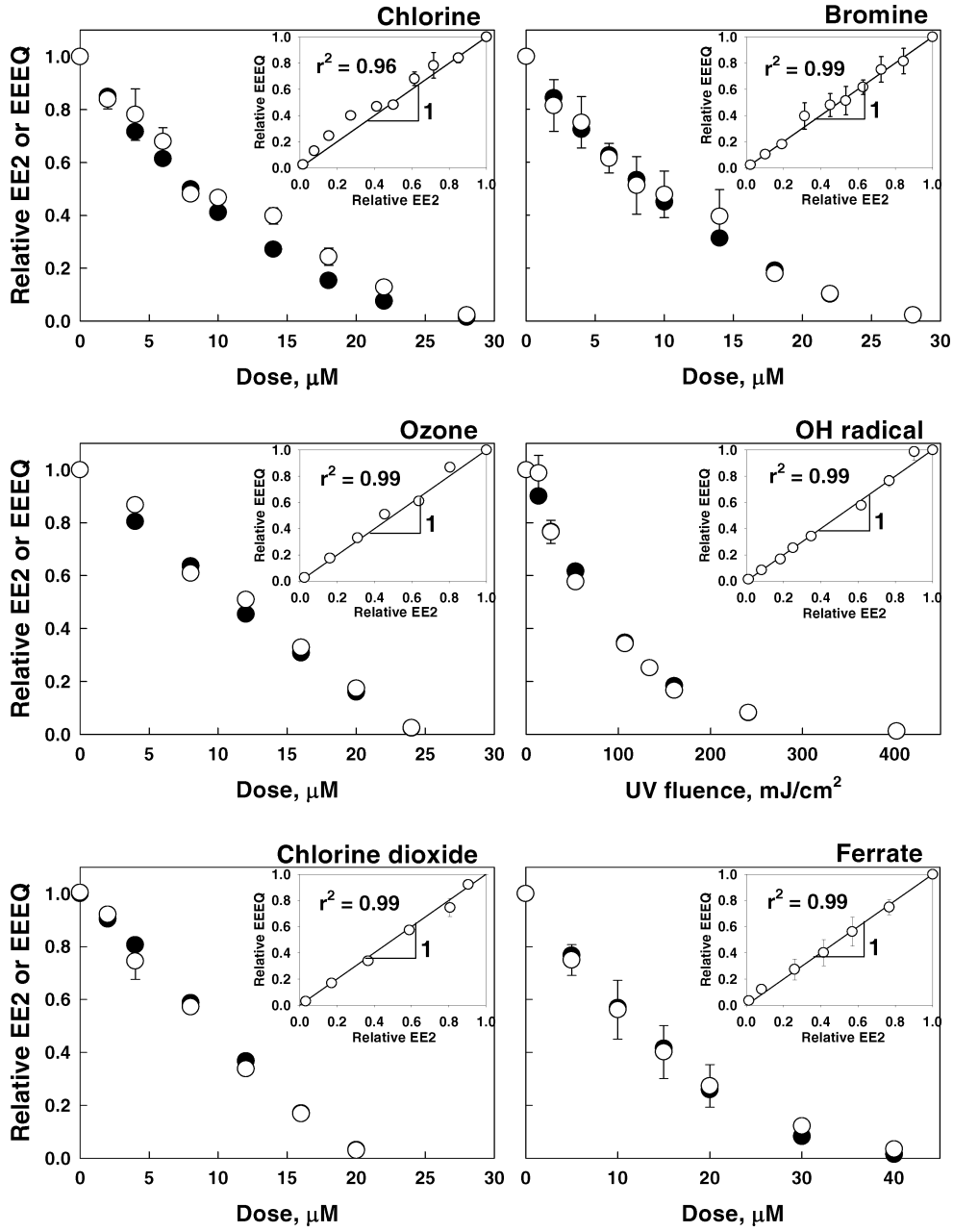

FIGURE 2. Decrease of the relative EE2 concentration (filled circles) and estrogenic activity expressed as EEEQs (open circles) as a function of different oxidant doses for chlorine, bromine, ozone, OH radical, chlorine dioxide, and ferrate. The estrogenic activity of a given sample is expressed quantitatively in 17α-ethinylestradiol equivalents (EEEQs). The insets show plots of the relative EEEQ vs the relative EE2 concentration. Experimental conditions: $[EE2]_0 = 10\ \mu M$, pH = 8 (5 mM phosphate buffer), $T = 23 \pm 2$ °C, ozonation was performed in the presence of *tert*-butyl alcohol (5 mM) to suppress oxidation of EE2 by OH radicals. If filled circles are not visible, the data overlap the open circles.

This can be shown by a kinetic model including both oxidation kinetics and relative EEEQ (see the modeling section in Supporting Information, Text S6). Therefore, it is important to understand not just the estrogenic potency of all the relevant products but also the rates of their formation and further transformation. It should be mentioned that much higher EE2 concentrations (3 mg L^{-1}) were applied in Figure 2 compared to those found in effluents of WWTPs (ng L^{-1} range). Therefore, the distribution of initial transformation products in wastewaters might be slightly different from those in Figure 2, especially for the oxidants such as ozone, chlorine dioxide, and ferrate, involving a phenoxyl radical as an intermediate.

Initial Products of Oxidative Transformation of EE2. In this section, (expected) initial transformation products and their reactivity toward further transformation during the reaction of EE2 with each oxidant are discussed.

Ozone (O_3). O_3 attacks the phenolic moiety of EE2 in the primary step. On the basis of previous studies on the ozonation of EE2 and phenol (*9*, *21*), the following compounds can be produced as initial oxidation products (Figure 3): 2-OH EE2 (**1**), 4-OH EE2 (**2**), 2,3-quinone EE2 (**3**), 3,4-quinone EE2 (**4**), and muconic-EE2 (**5**). Phenolic-coupling products of EE2 can be assumed to be produced only as minor products (below 1%). Because the two phenolic-hydroxylated products, **1** and **2**, have a higher reactivity with O_3 than the parent EE2, they will be quickly transformed further. Compounds **3**, **4**, and **5** will be also quickly transformed because O_3 is reactive toward the quinone or the carbon−carbon double bond (*5*).

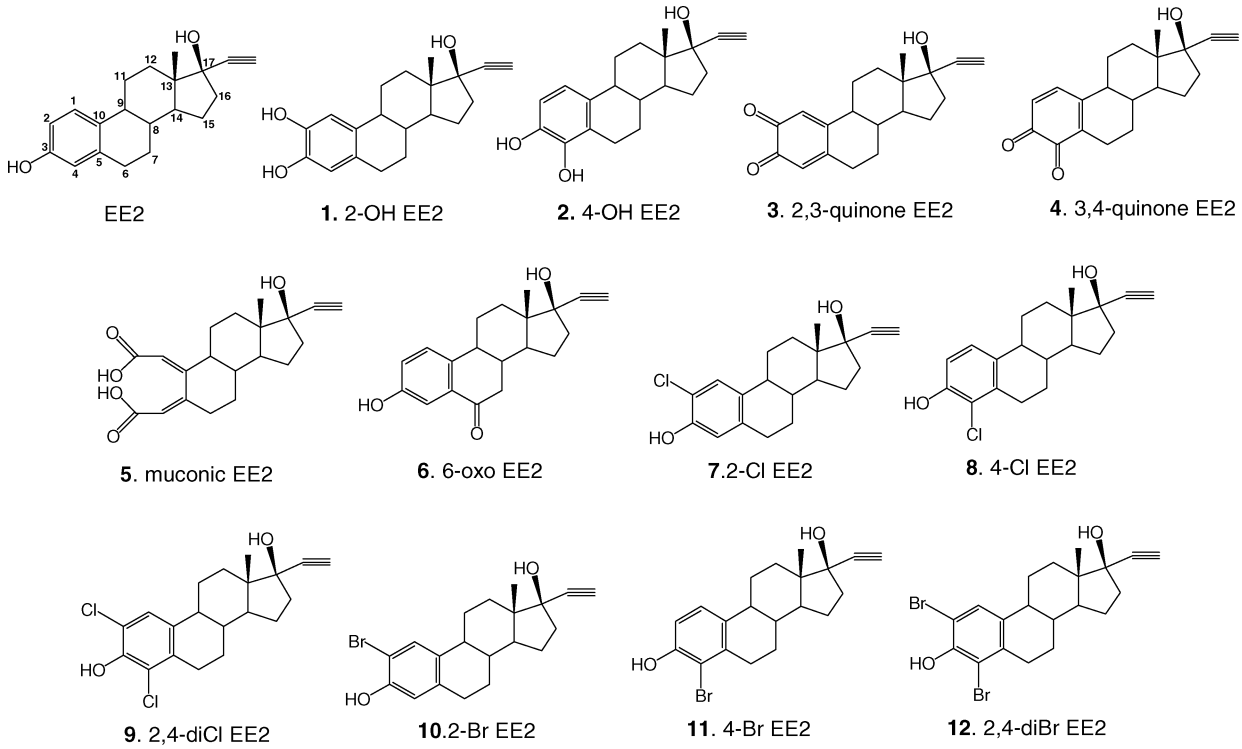

FIGURE 3. Initial transformation products during the reaction of EE2 with ozone (1, 2, 3, 4, and 5), chlorine dioxide (3 and 4), ferrate (1, 2, 3, and 4), hydroxyl radical (1, 2, and 6), chlorination (7, 8, and 9), and bromination (10, 11, and 12). Identification of the products, 1−6 was not attempted in this study. However, mechanistic considerations based on previous studies support the formation of these products. Halogenated products (7−12) were identified and quantified in this study.

Chlorine Dioxide (ClO_2). ClO_2 oxidizes phenol by electron-transfer generating a phenoxyl radical and ClO_2^-. The phenoxyl radical reacts rapidly with a second ClO_2 to generate a quinone. On the basis of a study on the oxidation of tyrosine (*para*-substituted phenol) by ClO_2 (*22*), **3** or **4** can be assumed to be produced as major initial oxidation products (Figure 3). The reactivity of **3** or **4** with ClO_2 is not known. Quinone−EE2s are unstable in water and can be transformed via transient quinone methide intermediates to several different products such as 6-oxo-2-hydroxyethinylestradiol (*23*).

Ferrate (Fe(VI)). Fe(VI) also oxidizes phenol by electron-transfer generating a phenoxyl radical and Fe(V) as a first step. The fate of the phenoxyl radical is not clear, yet. It has been proposed that the phenoxyl radical reacts with another ferrate (Fe(V) or Fe(VI)) generating 1,2- or 1,4-benzoquinone. 4,4′-biphenoquinone was detected as an unstable intermediate. It reacts with another phenol generating biphenols (*24*). Fe(V) produced from the reaction of Fe(VI) with phenol can oxidize another phenol directly to 1,2- or 1,4-hydroquinones (*25*). On the basis of these previous studies, **1**, **2**, **3**, and **4** are expected to be the main initial transformation products during the oxidation of EE2 by Fe(VI) (Figure 3). Phenolic-coupling products of EE2 can also be produced. Compounds **1** and **2** are not expected to accumulate significantly because these products have much higher reactivity toward ferrate than EE2.

Hydroxyl Radical (·OH). ·OH can react with EE2 (1) by addition to the phenolic ring, (2) by abstraction of hydrogen in the aliphatic rings, or (3) by addition to the ethinyl moiety of EE2. The kinetic ratio among the three pathways is expected to be 10:8:5 for paths 1:2:3. This is based on (1) the rate constant of ·OH with phenols ($k \approx 10^{10}\ M^{-1}\ s^{-1}$) (*26*), (2) the rate constant of ·OH with a single carbon-hydrogen bond ($k \approx 0.5 \times 10^9\ M^{-1}\ s^{-1}$) (*26*) and the number of available carbon-hydrogen bonds in the aliphatic rings of EE2 ($n = 15$), which corresponds to the overall rate constant of $k \approx 8 \times 10^9\ M^{-1}\ s^{-1}$, and (3) the rate constant of ·OH with acetylene ($k \approx 5 \times 10^9\ M^{-1}\ s^{-1}$) (*26*). ·OH adds preferentially to the ortho- and para-positions of phenol. In the case of EE2, the ortho-addition products, **1** and **2**, are expected to be the main products (Figure 3). The two products, **1** and **2**, might have similar reactivity with ·OH compared to EE2. When ·OH reacts with the aliphatic rings or the ethinyl moiety of EE2, a variety of structurally different oxidation products can be formed. 6-oxo EE2 (**6**) is one of them if ·OH abstract hydrogen at the six-position of the rings of EE2.

Chlorination and Bromination. Initial products of chlorination and bromination of EE2 were identified and quantified by HPLC/UV, HPLC/MS, and ^{1}H NMR (Table S1). For the structures of the initial product, see Figure 3. Chlorination of EE2 resulted in the formation of 2-Cl EE2 (**7**) with 20% yield and 4-Cl EE2 **(8)** with 80% yield as primary products. Further chlorination of these two primary products (2-Cl EE2 and 4-Cl EE2) resulted in the formation of 2,4-diCl EE2 (**9**) as a secondary intermediate. Products from further chlorination of 2,4-diCl EE2 were not identified in this study.

Bromination can be an important transformation pathway of EE2 during chlorination of water containing bromide due to (1) the rapid oxidation of bromide by chlorine to bromine ($HOCl + Br^- \rightarrow HOBr + Cl^-$, $k = 1550\ M^{-1}\ s^{-1}$ (*6*)) and (2) much higher reactivity of bromine than chlorine toward EE2 (*20*). Bromination of EE2 showed a similar trend of product formation compared with chlorination. As primary products, 2-Br EE2 (**10**) and 4-Br EE2 (**11**) were generated with 20 and 80% yields, respectively. 2,4-diBr EE2 (**12**) was a secondary product. Chlorinated or brominated products of EE2 are not stable and rapidly transformed due to their higher reactivity toward chlorine or bromine than EE2. Details on the kinetics, products, and pathways of the transformation of EE2 during chlorination or bromination are discussed in ref *20*.

Relative Estrogenic Potencies of Structural-derivatives of EE2. The YES was performed for a series of structural derivatives of EE2 with an intact steroid backbone (see Figure

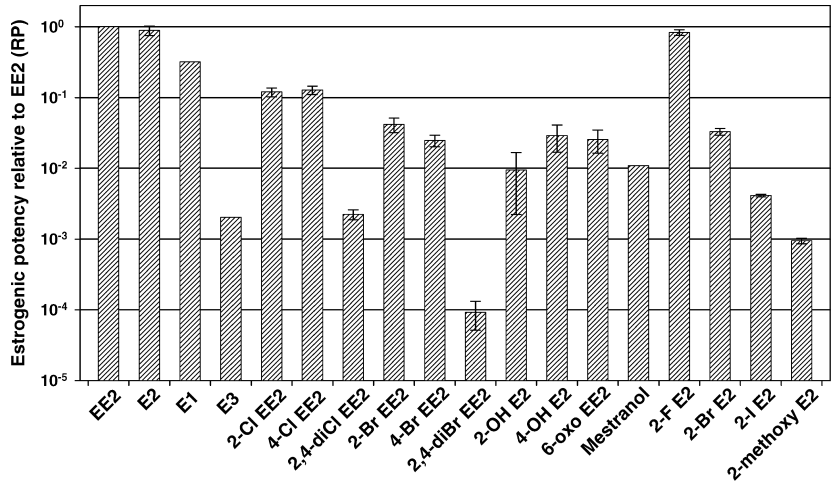

FIGURE 4. Estrogenic potencies relative to EE2 (relative potencies, RPs) of several structural derivatives of EE2 or E2. The RPs of E1, E3, and mestranol were taken from ref *15*.

S9 for their structures). These include products formed during chlorination or bromination of EE2 (i.e., **7**−**12** in Figure 3). The compounds **1**, **2**, and **6** (Figure 3), while not directly identified in this study, have been shown to be initial products of reaction of EE2 or E2 with ozone and OH radicals and, therefore, were also investigated. In addition, to test a broader range of substituent effects on the phenolic moiety, the YES was carried out for 2-fluoro E2 (2F-E2), 2-bromo E2 (2-Br E2), 2-iodo E2 (2-I E2), and 2-methoxy E2. The relative estrogenic activities of E1, E3 (estriol), and mestranol were taken from the literature (*15*).

Figure 4 shows the estrogenic potencies of diverse structural derivatives of EE2 relative to EE2 (relative potencies, RPs). The RPs were calculated as the ratio of the EC_{50} for EE2 to the EC_{50} for the structural derivatives of EE2. Most of the selected representative initial transformation products of EE2, such as **1**, **2**, **6**, and **9**−**12** in Figure 3 were found to have RPs of at least 1 order of magnitude lower than EE2 (or E2, which is equipotent to EE2 in the YES). These data support our previous observation (Figure 2) that there was little difference between the decrease of the relative EE2 concentrations and the decrease of the relative EEEQs in the oxidation experiments. Monochlorinated EE2s (**7** and **8** in Figure 3), however, still exhibited RPs of ∼13% of EE2, which does not match well with the results in Figure 2. Further rapid chlorination of **7** and **8** is responsible for the efficient removal of estrogenic activity during chlorination of EE2 (*20*).

The RP of 6-oxo EE2 is 2.6% of EE2. This shows that the structural modification of the aliphatic side chain as well as the phenolic moiety may lead to a significant reduction of the estrogenic activity of EE2. Chlorine dioxide, ozone, and ferrate transform the phenolic moiety of EE2 to a quinone, muconic acid, or biphenyl moiety (phenolic coupling product) in the initial reaction steps. Because the phenolic moiety is mainly responsible for the estrogenic activity of EE2 (*27*), the RPs of products without the phenolic moiety are expected to be much lower than that of EE2. For instance, the RP of mestranol, which has a methoxy- instead of hydroxy-substituent on the EE2 phenolic moiety, is 1.3% of EE2. Phenolic coupling products of the EE2 are expected to have low RPs (less than 1% of EE2) based on their structural resemblance with mestranol. The RPs of the phenolic-ring substituted EE2 or E2 will be further discussed in the following section.

Nakamura et al. (*13*) determined estrogenic activities of various 2- and 4-chlorinated/brominated steroid estrogens (EE2, E2, E1, and E3) using yeast cells into which the human (hERα) or medaka fish (medERα) estrogen receptor are incorporated. In their assays, estrogenic activities were measured as EC_{10} that were defined as the concentration producing 10-times higher chemiluminescent signals than that of a blank control. Therefore, the RPs from EC_{10} data of Nakamura et al. (*13*) should be cautiously interpreted because a very low effect end point was used and no concentration−effect model was applied in the data evaluation. From the data of Nakamura et al. (*13*), the RPs decrease up to a factor of ∼10 for mono-Cl steroid estrogens, ∼100 for mono-Br steroid estrogens, ∼500 for di-Cl steroid estrogens, and ∼10 000 for di-Br steroid estrogens compared to each of their parent steroid estrogens.

QSAR for the RPs of the Phenolic-ring Substituted Estradiols. The large difference in the RPs of the phenolic-ring substitutd EE2 or E2 can be rationalized by steric and electronic constraints resulting in a less efficient binding to the estrogen receptor (ER). The influence of steric and electronic parameters on the RP was explored with a QSAR analysis. A wide range of QSARs exist for the identification and prioritization of estrogenic compounds (*28*); however, because we wanted to explore the subtle differences caused by the substituents adjacent to the phenolic moiety, we used the incremental Hansch's approach (*29*).

Two types of physicochemical parameters of substituents were used to perform the QSAR regression analysis (see Supporting Information, Text S7): ΔMR (molar refractivity) as a steric parameter and I as an indicator parameter for any substituents containing oxygen atoms (i.e., $I = 1$ for the presence of oxygen and 0 for other substituents). ΔMR is defined as $\Delta MR = MR_{substituent} - MR_{hydrogen}$, and $MR = 0.1[(n^2 - 1)/(n^2 + 2)]\,(MW/d)$ where n is the refractive index, MW is the molecular weight, and d is the density of a compound. Because there is rather little variation in n, MR is largely a measure of volume with a small correction for polarizability. The values of ΔMR were taken from ref *29*. The RPs of all substituted EE2s and E2s were normalized to the RPs of parent EE2 and E2, respectively. These normalized RPs (NRPs) were used to establish the QSAR. Using NRPs is justified because we are interested in the substitution effect on the phenolic moiety. Table 1 summarizes the 2- and 4-substituted EE2s or E2s included in the QSAR analysis, their NRPs, and the corresponding substituent constants for ΔMR and I.

The NRPs of 2- and 4-substituted EE2 or E2 are related to the ΔMR and I values through eq 2. Figure 5 shows the

TABLE 1. 2- and 4-Substituted EE2s or E2s Included in the QSAR Analysis

No.	estradiols	substituents 2	substituents 4	log (NRP)[a] meas[b]	log (NRP)[a] pred[c]	ΔMR[d] (2 & 4)	I[e] (2 & 4)
1	EE2	H	H	0.00	0.00	0.00	0
2	E2	H	H	0.00	0.00	0.00	0
3	2F-E2	F	H	−0.03	0.00	0.00	0
4	4-Cl EE2	H	Cl	−0.89	−1.11	0.50	0
5	2-Cl EE2	Cl	H	−0.92	−1.11	0.50	0
6	2-Br EE2	Br	H	−1.38	−1.75	0.79	0
7	2-Br E2	Br	H	−1.43	−1.75	0.79	0
8	4-Br EE2	H	Br	−1.61	−1.75	0.79	0
9	2-I E2	I	H	−2.33	−2.86	1.29	0
10	2,4-diCl EE2	Cl	Cl	−2.65	−2.21	1.00	0
11	2,4-diBr EE2	Br	Br	−4.04	−3.50	1.58	0
12	4-OH E2	H	OH	−0.89	−1.37	0.18	1
13	2-OH E2	OH	H	−1.37	−1.37	0.18	1
14	2-OCH_3 E2	OCH_3	H	−2.97	−2.50	0.69	1

[a] Normalized relative potencies (see the main text), [b] Measured in this study, [c] Predicted by eq 2, [d] Values for Δ*MR* were from ref *29*, [e] *I* is an indicator parameter for any substituents containing oxygen atom (*I* = 1 for the presence of oxygen and *I* = 0 for the absence).

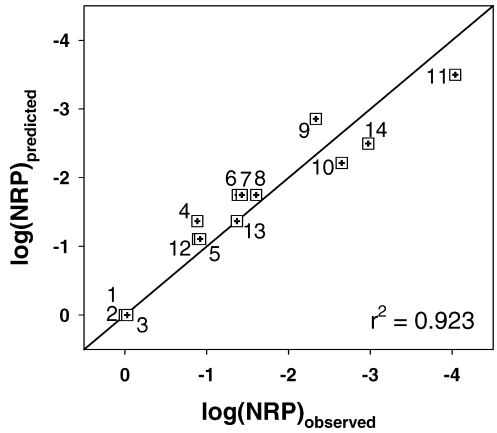

FIGURE 5. Correlation between the observed log (NRP) and the predicted log (NRP) based on eq 2. The numbers correspond to the estradiol derivatives in Table 1.

correlation between the observed log (NRP) and the predicted log (NRP) calculated using eq 2

$$\log(\mathrm{NRP}) = -2.21(\pm 0.28)\Delta\mathrm{MR} - 0.97(\pm 0.46)I \quad (2)$$

where $n = 14$, $r^2 = 0.923$, $s = 0.360$, and 95% confidence intervals are in parenthesis.

The negative coefficients for ΔMR and *I* in eq 2 point out that bulky and oxygen-containing substituents decrease the NRP. The QSAR results are consistent with the structure of the ligand-binding domain of the estrogen receptor (ERα) complexed with estradiol (*27*). First, the negative steric effect can be understood by considering that the phenolic ring of estradiol is tightly constrained within the ligand-binding pocket of ERα. Second, substituents containing oxygen atoms can act as a hydrogen-acceptor and, therefore, interfere with the hydrogen-bonding networks of the phenolic-hydroxyl group of estradiols. For estradiols chlorinated or brominated at the phenolic ring, the steric effect of substituents is mainly responsible for the decrease of the NRP. On the contrary, for estradiols hydroxylated at phenolic ring, the hydrogen-accepting effect of substituents is the main factor for their lower NRP.

Implications for Oxidative Treatment of Wastewater. Six oxidants were tested as a polishing step for enhanced removal of steroid estrogens during wastewater treatment. All oxidants are reactive enough to significantly transform EE2 at typical conditions of their doses (a few mg L^{-1}) and stabilities (from seconds to minutes) during oxidative wastewater treatments. Slight modifications of the chemical structure of EE2 induced by fast oxidation (from seconds to minutes) result in >87% reduction of their estrogenic activity as measured by a yeast in vitro assay. Nevertheless, this efficient removal is recommened to be confirmed by in vivo measurement systems because the toxicokinetics might vary substantially between in vitro and in vivo systems, whereas it can be expected that the toxicodynamics, that is, receptor affinity, is similar between in vitro and in vivo systems. In addition, initial transformation products are not stable and are prone to further fast transformation, yielding products with even lower estrogenic activity. These results from EE2 might be transferred to other structurally related steroid estrogens, such as E2, E1, and E3. Considering the high reactivity of water treatment oxidants toward steroid estrogens, such wastewater polishing processes might represent a powerful tool for removing estrogenic activity induced by steroid estrogens.

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Supporting Information Available

Seven texts, 2 tables, and 10 figures are available for further information addressing materials, experimental procedures, and estrogenic chemicals in WWTP effluents. This material is available free of charge via the Internet at http://pubs.acs.org.

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