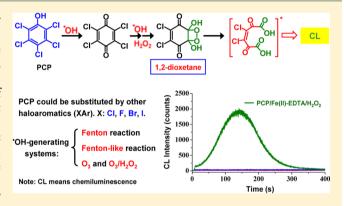


Intrinsic Chemiluminescence Generation during Advanced Oxidation of Persistent Halogenated Aromatic Carcinogens

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Supporting Information

ABSTRACT: The ubiquitous distribution coupled with their carcinogenicity has raised public concerns on the potential risks to both human health and the ecosystem posed by the halogenated aromatic compounds (XAr). Recently, advanced oxidation processes (AOPs) have been increasingly favored as an "environmentally-green" technology for the remediation of such recalcitrant and highly toxic XAr. Here, we show that AOPs-mediated degradation of the priority pollutant pentachlorophenol and all other XAr produces an intrinsic chemiluminescence that directly depends on the generation of the extremely reactive hydroxyl radicals. We propose that the hydroxyl radical-dependent formation of quinoid intermediates and electronically excited carbonyl species is responsible for this unusual chemiluminescence production. A rapid, sensitive,



simple, and effective chemiluminescence method was developed to quantify trace amounts of XAr and monitor their real-time degradation kinetics. These findings may have broad biological and environmental implications for future research on this important class of halogenated persistent organic pollutants.

INTRODUCTION

Halogenated aromatic compounds (XAr) have been widely used as pesticides, herbicides, disinfectants, wood preservatives, personal care agents, pharmaceuticals, flame retardants, and many other industrial products.^{1–4} Most of these compounds are poorly biodegradable, both in water and soil. This makes them persistent and widely distributed in the environment, and has earned them the name persistent organic pollutants (POPs) from UNEP (United Nations Environment Programme). 1-4 Their ubiquitous distribution coupled with their carcinogenicity has raised public concerns on the potential risks to both human health and the ecosystem posed by these recalcitrant and highly toxic XAr.^{3–13} Polyhalogenated phenols are an important class of XAr, and some of them, such as the widely used wood preservative pentachlorophenol (PCP) and 2,4,6-trichlorophenol, have been listed by the U.S. Environmental Protection Agency as priority pollutants. They are classified as group 2B human carcinogens by the International Agency for Research on Cancer.1,2

Among all the techniques used or tested so far, advanced oxidation processes (AOPs) have been increasingly favored for treating recalcitrant PCP and other XAr in the remediation of contaminated water or soil. 14-16 In these "environmentally green" AOP systems, which are based on hydrogen peroxide (Fenton and Fenton-like reactions) and ozone (O₃, O₃/H₂O₂), the most reactive radical intermediate formed at near-ambient temperature and pressure is the hydroxyl radical (*OH).¹⁴

Chemiluminescence (CL) is a phenomenon in which molecules in a chemically generated excited state liberate energy with light emission. CL frequently accompanies decomposition of organic peroxides and formation of free radicals. 17-21 Since the CL intensity is determined by the rate of the chemical reaction, it can be used to quantify any analyte whose concentration is rate-determining.²² CL intensity-based analytical assays are inherently highly sensitive, rapid, and simple to operate, without requiring sample pretreatment. Therefore, they are being increasingly used as a sensitive analytical method in various research fields. 22,23

We found, unexpectedly, that an intrinsic CL could be produced during our study of the advanced oxidation of XAr by OH-generating systems. Based on these findings, a novel

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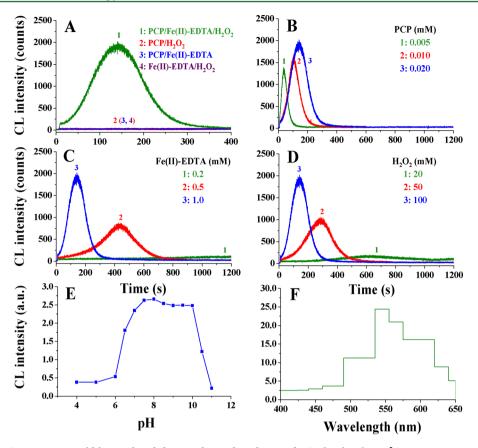


Figure 1. An intrinsic CL emission could be produced during advanced oxidation of PCP by the classic $^{\bullet}$ OH-generating Fenton system. (A) CL could be produced by PCP/Fe(II)–EDTA/H₂O₂; effects of PCP (B), Fe(II)–EDTA (C), H₂O₂ (D), and pH (E) on CL production; (F) emission spectrum of CL. PCP, 20 μ M; Fe(II)–EDTA, 1 mM; H₂O₂, 100 mM; all reactions were carried out in chelex-pretreated phosphate buffer (0.1 M, pH 7.4) and at room temperature.

mechanism underlying this unusual CL production was proposed, and a rapid, sensitive, simple, but effective CL-based method was developed to not only detect and quantify trace amounts of XAr, but also to monitor their degradation kinetics.

MATERIALS AND METHODS

Chemiluminescence (CL) Analysis. The CL emission from the reaction between XAr and an OH-generating system was measured by an ultraweak CL analyzer (Institute of Biophysics, Chinese Academy of Sciences, China) with a CR-120 red-sensitive photomultiplier tube (PMT, Hamamatsu, Japan). The CL analyzer was operated in pulse mode, and the CL signal was recorded by a computer equipped with a dataacquisition interface. Data acquisition and treatment were performed with BPCL software. The CL emission spectrum of PCP/Fe(II)-EDTA/H₂O₂ was obtained using a set of interference filters, with the wavelength from 400 to 640 nm, which were placed between the sample cuvette and the photomultiplier tube. During the determination of the CL emission wavelength, appropriate corrections were applied for both spectral response of the photomultiplier tube and transmissivity of filters. However, the CL emission spectrum obtained from the CL analyzer could only provide an approximate range, therefore a spectrofluorometer (Varian) set at CL mode was used to obtain a continuous CL spectrum and get a more accurate maximum wavelength.

Kinetic Study of *OH Formation in *OH-Generating Systems by both ESR and Fluorescent Methods. The

kinetics of OH formation in Fenton, Fenton-like, and ozonation systems were monitored by both ESR with DMPO (5,5-dimethyl-1-pyrroline N-oxide) as the spin trapping agent, and fluorescence with terephthalic acid (TPA) as an OH probe.²⁴ In Fenton and Fenton-like systems, the basic system consisted of 2 μ M PCP, 0.1 mM Fenton or Fenton-like agents, 10 mM H₂O₂, 4 mM TPA for fluorescence study, or 100 mM DMPO for ESR study, in 0.1 M chelex-treated phosphate buffer (pH 7.4) at room temperature. For the ozonation system, no Fenton or Fenton-like agents were added. ESR spectra were recorded either immediately after the interaction of PCP with Fe(II)-EDTA and H_2O_2 , or at indicated time intervals on a Bruker ER 200 D-SRC spectrometer operating at 9.8 GHz. Fluorescence detection was performed on a Cary Eclipse (Varian) spectrofluorometer. Samples with TPA as the OH probe were excited at 312 nm, and the resulting fluorescence was measured at 425 nm.

Quantitative Detection of Several Typical Classes of XAr by the *OH-Dependent CL Method Developed in This Study. The classic Fenton system was selected as the *OH-generating system. The solutions of Fe(II) (ammonium ferrous sulfate) and EDTA were mixed just before the CL experiment and stored under argon. The typical XAr were dissolved in diluted NaOH, CH₃CN, or DMF solution. H₂O₂ and XAr were added into phosphate buffer in advance, and the CL measurements were started by the injection of Fe(II)—EDTA. The total intensity of CL in the whole spectra range was integrated during the whole CL process. To determine the linear concentration range, 16 different concentrations of XAr

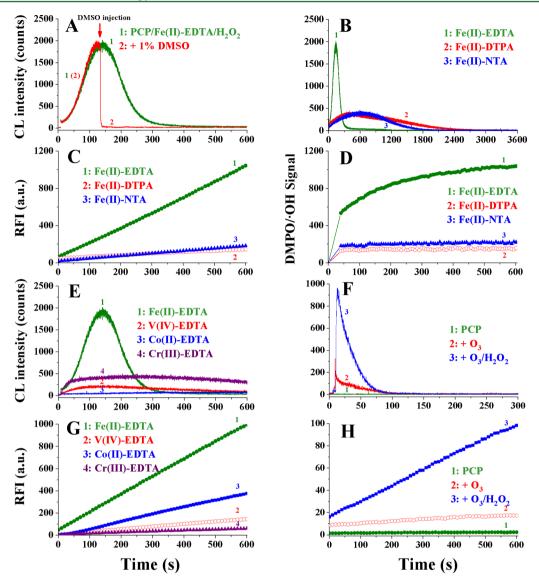


Figure 2. The CL production from PCP/Fe(II)–EDTA/H₂O₂ was directly dependent on OH generation. (A) CL production from PCP/Fe(II)–EDTA/H₂O₂ could be markedly quenched by the typical OH scavenger DMSO: PCP, 20 μM; Fe(II)–EDTA, 1 mM; H₂O₂, 100 mM; DMSO, 1%. (B) CL production was also observed with two other well-known OH-generating Fenton agents Fe(II)–DTPA and Fe(II)–NTA: PCP, 20 μM; Fe(II) complexes, 1 mM; H₂O₂, 100 mM. (C,D) Fluorescent and ESR kinetic study on OH generation by the three Fe(II) complexes and H₂O₂: PCP, 2 μM; Fe(II) complexes, 0.1 mM; H₂O₂, 10 mM; TPA, 4 mM; DMPO, 100 mM; (E,F) CL could also be produced when PCP was treated with other OH-generating systems including Fenton-like and ozonation systems. For Fenton-like systems: PCP, 20 μM; Fe(II)–EDTA, Co(II)–EDTA, V(IV)–EDTA, Cr(III)–EDTA, 1 mM; H₂O₂, 100 mM. For ozonation systems, phosphate buffer was purged with O₃ for 60 s before reaction, and then PCP (or PCP with H₂O₂) was quickly introduced into the reaction mixtures: PCP, 100 μM; H₂O₂, 100 mM. (G,H) Fluorescent kinetic study on OH generation by Fenton-like and ozonation systems. For Fenton-like systems: PCP, 2 μM; Fenton-like agents, 0.1 mM; H₂O₂, 10 mM; TPA, 4 mM. For ozonation systems: PCP, 100 μM; H₂O₂, 100 mM; TPA, 4 mM. All reactions were carried out in chelex-pretreated phosphate buffer (0.1 M, pH 7.4) and at room temperature. Co(II), cobalt(II); V(IV), vanadium(IV); Cr(III), chromium(III).

covering six orders of magnitude were selected. They were 0.003, 0.007, 0.01, 0.03, 0.07, 0.1, 0.3, 0.7, 1.0, 3.0, 7.0, 10, 30, 70, 100, and 300 μ M.

RESULTS AND DISCUSSION

Production of Intrinsic CL Emission during Advanced Oxidation of PCP by the Classic *OH-Generating Fenton System. During our study of the advanced oxidation of PCP, we observed an intrinsic CL emission produced during the oxidation of PCP by the classic Fenton system (Fe(II)–EDTA and H_2O_2) in the absence of any chemiluminescent or fluorescent agents (Figure 1A). In contrast, neither the three individual components alone, nor the combination of any two

components, could produce any CL. The intensity of CL was found to depend on the concentration of PCP, Fe(II)–EDTA, and H_2O_2 ; the higher is the concentration of any of the three components, the stronger is the CL (Figure 1B–D). The CL was also found to depend on the pH of the buffer. Thus, no CL was observed at pH \leq 5, but CL increased progressively with pH in the range of 5 to 8, remained steady between pH 8 and 10, and then declined at pH >10 (Figure 1E). The CL emission spectrum is characterized by a broad band (510–580 nm), which is centered at 535 nm (Figure 1F and Supporting Information Figure S1). It is worth noting that CL could be also generated from horseradish peroxidase (HRP) catalyzed decomposition of chlorophenol in the presence of H_2O_2 .

Scheme 1. Proposed Molecular Mechanism for *OH-Dependent CL Production by PCP/Fe(II)-EDTA/H₂O₂

Direct Dependence of CL Production from PCP/ Fe(II)-EDTA/H₂O₂ on *OH Generation. Since *OH is known to be produced by the classic Fenton system, we investigated whether or not the CL emission from PCP/ Fe(II)-EDTA/H₂O₂ is dependent on OH formation. Indeed, we found that the CL production by PCP/Fe(II)-EDTA/ H₂O₂ was directly dependent on *OH formation, as indicated by the following lines of evidence: (1) The CL produced by PCP/Fe(II)-EDTA/H2O2 was markedly inhibited by several typical *OH scavengers, such as dimethyl sulfoxide (DMSO), ethanol, and benzoate (Figure 2A and Supporting Information Figure S2); (2) not only the CL reaction and OH-generation could be markedly accelerated, but also the CL intensity and *OH yield were enhanced significantly as the concentration of Fe(II)-EDTA and H_2O_2 was increased (Figure 1C,D); (3) CL was also observed when Fe(II)-EDTA was substituted by two other well-known OH-generating Fenton agents Fe(II)-DTPA (diethylenetriaminepentaacetic acid) and Fe(II)-NTA (nitrilotriacetic acid) (Figure 2B); ^{26,27} and (4) CL emission correlated strongly with the rate of *OH generation from the three Fe(II) complexes and H₂O₂, as measured by both ESR spin-trapping and fluorescent methods (Figure 2C,D).

CL Produced by PCP with Other *OH-Generating Fenton-like and Ozonation Systems. We found that CL could be produced not only with the classic Fe(II)-mediated Fenton systems, but also with other redox-active metal-mediated Fenton-like systems including cobalt(II)-EDTA/H₂O₂, vanadium(IV)-EDTA/H₂O₂, and chromium(III)-EDTA/H₂O₂ (Figure 2E), as well as the well-known ozonation systems, including O₃ and O₃/H₂O₂ (Figure 2F). *OH was produced by all these systems (Figure 2G,H).

Chlorinated Quinoid Compounds and 2,3-Dichloromaleic Acid as the Major Transient Intermediates and Ring-Opened Product, Respectively. The aforementioned results suggest that, in the PCP/Fe(II)-EDTA/H₂O₂ system, the primary species responsible for CL emission are probably transient intermediates or final products of PCP during its degradation by OH. These may include the corresponding tetrachloroquinoid compounds, such as tetrachlorohydroquinone (TCHQ), tetrachloro-1,4-benzoquinone (TCBQ), tetrachlorocatechol (TCC), and tetrachloro-1,2-benzoquinone (O-TCBQ),6-8 as well as their hydroxylation products such as trichlorohydroxy-1,4-benzoquinone (TrCBQ-OH) and 2,5dichloro-3,6-dihydroxy-1,4-benzoquinone (DDBQ).²⁸⁻³¹ Indeed, we found by HPLC analysis that five of these six transient quinoid intermediates were formed in the PCP/ Fe(II)-EDTA/H₂O₂ system (Supporting Information, Figure S3). When reacted with H₂O₂, these tetrachloroquinoid compounds also produced weak CL that was greatly enhanced by the addition of Fe(II)-EDTA (Supporting Information, Figure S4). Further studies showed that these quinoid intermediates could be further decomposed to several ringopened products. Specifically, the major products were identified as 2,3-dichloromaleic acid (DCMA) (maximum yield, 22.7%) and CO₂ (and/or CO) (about 83.6% PCP can be mineralized to inorganic carbon after 2 h) (Supporting Information, Figure S3).

Possible Molecular Mechanism for CL Production during *OH-Dependent PCP Degradation. The fact that the emission spectrum of the CL in the PCP/Fe(II)–EDTA/ $\rm H_2O_2$ system is characterized by a broad band (510–580 nm) centered at 535 nm (Figure 1F and Supporting Information, Figure S1) suggests that the emitting species is the electronically excited carbonyls. The basis of the above results and our previous findings, 29,31 we proposed the following molecular mechanism for *OH-dependent CL emission from PCP/

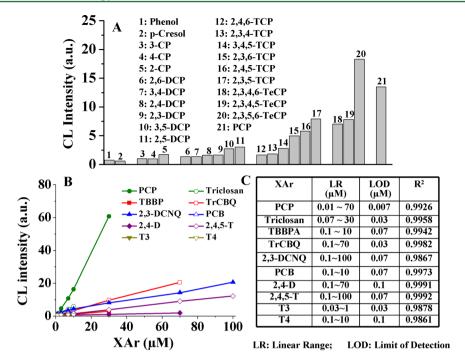


Figure 3. Detection and quantification of chlorinated phenols and other typical haloaromatics (XAr) by ${}^{\bullet}$ OH-dependent CL method. (A) CL production by 19 chlorinated phenols and nonchlorinated phenols in the presence of Fe(II)–EDTA/H₂O₂. Chlorinated phenols (or nonchlorinated phenols), 10 μ M; Fe(II)–EDTA, 1 mM; H₂O₂, 100 mM; room temperature. (B) Standard curves of CL intensity and XAr concentrations; and (C) linear range and detection limit for several typical XAr as measured by the CL method developed in this study. All reaction mixtures contained 1 mM Fe(II)–EDTA and 100 mM H₂O₂, and reaction temperature was 37 ${}^{\circ}$ C. The reactions were carried out in chelex-pretreated phosphate buffer (0.1 M, pH 7.4).

Fe(II)–EDTA/ H_2O_2 (Scheme 1): *OH first attacks PCP, via electron transfer and/or electrophilic addition, forming pentachlorophenoxyl radical and tetrachlorosemiquinone radical, 6,33,34 which would further convert to the initial tetrachloroquinoid intermediates. The nucleophilic reaction may take place between these tetrachloroquinoid intermediates and H_2O_2 , generating unusually highly energetic quinone-1,2-dioxetanes in the presence of *OH. These quinone-1,2-dioxetanes further decompose to form the electronically excited carbonyl species $[L]^*$. The CL at a broad band of 510–580 nm is emitted when the electronically excited state of $[L]^*$ returns to its ground state [L], and the subsequent decomposition of [L] leads to the formation of the final ring-opened products DCMA and CO_2 (and/or CO).

It should be noted that neither the excited CL-emitting carbonyl species [L]* nor its ground state [L] could be detected under our current experimental conditions, which might be due to their unstable nature and extremely short lifespan. Further investigations are needed to detect and identify these species.

*OH-Dependent CL Production as a General Phenomenon for All XAr Tested. It is interesting to know whether CL could also be produced by other chlorinated phenols with the classic Fenton system; and if so, what is the structure—activity relationship? Indeed, we found that the analogous *OH-dependent CL emission could be observed when PCP was substituted with all of the 19 chlorinated phenol congeners (Supporting Information, Figure S5), while the nonhalogenated phenol and methylated phenol, such as *p*-cresol, generated only very low CL under the same experimental conditions (Figure 3A and Supporting Information, Figure S6). The CL emission from the chlorinated phenols was found to depend not only on the chlorination level, but also on the chlorine substitution

position, and each compound showed a distinct CL profile. In general, the higher is the chlorination level, the stronger is the CL; however, there are clear exceptions. For example, the CL produced by PCP is weaker than that of 2,3,5,6-tetrachlorophenol (TeCP). Interestingly, the CL produced by chlorophenol isomers followed the general trend *ortho* > *meta* > *para*-substituted isomers. Furthermore, phenols with chlorine substitutions on both *ortho*- and *meta*-positions showed the highest CL emission. Thus, 2,5-dichlorophenol (DCP), 2,3,5-trichlorophenol (TCP), and 2,3,5,6-tetrachlorophenol (TeCP) produced the highest CL emission among their corresponding DCP, TCP, and TeCP isomers.

More importantly, we found that, besides chlorinated phenols, CL could also be produced by all other halogenated phenols, such as pentabromophenol (PBP), pentafluorophenol (PFP), the flame retardant 3,3',5,5'-tetrabromobisphenol A (TBBPA), and the broad-spectrum antibacterial personal care agent triclosan (Supporting Information, Figures S7 and S8). In fact, CL could be observed with all XAr tested (see Supporting Information, Table S1, Figure S7 and S8). These include the widely used chlorophenoxyacetic acid herbicides, such as 2,4dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), which are the major components of Agent Orange; iodinated aromatic pharmaceuticals, such as thyroxine (T4) and triiodothyronine (T3); new chlorination disinfection haloquinoid byproducts in drinking water, such as 2,6-dichloro- and trichloro-1,4-benzoquinone (TrCBQ);35 halogenated naphthoquinone pesticides, such as 2,3-dichloro-1,4-naphthoquinone (2,3-DCNQ); halogenated benzene biocides, such as pentachlorobenzene (PCB) and hexachlorobenzene; polyhalogenated diphenyl ether flame retardants, such as BDE 79 and BDE 209; polyhalogenated biphenyl dielectric and coolant fluids, such as trichlorobiphenyl (and their correspond-

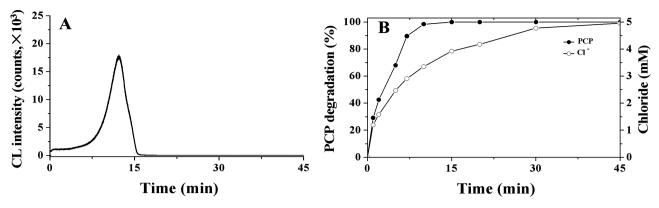


Figure 4. Good correlations were observed between CL emission (A) and degradation kinetics (B) during advanced oxidation of PCP. PCP, 1 mM; Fe(II)-EDTA, 3 mM; H_2O_2 , 300 mM. The reactions were carried out in chelex-pretreated phosphate buffer (0.1M, pH 7.4) and at room temperature.

ing diphenols and the quinones). These results indicate that OH-dependent CL production during advanced oxidation is a general phenomenon for all XAr tested, but each compound exhibits a distinct CL emission profile.

Potential Biological/Environmental Implications and Possible Practical Applications. The fact that not only PCP and other halogenated phenols, but also all other tested XAr, could produce OH-dependent CL during their advanced oxidation may have interesting chemical, biological, environmental, pharmaceutical, and toxicological implications. Many widely used XAr, which are considered persistent organic pollutants (POPs) and probable human carcinogens, including polyhalogenated phenols (such as PCP, triclosan, and 3,3',5,5'tetrabromobisphenol A), Agent Orange, hexachlorobenzene, and diphenyl ether flame retardants, can be degraded, detoxified, and mineralized in various *OH-mediated AOPs. 9-13 Recently, polychlorinated quinoid carcinogens were also identified as new chlorination disinfection byproducts in drinking water³⁵ and found in discharges from pulp and paper mills. 1,2 These XAr not only cause oxidative damage to DNA and other macromolecules, they also form protein and DNA adducts both in vitro and in vivo³⁶⁻³⁹ and therefore, are potentially carcinogenic toward mammalian organisms. Consequently, it is important to detect and measure these ubiquitous polyhalogenated pollutants or pharmaceutics both in the environment and in biological systems. It is well-known that CL-based analytical methods are inherently sensitive due to the relative ease with which low light emission can be quantified by photon-counting techniques. Therefore, we might use the CL-producing property to detect and measure trace amounts of several typical model XAr, including PCP, triclosan, 3,3',5,5'-tetrabromobisphenol A, TrCBQ, 2,3-DCNQ, PCB, 2,4-D, 2,4,5-T, T3, and T4 (see Materials and Methods, Figure 3B,C, and Supporting Information, Figure S8 and Tabels S1 and S2). Indeed, we found that as low as 1.8 ppb of PCP could be detected by this novel CL assay, and the linear range for PCP is 2.6-18620 ppb (Figure 3C). This is lower than the PCP concentration level of 40 ppb found in the body fluids of people who are not occupationally exposed to PCP, and much lower than the level of 19 580 ppb in the occupationally exposed individuals.1 Because of the low PCP detection limit, this CL-based method might be used to measure PCP both in our environment and in biological systems.

To test whether the CL-based method can be applied to detect and measure possible XAr present in real environmental

samples, the discharge from a paper mill was evaluated. We found that CL could be produced from the paper-mill discharge in the presence of Fe(II)–EDTA/H₂O₂ (Supporting Information, Figure S9), and further investigation indicated that the discharge indeed contained several XAr, including 4-chlorophenol, 2,4-dichlorophenol, and other chlorinated aromatics identified by GC–MS analysis coupled with enrichment through solid phase extraction (SPE). These results suggest that the CL-based method could be used to evaluate potential XAr contamination in real environmental samples.

Haloaromatics are degraded and detoxified during AOPs to low-toxic small molecules or even mineralized to CO_2 or CO . Therefore, during the practical remediation process, the kinetics of XAr degradation is often monitored to evaluate the efficiency of the remediation techniques. The traditional method, such as HPLC, $^{9-11,33,40}$ used to monitor the kinetics of XAr degradation during AOPs is not only relatively time-consuming, but also consumes large amounts of solvents. Thus, a rapid, sensitive, simple, and effective method to monitor the degradation kinetics of XAr is urgently needed.

We found that the new CL-based method we developed in this study could also be used for this purpose. In the PCP/Fe(II)-EDTA/H₂O₂ system, we found that the CL kinetics correlate well with the degradation kinetic curves of PCP as measured by HPLC. The time course of CL emission was accompanied concurrently with the degradation of PCP, and when the degradation level of PCP and the chlorine ions released reached the maximum, no further CL emission could be observed (Figure 4 and Supporting Information, Figure S10).

Another potential application for the CL-based method is to detect *OH production when coupled with typical *OH-scavenging agents since the CL produced by *OH-generating systems with XAr was found to be directly and specifically dependent on *OH production, but not on other reactive oxygen species, such as superoxide anion radical and singlet oxygen.

Distinguishing Characteristics and Advantages of This Novel CL-Dependent Method. When compared with previously reported analytical methods of measuring XAr, this novel CL-dependent method showed the following characteristics and advantages: (1) It is sensitive and selective since high intensity CL emission is observed from XAr, and each showed a distinct CL profile, while no or only extremely weak CL was obtained from nonhalogenated aromatics. (2) The CL emission

produced by XAr and an *OH-generating system is a direct and intrinsic CL, so no other fluorescent or chemiluminescent agents are needed. (3) It is simple and rapid to operate, and does not require time-consuming derivatization and sample pretreatment. (4) The chemical agents and the portable CL instrument used are quite inexpensive and also readily available commercially. Therefore, when compared to other methods, this CL-based method could reduce cost and would be very suitable for practical applications, especially on-site monitoring and analysis. (5) It could be used not only to detect and measure trace amounts of XAr in both pure and real samples, but also to monitor their real-time degradation kinetics, especially during advanced oxidation processes.

ASSOCIATED CONTENT

S Supporting Information

Experimental details; supporting figures. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b01227.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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