Photodegradation of the azole fungicide climbazole by ultraviolet irradiation under different conditions: Kinetics, mechanism and toxicity evaluation

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Abstract

Climbazole (CZ) has been known to persist in various environmental media, and may cause potential risks to aquatic organisms. This study investigated the photodegradation of CZ by ultraviolet (UV, 254 nm) under different conditions. The results revealed that CZ could be effectively degraded in aqueous solutions under UV-254 irradiation with a half-life of 9.78 min (pH=7.5), and the photodegradation followed pseudo-first-order kinetics. pH had almost no effect on its rate constants and quantum yields; but the water quality of natural waters could affect the photolysis of CZ, and the coexisting constituents such as Fe^{3+} , NO_3^- , and HA obviously inhibited its photolysis. The addition of different radical scavengers also inhibited the photodegradation of CZ due to the reduction of reactive oxygen species (ROS). CZ underwent direct and self-sensitized photolysis involving ROS. Based on the identified photodegradation by-products, the proposed pathways included hydroxylative dechlorination, dechlorination and de-pinacolone. Moreover, toxicity evaluation using

duckweed found significant toxicity reduction in the photodegradation system of CZ after the irradiation of UV-254, and the remaining by-products did not pose extra toxicity compared with CZ itself. These findings from present study suggest that CZ in effluent could be further reduced by applying UV photolysis treatment.

Keywords: Climbazole, Photodegradation, Reactive oxygen species, By-products, Toxicity

1. Introduction

The azole fungicide climbazole (CZ) is widely used in shampoos and other personal care products (PCPs) not only as an anti-dandruff active ingredient but also as an antimycotic preservative and an anti-aging agent [1,2]. The maximum usage in shampoos was up to 2.0%, equivalent to approximately 15×10^3 mg/L [3]. It was estimated that the annual consumptions of CZ in European Union and China were up to 100~1000 tons and 3800 tons, respectively [4,5]. After use of shampoos and other PCPs, CZ ingredient would be discharged into wastewater treatment plants (WWTPs) along with wastewater [6-8]. But the conventional WWTPs could not completely remove CZ, with its aqueous removal rates of 34~76%, and the removed CZ was mostly transferred into sludge [7,8]. Then through sewage effluent discharge and sludge application on land, the remaining CZ ended up in the receiving environments, including water compartment [8-10] and soil compartment [11,12] with an approximate distribution ratio of 93% in water and 7% in soil [13]. CZ has been reported to be quite toxic to aquatic and terrestrial organisms [12,14]. Moreover, azole fungicides like CZ have been regarded as potential endocrine disruptors due to their adverse effects on P450-regulated

steroidogenesis [15,16]. Therefore, it is essential to understand the environmental behavior and dissipation of CZ and its potential risks to organisms.

The frequent detection of CZ with high levels in various environmental media (influent, effluent, sludge, surface water and sediment as well as soil) indicated its limited dissipation ability or biodegradability in conventional WWTPs and in the natural environment [6,7,10]. For example, CZ showed persistence in soil with its half-lives of 175~179 d under the field condition [11]. Therefore, it is vital to remove or reduce this chemical in wastewater stream by selecting a suitable advanced treatment technique. Photolysis under ultraviolet (UV) irradiation may be an option for the removal of CZ in WWTPs, since previous studies have demonstrated that many pharmaceuticals and personal care products (PPCPs) could be degraded effectively under UV irradiation [17-19]. There are also limited reports about the UV photolysis of other azole fungicides, such as clotrimazole, ketoconazole and miconazole [20], and fluconazole [21]. However, few studies reported the UV photolysis of CZ, which only investigated the kinetics (different pH) and transformation products of CZ [22,23]. Therefore, a systematic research is clearly needed to explore the influencing factors, degradation by-products, pathways and the toxicity changes during the photolysis of CZ.

The objectives of this study were to investigate the photodegradation kinetics of CZ under the UV-254 irradiation in aqueous solutions under various conditions for elucidating its primary influencing factors, and to identify the photodegradation by-products of CZ and propose its photolysis mechanism. Moreover, the toxicity changes of CZ and its by-products during the photodegradation process were evaluated by duckweed toxicity test and software prediction.

2. Experimental section

2.1 Chemicals and reagents

CZ (99.9%), Furfuryl alcohol (98%) and 1, 4-Benzoquinone (99%) and humic acid (HA, 98%) were obtained from Dr. Ehrenstorfer, Alfa Aesar and J&K Chemical, respectively. Reagents of HPLC grade (methanol, acetonitrile, ethyl acetate, dichloromethane and isopropanol) were purchased from Merck and CNW Technologies. The other chemicals and reagents were of analytical grade. Stock solutions of CZ were prepared in methanol at a concentration of 1×10^3 mg/L and stored in amber bottle and kept at -18 °C. The freshwater duckweed (*Lemna minor*) for toxicity testing was obtained from the Sydney Ecotoxicology Laboratory of the Commonwealth Scientific and Industrial Research Organization (CSIRO, Australia).

2.2 Photodegradation experiments

A photo-reactor with a 10 W low-pressure mercury lamp ($\lambda = 254$ nm) (Beijing Cel Sci-tech Co., Ltd., China) was used for all the photodegradation experiments of CZ. The lamp was inserted into the center of a cylinder reactor with Pyrex outer walls. The light was filtered through a quartz jacket, situating between the lamp and the reaction vessel, and the light intensity of the lamp was 4.36 ± 0.14 mW/cm² (n=10) through the quartz jacket containing circulating water which was used for maintaining a constant temperature of 25 ± 1 °C. The reaction solution was stirred to ensure complete mixing by a magnetic mixer at a speed of 1500 rpm. Then the lamp was turned on to initiate the reaction. In order to avoid sunlight influence, the photo-reactor was covered with double tin foil, and the diagram of photo-reaction device was shown in the Supplementary Information (Fig. S1).

Dark controls were included in the experiments, and all the kinetic experiments were conducted in duplicate. The initial concentration of CZ was 0.5855 mg/L, and methanol contained in the CZ stock solution were dried under a gentle nitrogen stream, then redissolved in 400 mL ultrapure water, collected natural waters or phosphate solution (isometric mixture of 680 mg/L monopotassium phosphate and 1,141 mg/L dipotassium phosphate). The latter was just used for the pH effect study (pH 5.0, 7.0 and 9.0). Other kinetic experiments were carried out in pH 7.5 aqueous solutions (nearly the pH value of the secondary effluent). Appropriate concentration of HCl and NaOH solutions were employed for pH adjustment. The accurate pH values were determined using a pH meter (Thermo Fisher Scientific, USA). The Quantum yields of CZ at 254 nm under different pH conditions (5.0, 7.0 and 9.0) were determined by chemical actinometry for weakly absorbing systems using atrazine as an actinometer [21], and the details of the method is described in Supplementary Information (Text S1).

The effects of natural waters (tap water, river water and secondary effluent) with different water quality and different concentrations of coexisting constituents (Fe^{3+} , NO_3^- and HA) on the photodegradation of CZ were also investigated. In order to remove the influence of microorganisms and particles to photodegradation, natural waters were filtered through 0.22 μm sterilized membrane before use, and their basic information could be found in Table S1.

The effect of oxygen (O_2) was also examined in ultrapure water (contains O_2) and N_2 aeration solution (2 L/min) [21]. The roles of reactive oxygen species (ROS) were monitored by adding radical scavengers into reaction solutions. Isopropanol (120 mg/L), furfuryl alcohol (196 mg/L) and benzoquinone (216 mg/L) were respectively used to remove the hydroxyl radical ($\bullet\text{OH}$), singlet oxygen ($^1\text{O}_2$) and superoxide anion radical ($\text{O}_2^{\bullet-}$), forming in the photodegradation process, as the

previous reports [24,25]. In addition, the aqueous solution was replaced with acetonitrile to studying the effect of H₂O on the forming of •OH [26].

In the process of reaction, 1 mL solution was collected at different reaction time intervals (0, 2, 5, 8, 12, 16, 20, 30, 40, 50 and 60 min) to measure the concentrations of residual CZ in the reaction solution by high performance liquid chromatograph system equipped with a diode array detector (HPLC-DAD, Agilent 1200, USA). The detailed instrumental method is described in Text S2.

In addition, for comparing the removal efficiency of 0.5855 mg/L CZ aqueous solutions (pH=7.5) in different light sources, the 10 W low-pressure mercury lamp was replaced by the 250 W high-pressure mercury lamp (λ =225~425 nm); Meanwhile, the chlorine oxidation of CZ was also performed for the comparison with photolysis, and the detailed experiment method is given in Text S3.

2.3 Identification and semi-quantitation of photodegradation by-products

For photodegradation by-products identification, another photodegradation experiment was performed at a higher initial concentration of 1.1710 mg/L for CZ in ultrapure water, and the other reaction conditions were same as kinetic experiment. At the time of 0, 5, 10, 20, 40 and 60 min, every 400 mL reaction solution was transferred into a 500 mL amber bottle, and a control experiment without the addition of CZ but irradiated 60 min by UV-254 was also conducted. The CZ and its photodegradation by-products in the reaction solutions were extracted by solid phase extraction (SPE). Briefly, The reaction solutions were loaded at a flow rate of 5 mL min⁻¹ on the SPE cartridges (Waters Oasis HLB, 500 mg, 6 mL), which had been previously preconditioned each with 2×5 mL methanol and 2×5 mL ultrapure water. The cartridges were eluted with 2×3 mL dichloromethane,

2×3 mL ethyl acetate and 2×3 mL methanol in sequence after drying under vacuum. Three eluates from each cartridge were combined, then dried under a gentle nitrogen stream and finally redissolved in 1 mL methanol into an amber vial (Agilent). Photodegradation by-products were identified using MS² scan mode for preliminary screening and using product ion scan mode for confirmation by an Agilent 1290 ultra high performance liquid chromatograph coupled to an Agilent 6500 quadrupole time-of-flight mass spectrometer (UHPLC-Q-TOF). The detailed instrumental parameters are given in Table S2. Two high responses fragment ions observed by UHPLC-Q-TOF were selected as quantification and qualification ions, which used for the semi-quantitation of by-products and confirmed the results of identification again by an Agilent 1200 ultra high performance liquid chromatograph coupled to an Agilent 6460 triple quadrupole mass spectrometer (UHPLC-MS/MS) with electrospray ionization (ESI) source under the positive mode of multiple reaction monitoring (MRM). The detailed instrumental parameters are given in Table S3, and the information of qualitative and quantitative ions are listed in Table S4. In addition, the samples of semi-quantitation were from the previous kinetics experiments, including different pH (5.0, 7.0 and 9.0), different radical scavengers (isopropanol, furfuryl alcohol and benzoquinone), N₂ aeration, acetonitrile solution and ultrapure water.

2.4 Toxicity evaluation of photodegradation by-products

It has been reported that *Lemna minor* is very sensitive to CZ with its EC₅₀ (the concentration for 50% of maximal effect) of 0.0339 mg/L for the growth rate of frond number [14]. So *Lemna minor* was adopted to test the toxicity changes of the CZ photolysis system in present study. Based on the CZ toxicity and considering the convenience of toxic effect observation, a high initial concentration of CZ (1.1710 mg/L) was chosen for the toxicity evaluation of CZ and its photodegradation by-products.

The photodegradation experiments were performed in ultrapure water at pH 6.5 and 25 ± 1 °C under UV-254 irradiation. At the time of 0, 10, 20, 40, 70 and 110 min, 30 mL solutions were collected for the toxicity test (three replicates, about 10 mL for each). Another 1 mL was used for the determination of residual CZ concentrations and the semi-quantitation of its by-products as the previous methods. Corresponding to the residual CZ concentrations at different stages of photodegradation, the same CZ concentrations without any photodegradation by-products were prepared as the standard control by redissolving in ultrapure water after evaporating the methanol of CZ stock solutions or diluent. In addition, different concentrations of KCl solutions (0, 2.5, 5, 10, 20 and 40×10^3 mg/L) and just ultrapure water were included as positive controls and negative control, respectively. Then, the *Lemna minor* toxicity testing and evaluation for the CZ photolysis system were performed according to the OECD guideline 221 [27], with the detailed method given in Text S4.

3. Results and discussion

3.1 UV photodegradation compared to chlorination

Given that UV and chlorine oxidation disinfection are applied widely in the advanced treatment for wastewater [28,29], the degradation behaviour of CZ by UV-254 nm, UV-225~425 nm and chlorine oxidation were compared (Fig.1). CZ in aqueous solution was found stable in the dark controls (Fig. S2), demonstrating that hydrolysis can be omitted in CZ photodegradation process. As shown in Fig.1a, CZ was degraded fast under UV-225~425 with the removal rate of 99.32% at 4 min, but the result could not followed the pseudo-first-order kinetic equation well ($R^2=0.6797$, $p=0.0063$). Comparatively, CZ was degraded slower under UV-254 than UV-225~425, but the removal rate of

CZ was also up to 98.41% at 60 min, and it followed the pseudo-first-order kinetic equation well ($R^2=0.9985$, $p<0.0001$) [30]. The large difference in degradation rate of CZ under UV-225~425 and UV-254 was mainly caused by the power disparity of light source [31,32]. A nearest literature reported that the half-life ($t_{1/2}$) of CZ under UV-254 was 3.95 min [23], slightly lower than present study (9.78 min), which might cause by the different photodegradation conditions, such as light source (λ), photo-reactor, initial concentration etc. [19,31,33].

When compared to photodegradation, the chlorine oxidation of CZ was very poor (Fig.1b). The degradation of CZ was very slow and even tended to be stable after 32 h when the concentration of available chlorine was below 2.3 mg/L. The removal rate of CZ at 60 h was just 71.32%. However, the process followed the pseudo-first-order reaction kinetics ($R^2 = 0.8729$, $p < 0.0001$) with its half-life of 30.14 h. It was clear that the degradation of CZ under UV-225~425 and UV-254 was much faster than the chlorine oxidation, thus UV photolysis deserves to be further explored and applied in practicality. Considering sampling difficulty in photolysis under UV-225~425, further experiments were only carried out under UV-254 for photolysis mechanism study.

3.2 Effect of pH on the photodegradation of CZ

The effect of different pH values on photodegradation of CZ and its estimated quantum yield at 254nm was investigated, and the results are listed in Table 1. The photodegradation rate constants (k) of CZ at pH 5.0, 7.0 and 9.0 almost kept stable, ranging from 0.0635~0.0665 min^{-1} with the half-lives of 10.43~10.92 min. A similar law was also observed for the estimated quantum yields (Φ) and molar absorption coefficient (ϵ), with the values of 0.1716~0.1765 mol einstein^{-1} and 616.16~627.67 $\text{M}^{-1} \text{cm}^{-1}$ (Fig. for Text S1). According to the pKa value (7.50) of CZ, existing two species in aqueous solution [14], CZ^+ and CZ are the dominant species at pH =5.0 and 9.0, respectively (Fig. S3). But

different species of CZ have no significant influence on the photodegradation rate of CZ. From the above results, we can infer that the pH of secondary effluent at 5.0~9.0 almost have no influence on the photodegradation of CZ under UV-C irradiation. However, an early report pointed out that the photodegradation of CZ (15.8 mg/L) under UV-A and UV-B appeared to be pH-dependent (pH =5.0, 7.0 and 9.0) with the k values range of 0.0099~0.0165 min⁻¹ [22]. Similarly, this contrast may also explain by the difference of photodegradation conditions [19,31,33].

3.3 Effect of water quality and coexisting constituents on the photodegradation of CZ

It was reported that the water quality and coexisting constituents of natural waters had some influence on the photodegradation of organic pollutants [33-35]. So the effects of natural waters, Fe³⁺, NO₃⁻ and HA on the photodegradation of CZ under UV-254 were investigated (Fig.2a). In comparison with ultrapure water, natural waters showed obvious inhibition to the photodegradation of CZ, and the k values had the following order: ultrapure water (UW) > tap water (TW) > river water (RW) > secondary effluent (SE), showing a positive correlation with the pure degree of water quality (Table S1). The alone existence of Fe³⁺ or HA showed inhibition to the photodegradation of CZ, which was different from the promoting effects of Fe(III)-HA complexes [36]. Because the absence of HA could cause the damage of the iron cycle involving in the photo-reaction [37]. While HA scavenged radicals as light-absorbing species and result in a quenching effect on the reaction system [33,38], so the higher of the HA concentration, the more evident of the inhibitory effects, which was consistent with the previous reports [19,39]. In contrast, a promoting effect of 10 mg/L NO₃⁻ on the photodegradation of CZ was discovered, which might be caused by the generation of nitrogen reactive species (NRS) and ROS from NO₃⁻ under the UV- 254 nm [40,41]. However, 5 or 50 mg/L NO₃⁻ almost had no effects on the photodegradation of CZ, this was probable due to the insufficient

of NO_3^- or the competitive photoabsorption by the high concentration of NO_3^- [19]. In fact, the effect of poor water quality on photodegradation of CZ was a comprehensive result from coexisting constituents such as Fe^{3+} , NO_3^- , HA, and dissolved organic matter in natural waters [34,42].

3.4 Effect of O_2 and ROS on the photodegradation of CZ

The ROS (includes $\bullet\text{OH}$, $^1\text{O}_2$ and $\text{O}_2^{\bullet-}$) could be generated during the photodegradation of PPCPs, and contributed to the degradation of PPCPs [18,19,26,34]. Fig.2b presented the effect of O_2 and ROS on the photodegradation of CZ under UV-254. The k value of CZ in N_2 aeration solution was the highest, and the rest conditions (adding different radical scavengers, especially benzoquinone) displayed a significant inhibition to the photodegradation of CZ compared to the ultrapure water. Our previous study suggested that O_2 played an important role in the photodegradation of fluconazole by inducing the generation of ROS [21], but no similar phenomenon was observed in this study, which suggests that O_2 was not the main factors influencing the photodegradation of CZ. In contrast, agitation caused by nitrogen stream was in favour of the contact of CZ and ROS. The effect of isopropanol on the photodegradation rate has commonly been used to estimate the oxidation mechanism [24,26]. Azimi et al. [43] have been found that the addition of isopropanol significantly inhibited the photocatalytic degradation of tetracycline and cephalexin due to the excellent quenching effect of isopropanol to $\bullet\text{OH}$. Similarly, furfuryl alcohol has been usually used as the quencher of $^1\text{O}_2$ due to its well performance [25,44]. While benzoquinone not only can scavenge the $\text{O}_2^{\bullet-}$ but also screen the light due to the brown of itself [19,26,44]. In addition, the k value of CZ in acetonitrile was distinctly lower than in the ultrapure water, suggesting that H_2O played a vital role in the forming of $\bullet\text{OH}$ [26], and indirectly indicating that $\bullet\text{OH}$ was one of the most important ROS in the photodegradation of CZ. In a word, three ROS ($\bullet\text{OH}$, $^1\text{O}_2$ and $\text{O}_2^{\bullet-}$) produced during the

photodegradation of CZ and promoted the degradation of it, while O₂ seemed to have no influence on the generation of the ROS. These results suggest that CZ could undergo both direct and self-sensitized photodegradation like other PPCPs [19,21,25].

3.5 Photodegradation mechanism of CZ

3.5.1 Identification of photodegradation by-products

A previous study from Castro et al. [23] suggested that the photodegradation of CZ under the UV-254 irradiation could produce a variety of by-products (BPs), just as demonstrated in the chromatograms acquired from UHPLC-MS/MS analysis in the present study (Fig. S4). Five photodegradation by-products of CZ (CZ-BP1~CZ-BP5) were identified and confirmed by the UHPLC-QTOF and UHPLC-MS/MS (Table S4), which was less than the reported number (twelve) from Castro et al. [23], but two of them were same (CZ-BP4 and CZ-BP5). This difference was probably caused by the differences in photolysis conditions, the pre-treatment methods for samples, the identification conditions for instruments and the acceptance or rejection of identification results. The by-products confirmed in the present study all obtained the high overall scores (>93.43, Table S4) based on the MFG (Molecular Formula Generation) and PCDL (Personal Compound Database Libraries) of the MussHunter MSC (Molecular Structure Correlator) software (Agilent, Version B.05.00). To elucidate the chemical structures of by-products from the photodegradation of CZ, the MS² spectra extracted from their chromatograms of product ion scan mode provided the basis of interpretation (Fig. S5). For instance, the fragment of 69 m/z (Fig. S5 (a, c and e)) indicated the presence of the imidazole ring, which had not been opened in all of the photodegradation by-products (Fig. S5). Similarly, 141 m/z and 123 m/z suggested the 4-chlorophenoxy group and the 4-hydroxyphenoxy group contained in the CZ-BP3 (Fig. S5c) and CZ-BP5 (Fig. S5e), respectively.

Based on these fragments and its structure provided by the MussHunter MSC software, the molecular structures of the corresponding by-products could be confirmed [20]. As to the proposed fragmentation pathway, we could take CZ-BP5 as an example to illustrate it (Fig. S5e). The precursor CZ-BP5 fractured to a fragment containing 4-hydroxyphenoxy group and pinacolone group (207 m/z) and imidazole ring (69 m/z), with the breakage of the chemical bond C-N in the CZ-BP5. Then followed a continued fragments transformation: from 207 m/z to 179 m/z (-CO), 179 m/z to 123 m/z (-C₄H₈) and 123 m/z to 109 m/z (-CH₂).

3.5.2 Semi-quantitation of photodegradation by-products

Semi-quantitation of the photodegradation by-products during the process of photolysis could express their estimated yields, which were calculated using their peak areas at different times versus that of their precursor compound at 0 min [20,21]. So the estimated yields of CZ-BP1~CZ-BP5 during the process of CZ photolysis were calculated, as shown in Fig. 3. No matter in any reaction conditions, CZ-BP1~CZ-BP5 were all found with low estimated yield (<23%, 60 min), and most of them showed an increasing tendency with the decrease of CZ. CZ-BP5 had a higher estimated yield than the other by-products, especially at pH 5.0 and 7.0 (>12%, 60 min) (Fig. 3a and Fig. 3b). Under the acidic conditions, the CZ⁺ is the main species of CZ (Fig. S3), which suggests that CZ⁺ was conducive to the generation of CZ-BP5 compared to CZ. The condition of pH affected the generation of certain by-products, which is consistent with our previous finding for fluconazole photodegradation [21]. For the common by-products (CZ-BP4 and CZ-BP5) of this study with report from Castro et al. [23], their estimated yields were comparative in ultrapure water (pH=7.5) (Fig. 3d). In addition, the estimated yield of CZ-BP5 presented some visible increase with the slow decrease of the CZ-BP4 after irradiation for 30min (Fig. 3(a, b, d and f) and Fig 5b), which indicated CZ-BP4

could partly transform into CZ-BP5.

Compared with the ultrapure water (Fig. 3d), the estimated yield of by-products slightly decreased in the solutions of N₂ aeration (Fig. 3e), acetonitrile (Fig. 3i) and other solutions with addition of radical scavengers (Fig. 3g , Fig. 3h), except for the solution with furfuryl alcohol (scavenging ¹O₂) (Fig. 3f) which displayed a slight increase for CZ-BP5. These phenomena revealed the involving of ROS in the photodegradation process, and the decrease of O₂^{-•} and •OH significantly influenced the generation of by-products (Fig. 3g and Fig. 3i), but the decrease of ¹O₂ seemed to be beneficial to the formation of CZ-BP5 (Fig. 3f). Finally, CZ-BP1~CZ-BP5 were all detected during the photodegradation process under both in oxygenated water (ultrapure water, forming ROS) and anoxic water (N₂ aeration) (Fig. 3d and Fig. 3e), indicating that they could be generated in both direct photodegradation and self-sensitized photodegradation involving with ROS.

3.5.3 Proposed degradation pathway

Based on the results of by-product identification and semi-quantitation, a plausible degradation pathway of CZ is proposed in Fig. 4. Firstly, CZ was excited to the excited state (CZ*) by UV-254. Then two routes were included in the proposed mechanism for the photodegradation of CZ. Through route (1) direct photodegradation and route (2) self-sensitized photodegradation involving with ROS, CZ was photodegraded to five by-products (CZ-BP1~CZ-BP5). The generation of these by-products proved that the photodegradation of CZ was subject to hydroxylative dechlorination (for CZ-BP5), dechlorination (for CZ-BP4), de-pinacolone (for CZ-BP3), de-chlorophenyl (for CZ-BP2) and rearrangement after de-chlorophenyl (for CZ-BP1). Among them, hydroxylative dechlorination and dechlorination for CZ photolysis have been reported by Castro et al. [23]. Meanwhile, hydroxylative dechlorination was similar to the hydroxylative defluorination for fluconazole [21], and the

dechlorination has been found in the photolysis of clotrimazole, ketoconazole and miconazole under UV exposure [20]. These results revealed the similarity of photodegradation mechanism of azole fungicides under UV irradiation. In addition, CZ-BP4 could be converted to CZ-BP5 by the hydroxylation, as found in the photodegradation of some other PPCPs [19,21,26].

3.6 Toxicity change of the CZ photodegradation system

As shown in Fig. 5a, the growth inhibition rates (GIRs) for the chlorophyll content of *Lemna minor* did not exhibit significant differences between the treated samples and the standard samples at most irradiation time ($p>0.05$), except for 10 min and 70 min (the treated samples were lower than the standard samples, $p<0.05$). However, most of GIRs manifested the statistically variations among different irradiation time no matter for the treated samples or the standard samples, and the values presented a visible decline tendencies with the extension of irradiation time, but all more than 63%. Similar results were found through biomass yield (frond numbers or wet weight) (Fig. S6). These findings revealed that the toxicity of CZ to *Lemna minor* was reduced after the irradiation of UV-254, but *Lemna minor* was indeed an easily sensitive species to CZ [14]. During the process of photodegradation, the concentrations of CZ decreased continuously with the extension of irradiation time (the removal rate up to 97% at 110 min); while the estimated yields of its five by-products (CZ-BP1~CZ-BP5) increased slowly, but always lower than 6% (Fig. 5b). However, during the cultivation process of *Lemna minor* for toxicity test, CZ was found extraordinary stable for both treated samples and CZ standards (Fig. S7) which were not detected with any by-products; while the estimated yields of CZ-BP1~CZ-BP5 were changed with the elongation of incubation time, showing a distinct decrease tendency on the whole (all lower than 6%), except the low-producing CZ-BP3 (Fig. S8). These results suggested the by-products almost had no contribution to the total toxicity of

photolysis system, and the remaining toxicity was posed mainly by CZ itself.

Moreover, the toxicity of CZ and its five by-products (CZ-BP1~CZ-BP5) were predicted by a toxicity prediction software ECOSAR V1.11 [45], developed by the US Environmental Protection Agency, and the results is listed in Table S5. The predicted 96 h EC50 value of CZ (0.144 mg/L) for green algae was very close to the experimental 72 h EC50 values: 0.1536 mg/L for *Navicula pelliculosa* and 0.2144 mg/L for *Pseudokirchneriella subcapitata* (both taking biomass yield as endpoint)[14], which proved the relative accuracy of prediction, with great reference significance for evaluating the toxicity of CZ-BP1~CZ-BP5. As shown in Table S5, all the predicted EC50 values of CZ-BP1~CZ-BP5 for green algae were higher than that of CZ, which indicated that the CZ was more toxic than all its photodegradation by-products for green algae. It was a pity for the absence of predicted EC50 values for *Lemna*, except for the CZ-BP5. When CZ-BP5 was classed as phenols, it exhibited much lower toxic than CZ for *Lemna*, with a predicted 7 d EC50 value of 9.523 mg/L for *Lemna gibba*, while the experimental value of CZ for *Lemna minor* was 0.0190 mg/L for the biomass yield [14]. Overall, the predicted toxicity of photodegradation by-products for CZ was lower than CZ itself, coupling with their low estimated yields (Fig. 5b), which provided a plausible explanation for that the photodegradation by-products of CZ did not pose extra toxicity compared with CZ itself, just as observed in the toxicity test of *Lemna minor*.

4. Conclusions

The results from this study revealed the rapid photodegradation of CZ under UV irradiation (UV-225~425 and UV-254), which was more effective than the chlorine oxidation. The pH value of aqueous solutions had almost no influence on the photodegradation of CZ under UV-254. Natural

waters was found to have inhibitory effects on the photodegradation of CZ when compared to the ultrapure water, this could be the combined results from the coexisting constituents in natural waters including Fe^{3+} , NO_3^- and HA. The addition of different radical scavengers (isopropanol, furfuryl alcohol and benzoquinone) also inhibited the photodegradation of CZ, which indicated the production of the ROS ($\bullet\text{OH}$, $^1\text{O}_2$ and $\text{O}_2^{\bullet-}$) during the photolysis process; while the absence of O_2 seemed to have no influence on the generation of the ROS. Thus, CZ could be photodegraded effectively via a direct and a self-sensitized mechanism involving ROS. Based on the identification and sem-quantitation of five photodegradation by-products, a photodegradation pathway of CZ was tentatively proposed, and involved hydroxylative dechlorination, dechlorination, and de-pinacolone. Moreover, photodegradation of CZ was found to be associated with toxicity reduction. The findings from present study suggested that UV photolysis could be applied as advanced treatment technology for further removal of azole fungicides like CZ in wastewater treatment plants in order to reduce their potential risks to the environment.

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Table 1 Effect of different pH values on the photodegradation of CZ and its quantum yield

pH	k (min ⁻¹)	$t_{1/2}$ (min)	ε (M ⁻¹ cm ⁻¹)	Φ (mol einstein ⁻¹)	Removal rate ^a
5.0	0.0665±0.0013	10.43±0.21	627.67	0.1765±0.0036	98.25±0.21
7.0	0.0635±0.0005	10.92±0.09	616.16	0.1717±0.0013	97.39±0.50
9.0	0.0663±0.0001	10.45±0.02	644.42	0.1716±0.0004	98.07±0.01

^a The removal rate of CZ after UV-254 irradiation for 60 min ([CZ]₀=0.5855 mg/L, in phosphate solution).

Figure captions

Fig. 1 The degradation effects of CZ by UV-254 nm, UV-225~425 nm and chlorine oxidation ($[CZ]_0=0.5855$ mg/L, in $pH_0=7.5$ aqueous solution): (a), UV-254 +UV-225~425; (b), Chlorine oxidation ($[Cl]_0=10$ mg/L).

Fig. 2 Effect of water quality and coexisting ions (a) & O_2 and ROS (b) on photodegradation of CZ: $[CZ]_0=0.5855$ mg/L, in $pH_0=7.5$ aqueous solution; UW, ultrapure water; TW, tap water; RW, river water; SE, secondary effluent.

Fig. 3 The presence rate of CZ and the estimated yield of its photodegradation by-products (BPs) in different irradiation times under different conditions: $[CZ]_0=0.5855$ mg/L, in 5 mM phosphate solutions (for $pH_0=5.0$, $pH_0=7.0$ and $pH_0=9.0$) or $pH_0=7.5$ aqueous solution; while the acetonitrile was a substitute for aqueous solution; the flow of N_2 aeration was 2 L/min; the initial concentrations of isopropanol, furfuryl alcohol and benzoquinone were all 2 mM.

Fig. 4 Proposed pathways for the identified photodegradation by-products (BPs) of CZ under the UV-254 irradiation.

Fig. 5 Toxicity evaluation results obtained from *Lemna minor* growth inhibition test (by chlorophyll content) for CZ standard samples and treated samples (a), and the changes of CZ concentrations and estimated yield of its photodegradation by-products (BPs) (b) under UV-254 at different irradiation times: the concentrations of CZ standard samples were same with the corresponding treated samples but without any by-products; the treated samples were obtained from the photodegradation of CZ ($[CZ]_0=1.1710$ mg/L, in $pH_0=7.5$ aqueous solution) in different irradiation times; letters a, b, c, d and e (or a', b', c', d' and e') indicated that the growth inhibition rate show significant difference from each other by Student-Newman-Keuls test, $p < 0.05$, while b and bc or c and bc suggested no statistically variations.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5