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The degradation and environmental risk of camptothecin, a promising marine antifoulant

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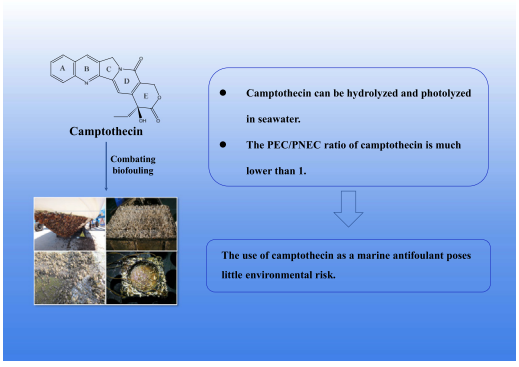
HIGHLIGHTS

- The antifouling active camptothecin is degradable in the marine environment.
- The fastest degradation pathway for camptothecin in seawater is photodegradation.
- The PEC/PNEC ratio of camptothecin is much lower than 1.
- The use of camptothecin as a marine antifoulant poses little environmental risk.

GRAPHICAL ABSTRACT



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ABSTRACT

Given the adverse environmental impacts of the antifoulants currently used in marine antifouling paints, such as copper and booster biocides, it is urgent to identify potential substitutes that are environmentally benign. Here, we examined the degradation of camptothecin (a natural product previously identified as an efficient antifoulant in the laboratory and in the field) under various conditions and evaluated the environmental risks associated with its use as a marine antifoulant. We found that camptothecin was rapidly photolyzed in seawater: the half-life of camptothecin was less than 1 d under a light intensity of 1000–20,000 lx and was approximately 0.17 d under sunlight irradiation. At pH 4 and pH 7, camptothecin had half-lives of 30.13 and 16.90 d, respectively; at 4 °C, 25 °C, and 35 °C, the half-lives of camptothecin were 23.90, 21.66, and 26.65 d, respectively. Camptothecin biodegradation in seawater was negligible. The predicted no-effect concentration (PNEC) of camptothecin was 2.19×10^{-1} μg L^{-1}, while the average predicted environmental concentrations (PECs) in open seas, shipping lanes, commercial harbors, and marinas were 6.14×10^{-7}, 9.39×10^{-7}, 6.80×10^{-3}, and 5.03×10^{-2} μg L^{-1}, respectively. The PEC/PNEC ratio of camptothecin was much lower than 1 (i.e., 2.80×10^{-6}, 4.29×10^{-6}, 3.11×10^{-2}, and 2.30×10^{-1} for open seas, shipping lanes, commercial harbors, and marinas, respectively), indicating that the use of camptothecin as a marine antifoulant posed little environmental risk.

1. Introduction

Marine biofouling, the undesirable settlement and growth of marine fouling organisms on artificial structures (e.g., ship hulls), is a worldwide problem that leads to enormous economic losses and environmental risks (Yebra et al., 2004; Fitridge et al., 2012; Bannister et al., 2019). Marine biofouling can reduce ship speed, increase fuel cost, increase emissions of carbon dioxide and sulfur dioxide, and introduce invasive species (Bressy and Lejars, 2014). Marine paints containing antifoulants are applied to combat biofouling. Metal-based compounds such as tributyltins (TBT) and copper have been widely used as antifoulants. However, these compounds are

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toxic to non-target organisms, difficult to degrade, and accumulate in the marine environment (Dimitriou et al., 2003; Blanca, 2008; Bao et al., 2011). The use of metal-based compounds may benefit certain marine species with greater tolerances to pollutants and therefore provide a competitive advantage, causing significant ecological impacts (González-Ortegón and Moreno-Andrés, 2021). These adverse environmental impacts have led governments to ban the use of tin-based compounds (IMO, 2008). Some metal-free synthetic organic biocides, such as Diuron and Irgarol 1051, have been developed for use in antifouling paints. However, due to their nonselective toxicity and slow degradation, the use of these biocides as antifouling agents has also been restricted in some countries (Thomas et al., 2002; Price and Readman, 2013). To avoid repeating history, the non-specific toxicity and environmental degradation of active antibiofouling compounds should be studied before they are approved for use in antifouling paints (Wang et al., 2014). Indeed, such studies are now required by regulatory agencies in many countries (Liu et al., 2016).

One important source of environmentally friendly antifoulants is natural product antifoulants (NPAs) (Qian et al., 2015). Many NPAs have been found to show high antifouling toxicity and low toxicity (Liu et al., 2020). However, the degradation of few NPAs has been investigated under marine environmental conditions (Wang et al., 2014). Camptothecin (CPT; chemical structure shown in Supplementary Fig. S1) is a natural product found in many plants, including *Camptotheca acuminata* (Wall et al., 1966), *Nothapodytes nimmoniana* (Govindachari and Viswanathan, 1972), *Pyrenacantha klaineana* (Zhou et al., 2000), *P. volubilis* (Suma et al., 2014), and *Dysoxylum binectariferum* (Jain et al., 2014). This compound is also found in endophytic fungi isolated from CPT-yielding plants (Puri et al., 2005; Bhalkar et al., 2016). While CPT is well known for its antitumor activity (Lin et al., 2014), we recently showed that CPT efficiently inhibited the settlement of marine fouling organisms in the laboratory and in the field (Feng et al., 2018). Furthermore, CPT was less toxic than several commonly-used commercial antifoulants to three non-target organisms from different trophic levels (the planktonic microalgae *Phaeodactylum tricornutum* and *Isochrysis galbana,* and the crustacean *Artemia salina*) (Feng et al., 2018). Although CPT shows promising application potential as a novel antifoulant, it remains unclear whether CPT is readily degraded under marine environmental conditions. This uncertainty impedes the environmental risk assessment of CPT as an antifoulant.

Information on degradation in the marine environment is required for estimations of the persistence of antifouling compounds and to identify the factors that influence antifouling-compound behavior (Harino and Langston, 2009). The aim of this study was to investigate the degradation kinetics of CPT under different conditions (hydrolysis, photolysis, and biodegradation). The environmental risks associated with the use of CPT as a marine antifoulant were evaluated; this evaluation is important for promoting the application of this novel antifouling agent to submerged marine structures.

2. Materials and methods

2.1. Chemicals

CPT with purity >98% was purchased from Chendu Dsiter High-Tech Co., Ltd. (Chendu, China). CPT was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution with a concentration of 2 mg mL^{-1}.

2.2. Degradation experiments

2.2.1. Hydrolysis at various pH values

CPT hydrolysis experiments were performed following the Organization for Economic Cooperation and Development (OECD) guideline 111 (OECD, 2004) and Chen et al. (2015). As suggested by OECD guideline 111, CPT hydrolysis was tested in buffer solutions with pH values of 4, 7, and 9. Aqueous buffers were prepared at pH 4 (0.40 mL of 1 N NaOH and 100 mL of 0.5 M potassium biphthalate in 1000 mL of ultrapure water, salinity 10‰), pH 7 (29.63 mL of 1 N NaOH and 100 mL of 0.5 M monopotassium phosphate in 1000 mL of ultrapure water, salinity 7‰), and pH 9 (21.30 mL of 1 N NaOH and 100 mL of 0.5 M boric acid (in 0.5 M KCl) in 1000 mL of ultrapure water, salinity 5‰). These buffers were sterilized at 121 °C under 2 atm for 30 min. Then, 1.25 mL of the CPT stock solution was added to 250 mL of each buffer in a 500-mL capped glass reservoir to obtain a final concentration of 10 μg mL^{-1} (this concentration of CPT was chosen based on its solubility in seawater and antifouling activity). Three replicate reservoirs were used to represent each pH value. All reservoirs were sealed and incubated at 25 °C in the dark for 30 d. At each sampling point (0 h, 2 h, 6 h, 12 h, 1 d, 4 d, 7 d, 15 d, and 30 d), 6 mL of buffer solution from each reservoir was transferred to a container (a 15-mL centrifuge glass tube with a cap) and stored at −20 °C for further analysis.

2.2.2. Hydrolysis at various temperatures

CPT hydrolysis experiments at different temperatures were performed following OECD guideline 111. CPT hydrolysis was tested in artificial seawater at 4 °C, 25 °C, and 35 °C. Artificial seawater (salinity 31‰, pH 8.2) was prepared using the formula shown in Table S1 and sterilized at 121 °C under 2 atm for 30 min. The solution of 10 μg mL^{-1} CPT in capped glass reservoirs was prepared with artificial seawater as described above. Three replicate reservoirs were used to represent each temperature. All reservoirs were sealed and incubated in the dark at 4 °C, 25 °C, or 35 °C. The sampling from each reservoir and storage of the samples were performed according to the procedures described above.

2.2.3. Photodegradation

CPT photodegradation was investigated following Okamura and Sugiyama (2004), with modifications. This experiment was performed in sterilized artificial seawater (prepared as described above) under natural and artificial light. We prepared a solution of 10 μg mL^{-1} CPT in capped glass reservoirs with artificial seawater as described above. The sunlight-exposed reservoirs were placed in an outdoor corridor on the 4th floor of the Zhoulongquan Building of Xiamen University (Xiamen, China) from the middle of February to the middle of March 2018. Sunlight radiation and temperature were monitored every day during the photolysis period. The light intensity was 3000–14,000 lx and the temperature was 18–22 °C. The artificial light-exposed reservoirs were placed in an incubator with fluorescent lamps (28 W) at 25 °C. The tested light intensities were 0 (the dark control), 1000, 5000, 1×10^4, and 2×10^4 lx (see the emission spectrum and intensity in W m^{-2} unit of each light treatment in the Supplementary Figs. S2–S5). The dark control reservoirs were wrapped in aluminum foil. For other treatments, the light intensity was adjusted by changing the distance between the light source and the reservoir. To ensure that distances were correct, light intensities were measured using an illuminance meter (TES-1300A, TES, Taiwan). There were three replicates of all the treatments. The sampling from each reservoir and storage of the samples were performed according to the procedures described above.

2.2.4. Biodegradation

CPT biodegradation was assessed following the OPPTS 835.3160 guideline "Biodegradability in sea water" (USEPA, 1998). In this experiment, we used natural seawater collected from Xiamen Bay, China (salinity, 29‰; pH 8.0; chemical oxygen demand (COD), 0.92 mg/L; bacterial density, 4.13×10^3 colony-forming units mL^{-1}). A stock solution of mineral nutrients (details shown in Supplementary Table 1) was added to the collected natural seawater at a ratio of 1 mL:1 L. The mixture was then filtered through a 1-μm membrane to remove large particles and zooplankton but not microorganisms. We then prepared a solution of 10 μg mL^{-1} CPT in capped glass reservoirs with filtered natural seawater (FNS) as described above. Two treatments were established: (1) FNS with 10 μg mL^{-1} CPT, and (2) FNS with 10 μg mL^{-1} CPT and 80 μg mL^{-1} HgCl to inhibit microbial activity (Liu and Liu, 2018) (used as a control). Each treatment consisted of three replicate reservoirs, which were incubated in a shaker at 100 rpm in the dark at 25 °C. The sampling from each reservoir and storage of the samples were performed according to the procedures described above.

2.3. Extraction and high performance liquid chromatography (HPLC) analysis

The CPT degradation samples were extracted using solid phase extraction (SPE) and analyzed by HPLC following Boyd et al. (2001) with modifications. For SPE, we used CNWBOND LC-C18 columns for the samples with different pH values, conditioned with the corresponding buffer solution as described in Section 2.2.1. CNWBOND HC-C18 columns were used for all the other samples, conditioned with 6 mL of methanol and 10 mL of methanol (5% v/v) in ultrapure water. After conditioning, water samples were loaded, and the SPE columns were rinsed with 6 mL of methanol (5% v/v) in ultrapure water at a rate of 1 mL min^{-1}. The extracts were eluted using 3 mL of methanol and evaporated to dryness using an evaporator (Genevac EZ-2, Ipswich, United Kingdom). The residue was redissolved in 1 mL of methanol for HPLC analysis. HPLC analysis was carried out on a Hypersil Phenyl-2 column (250 mm × 0.5 mm, 2.6 μm) using an Ultimate 3000 HPLC system (Dionex, Germany) equipped with a diode array detector (DAD). The mobile phase consisted of methanol and water, with the following proportions of methanol: 10–40% (0–12 min), 40–60% (12–35 min), 60–100% (35–50 min), 100–10% (50–55 min), and 10% (55–58 min). The flow rate was set to 1 mL min^{-1}. The sample injection volume was 20 μL. CPT was identified based on its retention time (14.3 min). CPT concentrations were calculated based on its standard curve (shown in Supplementary Fig. S6) using peak areas plotted against known quantities of the CPT standard. The limit of detection for CPT was 0.1 μg mL^{-1}.

2.4. Calculation of half-life

The first-order equation (Chen et al., 2015) was used to calculate the half-life as follows:

$$C_t = C_0 \cdot e^{-kt},$$

where C_t represents the concentration at time t, C_0 represents the initial concentration, and K is the degradation constant calculated from the regression curve. Solving the above equation for the half-life ($t_{1/2}$) yields the following expression:

$$t_{1/2} = \ln(2)/k = 0.693/k.$$

2.5. Methods for the calculation of predicted environmental concentration (PEC) and the predicted no-effect concentration (PNEC)

The environmental risk assessment for antifoulants in marine environment can be evaluated by comparing the PEC to the PNEC (Bellas, 2006; Gallo and Tosti, 2015). The PEC of CPT was calculated using the MAMPEC model (Van Hattum et al., 2006; Wang et al., 2014), which is used for antifoulants in OECD countries and is recommended in ISO 13073-1 (Ships and marine technology — Risk assessment on anti-fouling systems on ships — Part 1: Marine environmental risk assessment method of biocidally active substances used for anti-fouling systems on ships). The MAMPEC model is a standardized and widely applicable modelling tool for the assessment of PECs of antifouling biocides in marine environments. The MAMPEC model runs are executed after entry or editing of input data of compound properties, emission factors, and environmental and hydrodynamical parameters (Van Hattum et al., 2006). The physio-chemical properties of CPT used in the calculation are listed in Table 1. The rate at which CPT leached from the antifouling paint was calculated using the mass-balance calculation method recommended in ISO 13073-1, based on the properties of an antifouling paint containing 20% CPT (Table S2) that showed outstanding antifouling performance in the field (Feng et al., 2018). Four generic emission scenarios were chosen: commercial harbor, marina, shipping lane, and open sea. The CPT emissions in these four scenarios were estimated using the default values of the emission-related parameters in the MAMPEC 3.1.0.5 software.

Table 1
Physio-chemical properties of camptothecin.

Physio-chemical properties	Values	Notes
Molecular mass (g/mol)	348.36	
Melting point (°C)	270	
Vapor pressure (Pa)	5.01×10^{-13}	EPI Suite™ v4.0, use the value at 25 °C
Water solubility (mg/L)	<10	EPI Suite™ v4.0, use the value at 25 °C
Henry's law constant (Pa $m^3 mol^{-1}$)	2.83×10^{-12}	EPI Suite™ v4.0, use the value at 25 °C
LogKow (octanol-water coefficient)	1.74	EPI Suite™ v4.0, use the value at 25 °C
LogKoc	1.363	EPI Suite™ v4.0, use the value at 25 °C

According to ISO 13073-1, PNEC is calculated by dividing the lowest L(*E*)C_{50} value by an assessment factor (AF). An AF is incorporated into the PNEC to adjust the uncertainty in calculating the PNEC that results from testing on a limited set of potential aquatic organisms. Of the toxicity data for CPT against non-target marine organisms from Feng et al. (2018), the lowest L(*E*)C_{50} was 6.29 μM (i.e., 2.19×10^3 μg L^{-1}). Guided by ISO 13073-1, a conservative and protective AF of 10,000 was used when calculating PNEC.

3. Results

3.1. Hydrolysis at various pH values

CPT hydrolysis was faster at pH 7 than at pH 4 (Fig. 1, Table 2). The half-lives of CPT at pH 7 and pH 4 were calculated to be 16.90 d and 30.13 d, respectively (Table 2). In the buffer solutions at pH 9, CPT was not detected, probably because the CPT lactone ring (ring E) opened under alkaline conditions.

3.2. Hydrolysis at various temperatures

The effects of temperature on CPT hydrolysis is shown in Fig. 2. CPT degraded rapidly in artificial seawater at 25 °C: up to 73.1% in 30 days. At 4 °C and 35 °C, CPT degraded only 48.3% and 46.2% in 30 days, respectively. CPT degraded relatively quickly at 25 °C ($t_{1/2}$ = 21.7 d) as compared to 4 °C ($t_{1/2}$ = 23.90 d) and 35 °C ($t_{1/2}$ = 26.65 d; Table 2).

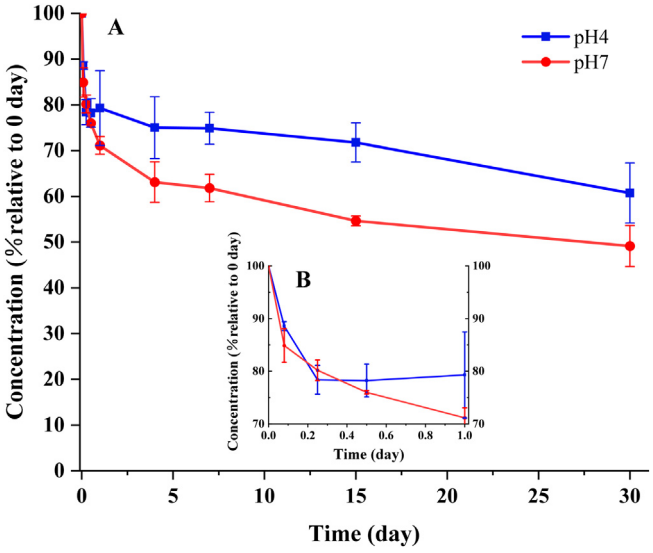

Fig. 1. The hydrolysis kinetics of camptothecin at various pH values. Data shown are the means of three replicates ± standard error. (B) is an enlarged view of (A) during the first 1 d.

Table 2
Kinetic data of the degradation of camptothecin under various conditions.

Degradation condition		K (Degradation constant, d^{-1})	$t_{1/2}$ (Half-life, d)	R^2 (Goodness of fit)
Hydrolysis	pH 4	0.023	30.13	0.9087
	pH 7	0.041	16.90	0.6732
	pH 9	/	/	/
	4 °C	0.029	23.90	0.7875
	25 °C	0.032	21.66	0.9065
	35 °C	0.026	26.65	0.7993
Photolysis	Sunlight	4.115	0.17	0.9853
	1000 lx	0.75	0.92	0.9901
	5000 lx	0.643	1.08	0.9801
	10,000 lx	2.091	0.33	0.9970
	20,000 lx	2.218	0.31	0.9507
Biodegradation	Bio	0.042	16.50	0.8139
	Hg	0.035	19.80	0.8872

/: CPT was not detected, probably due to the opening of the CPT lactone ring under alkaline condition. Hg: Filtered natural seawater (FNS) was treated with 10 μg mL^{-1} CPT and 80 μg mL^{-1} HgCl. Bio: FNS was treated with 10 μg mL^{-1} CPT.

3.3. Photodegradation

Light irradiation substantially accelerated CPT degradation (Fig. 3, Table 2). More than 98% of the CPT degraded within 4 d under sunlight and artificial irradiation, while only 11.5% of the CPT degraded within 4 d in the dark control (Fig. 3). Degradation was fastest under sunlight irradiation, with 97% degradation observed after 12 h. The half-life of CPT under sunlight irradiation was calculated to be 0.17 d (Table 2). Under artificial irradiation, CPT degradation rate increased with light intensity (Fig. 3, Table 2). Overall, the half-life of CPT was less than 1 d under light intensities of 1000–20,000 lx (Table 2).

3.4. Biodegradation

The degradation rate of CPT in natural seawater (with a degradation constant of 0.042 d^{-1}) was slightly higher than that in seawater sterilized with HgCl (with a degradation constant of 0.035 d^{-1}; Fig. 4). The half-life of CPT in natural seawater (16.50 d) was similar to that of CPT in the HgCl-sterilized seawater (19.80 d). The similar degradation kinetics between these two treatments indicated that biodegradation did not play a significant role in CPT degradation.

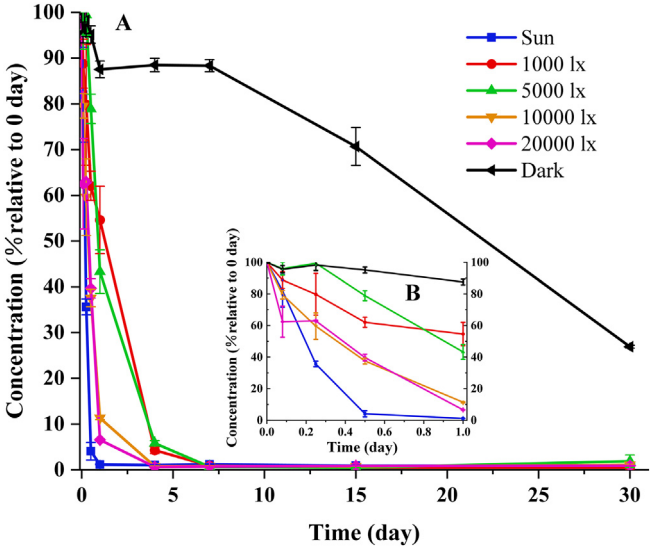

Fig. 3. The photodegradation kinetics of camptothecin in seawater. Data shown are the means of three replicates ± standard error. (B) is an enlarged view of (A) during the first 1 d.

3.5. PEC and PNEC

Using the mass-balance calculation method recommended in ISO 13073-1, the rate at which CPT leached from the antifouling paint was calculated to be 0.138 μg cm^{-2} d^{-1}. This figure and the physio-chemical properties of CPT listed in Table 1 were used for the calculation of PEC by the MAMPEC model. The estimated average PEC for the four generic scenarios (open sea, shipping lane, commercial harbor, and marina) were 6.14×10^{-7}, 9.39×10^{-7}, 6.80×10^{-3}, and 5.03×10^{-2} μg L^{-1}, respectively. The PNEC was 2.19×10^{-1} μg L^{-1}. This was calculated by dividing the lowest L(*E*)C_{50} value (2.19×10^{3} μg L^{-1}) of CPT against non-target marine organisms (Feng et al., 2018) by an assessment factor of 10,000 (see the

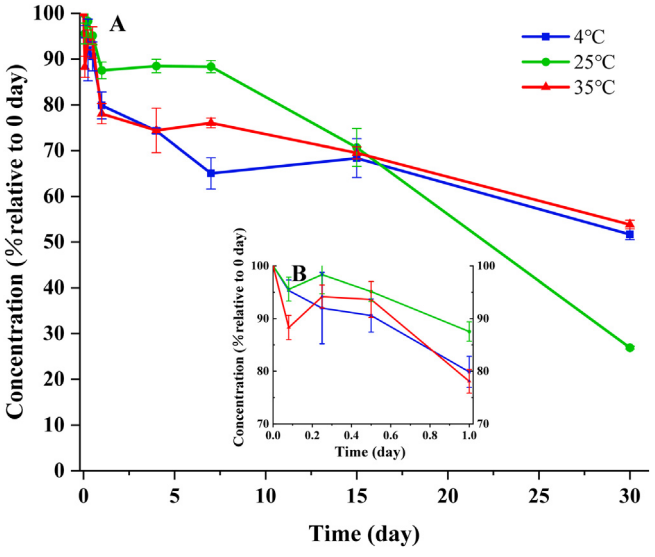

Fig. 2. The hydrolysis kinetics of camptothecin at various temperature. Data shown are the means of three replicates ± standard error. (B) is an enlarged view of (A) during the first 1 d.

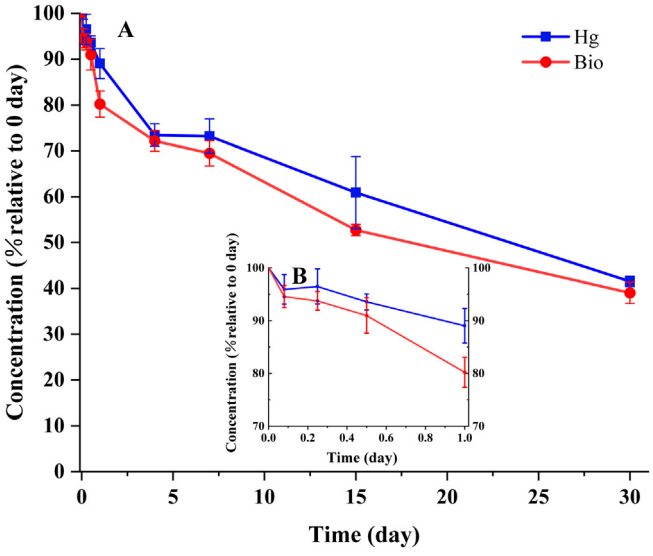

Fig. 4. The biodegradation kinetics of camptothecin in seawater. Data shown are the means of three replicates ± standard error. (B) is an enlarged view of (A) during the first 1 d. Hg: Filtered natural seawater (FNS) treated with 10 μg mL^{-1} CPT and 80 μg mL^{-1} HgCl. Bio: FNS treated with 10 μg mL^{-1} CPT.

detailed explanations for methods for calculation in the materials and methods section). These data demonstrated that the PEC/PNEC ratio (a quantitative index for risk assessment) for CPT in antifouling paint was much lower than 1, i.e., 2.80×10^{-6}, 4.29×10^{-6}, 3.11×10^{-2}, and 2.30×10^{-1} for open seas, shipping lanes, commercial harbors, and marinas, respectively (Fig. 5), indicating that CPT is an environmentally acceptable antifoulant.

4. Discussion and conclusion

Despite the large number of studies screening and isolating NPAs (Liu et al., 2020), few studies have examined NPA degradation and performed environmental risk evaluations. However, such assessments are important for the approval of NPA use as environmentally-friendly antifoulants in antifouling paints. To the best of our knowledge, experimental data on degradation under marine environmental conditions have only been reported for two NPAs: capsaicin and butenolide (Wang et al., 2014; Chen et al., 2015). In this study, the degradability of CPT under various conditions was evaluated. The results suggested that CPT would be suitably degraded in the marine environment. The fastest degradation pathway for CPT in seawater was photodegradation: CPT rapidly photolyzes under direct exposure to sunlight radiation, with a half-life of only 0.17 d (i.e., 4.08 h). CPT contains a tetracyclic A-D ring planar chromophore which adsorbs light. The absorbance spectrum of CPT (Supplementary Fig. S7) indicated that CPT had absorption in the UV range (190–400 nm), which may possibly have caused its rapid degradation under sunlight radiation. Irinotecan, a semi-synthetic analogue of CPT that is also a widely-used anticancer drug (Besse et al., 2012; Babu et al., 2012), also easily photodegrades in an aqueous system (ultrapure water solution) exposed to simulated sunlight irradiation, with a half-life of 8.24 h (Gosetti et al., 2020). Furthermore, for the artificial light tested in this study, the emission spectra in the UV and visible regions were recorded (Supplementary Figs. S2–S5). The possible UV action may also have played a role in the degradation of CPT in the treatments with artificial light. The light intensity upon the ocean surface depends on the time, geographical location and atmospheric condition. The intensities of artificial light tested in this study (2.69–42.84 W m^{-2}, see Supplementary Figs. S2–S5) are in the range of those existing at the sea surface (personal communication with Dr. Xiaolong Yu from College of Ocean & Earth Sciences, Xiamen University, whose major is marine optics and whose field record of coastal oceans of China and western Pacific oceans shows that irradiance intensities that reach ocean surface vary from 0 to 810 W m^{-2}). With regard to the natural sunlight tested in this study, these intensities of sunlight should also exist at the sea surface since the location of our college, where we performed the photodegradation experiment, is close to the sea with a distance of only 2.3 km. Overall, the present result of the rapid removal of CPT from seawater via photodegradation supports its potential use as an environmentally-friendly antifoulant.

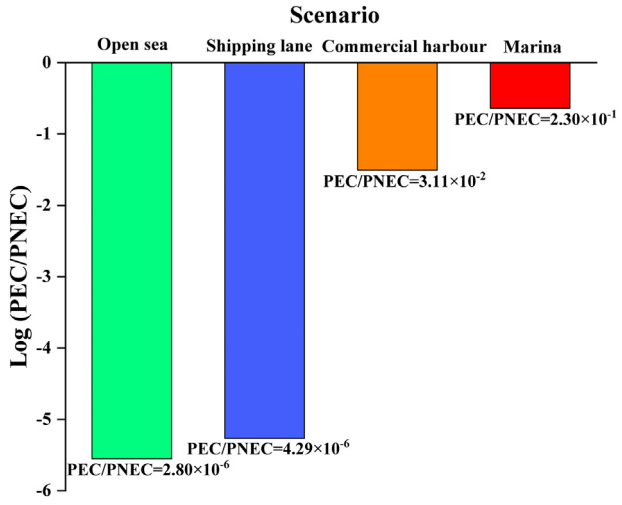

Fig. 5. The PEC/PNEC ratio of CPT under different scenarios.

In an alkaline aqueous system, the CPT lactone ring opens due to hydrolysis and is converted to the carboxylate form (Fassberg and Stella, 1992). This explains why we did not detect CPT in the solutions at pH 9. The gradual degradation of CPT at pH 7 further indicated its potential application in marine antifouling paints under ocean acidification. It was noted that the degradation of CPT under different temperatures was somewhat complex. CPT hydrolysis was slower at 25 °C than at the other two temperatures (4 °C and 35 °C) during the initial 15 days, but after that, the degradation of CPT was faster at 25 °C than those at 4 °C and 35 °C. In the weak alkaline seawater (pH 8.2) used in this experiment of hydrolysis at various temperatures, some CPT might have underwent a reversible reaction of opening the lactone ring to the carboxylate form (Mano and Tong, 2019). It has been suggested that the hydrolysis rates of CPT and its carboxylate form are different (Akimoto et al., 1994). The impact of temperature on the lactone-carboxylate equilibrium and that on the hydrolysis of CPT and its carboxylate form may jointly cause the complexity mentioned above, and this requires further exploration in the future. Because our results demonstrated CPT hydrolysis between 4 °C and 35 °C, CPT can potentially be used in a wide range of sea areas, including both polar and tropical seas.

The small increase in CPT degradation rate between natural seawater and HgCl-sterilized seawater indicated that biodegradation contributed little to the elimination of CPT in seawater and again indicated that hydrolysis played a major role. Similarly, biodegradation has little effect on the antifoulants zine pyrithione and copper pyrithione (Maraldo and Dahllöf, 2004). Here, the capped glass reservoirs were used in the biodegradation test of CPT. Whether biodegradation still showed little effect if using open equipment was not known (the bacterial species and community may be different between a closed and open system). This is an interesting aspect that requires further exploration in the future.

Comparison of hydrolysis and photolysis among CPT and other antifoulants (Table 2 and Table S3) suggested that the degradation behaviors of antifouling compounds differ widely, and that CPT photodegrades relatively easily, with a shorter half-life, as compared to most other antifoulants under similar conditions. Previously, we showed that the toxicity of CPT to non-target aquatic organisms was low; in particular, CPT was less toxic to the crustacean *Artemia salina* than several commonly used antifoulants (tributyltin, irgarol, copper pyrithione, zinc pyrithione, and chlorothalonil) and less toxic to microalgae than diuron and copper (Feng et al., 2018). Furthermore, our preliminary test showed that the toxicity of its degradation product(s) against *A. salina* was lower than that of CPT (Supplementary Fig. S8). The decrease in antitumor activity caused by the hydrolysis of CPT has also been previously reported (Lazareva et al., 2018; Martino et al., 2017). The octanol-water partition coefficient (Log*Kow*) can be used to predict the bioaccumulation potential of a given compound (Vilas-Boas et al., 2017). It has been suggested that compounds with a log Log*Kow* >3 are prone to bioaccumulate (Thomas and Brooks, 2010). CPT has a low Log*Kow* value of 1.74, indicating that the bioaccumulation potential of this compound is low. In addition, the Estimation Programs Interface (EPI) Suite (v4.0, 2009, US EPA), a Windows-based suite of physical/chemical properties and environmental fate estimation programs developed by the EPA and the Syracuse Research Corporation, predicted a bioconcentration factor (BCF, another parameter that reflects bioaccumulation potential) for CPT as low as 0.815, indicating that CPT was not expected to bioaccumulate. Overall, the rapid degradation of CPT, in conjunction with its relatively low toxicity to non-target organisms and its low potential for bioaccumulation, highlights the low risk represented by this compound to the marine environment. The antitumor activity of CPT has been revealed to act through its inhibition of topoisomerase I (an enzyme that reduces the torsional stress of supercoiled DNA, Garcia-Carbonero and Supko, 2002), and this may provide clue(s) for exploring the mechanism of its antifouling activity. The action mode of CPT against fouling organisms is still unknown and requires further investigations. As Qian et al. (2009, 2015) suggested, studies of the modes of action of

potential antifoulants are important for developing effective, environmentally friendly antifouling products with long market lifespans and for protecting marine environments.

The PEC/PNEC ratio calculated in this study further suggested that using CPT as an antifoulant was environmentally friendly. Current commercial antifoulants, including Cu_2O and other antifouling biocides (e.g., diuron, Irgarol 1051, Sea-Nine 211, dichlofluanid, and chlorothalonil) generally have PEC/PNEC ratios much greater than 1, indicating that these compounds pose risks to the marine environment (Campos et al., 2021). Hence, alternatives that are more environmentally safe are urgently needed. CPT, which has a PEC/PNEC ratio much lower than 1, may represent such an alternative.

Recent studies of the genomes of *Ophiorrhiza pumila* and *Camptotheca acuminate*, two CPT-producing plants, provided insights into the pathway and evolution of CPT biosynthesis, laying a foundation for CPT yield improvement through synthetic biology and biotechnology (Rai et al., 2021; Kang et al., 2021). In addition to current CPT-production methods, which rely on extraction from source plants, other avenues have been explored for the large scale production of CPT, including total chemical synthesis (Stork and Schultz, 1971; Blagg and Boger, 2002), identification of CPT-yielding fungi suitable for fermentation (Puri et al., 2005; Pu et al., 2015), plant tissue culture (Pan et al., 2004), and improved source plant cultivation techniques (Jain and Nessler, 1996). Increasing the scale of CPT production would facilitate its practical application as an antifoulant.

In conclusion, our results suggested that photolysis is the dominant degradation pathway for CPT in the marine environment, and that photolysis is 100 times faster than hydrolysis. This rapid photolysis indicated that CPT would not persist in the marine environment following its release from painted surfaces. Furthermore, our evaluation of the environmental risk posed by the use of CPT as an antifoulant showed that the PEC/PNEC ratio of CPT was much lower than 1. The results of this work highlight the promising application of CPT as eco-friendly antifouling agent to protect immersed artificial structures from marine biofouling.

CRediT authorship contribution statement

Huanhuan Hao: Validation, Formal analysis, Writing – original draft, Visualization. **Siyu Chen:** Conceptualization, Methodology, Investigation. **Zhiwen Wu:** Data curation, Software. **Pei Su:** Resources. **Caihuan Ke:** Supervision. **Danqing Feng:** Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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