ECOTOX BRASIL
SOCIEDADE BRASILEIRA DE ECOTOXICOLOGIA

Ecotoxicol. Environ. Contam.,v. 19, n. 1, 2024, 39-49
doi: 10.5132/eec.2024.01.05

EEC

Technical Note

Degradation Kinetics of Antifouling Biocides in Sediment During the Spiking Equilibrium Phase

Bruno Galvão de Campos[1], Fernando Cesar Perina[2], Fiamma Eugênia Lemos Abreu[3,4], Gilberto Fillmann[3], Denis Abessa[1]*

[1] Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), São Vicente, SP, Brazil

[2] Centro de Estudos do Ambiente e do Mar (CESAM) e Departamento de Biologia (Dbio), Universidade de Aveiro, Aveiro, Portugal

[3] Universidade Federal do Rio Grande (FURG), Rio Grande, RS, Brazil

[4] Universidade Federal de Pernambuco (UFPE), Recife, PE, Brazil

Received January 04, 2024; Accept April 19, 2024

Abstract

The new generation of antifouling biocides, as well as other emerging contaminants, has not yet been included in sediment quality guidelines or present standard protocols for sediment spiking. Most of these biocides have short half-lives in water, but little information is available regarding their degradation in sediments. Thus, there is a need to establish a reliable duration of the equilibrium phase for sediment spiking prior to sediment toxicity testing to determine the actual exposure concentrations during ecotoxicological tests. This study aimed to evaluate the degradation of DCOIT, Irgarol, Diuron, and Dichlofluanid during a spiking equilibrium phase of 24 h at three different time intervals (0, 6, and 24 h) and concentrations (10, 100, and 1000 ng g^{-1}), by applying kinetic degradation models. The models presented a better fit for the 1000 ng g^{-1} treatments, in which the half-lives of DCOIT and Diuron were < 5 h, Dichlofluanid < 2 h, and Irgarol < 6h. Our results also indicate that, except for Dichlofluanid, the degradation rates of the other antifouling biocides were reduced dramatically after 6 h of equilibrium. Therefore, an equilibrium phase of 24 h (or greater than 6 h) was considered viable for sediment spiking. Our findings provide valuable information to guide future sediment toxicity tests using these compounds.

Keywords: antifouling biocides, DT_{50}, ecotoxicology, pollution, sediment spiking

INTRODUCTION

The marine biological fouling, or biofouling, is a natural ecological succession process in which different species colonize anthropogenic surfaces immersed into the seawater (Yebra *et al*., 2004; Campos *et al*., 2022). Biofouling organisms involve species of different trophic levels, such as bacteria, diatoms, macroalgae, tunicates, barnacles, mussels or tubeworms, and others (Chen & Lam, 2017). Biofouling in human-made structures is related with extensive socioeconomic and environmental impacts (Dafforn *et al.*, 2011) in the shipping industry, bioinvasion (Fernandes *et al.,* 2016), and structural instability and failure of fixed structures such as bridges, buoys, and aquaculture cages (Maki & Mitchell, 2003). The most employed strategy to deal with biofouling in human-made structures is the application of antifouling paints containing toxic biocide substances that form a protective chemical layer against foulers settlement (Voulvoulis, 2006).

Antifouling paints are specially designed to cope with biofouling in human-made structures through the continuous release of biocides, thus creating a protective chemical barrier against the target species (Yebra *et al.*, 2004). The lack of a systematic assessment of the environmental risk of these biocides may allow the emergence of new threats to the marine environment, as recently recognized for Irgarol 1051, which was banned from the European Union

*Corresponding author: denis.abessa@unesp.br

(EU) in 2017 due to its persistence and high toxicity (Campos *et al*., 2022). Along with Irgarol, the literature also reports that other biocides such as 4,5-Dichloro-2-octyl-3-isothiazolone (DCOIT), Dichlofluanid, Chlorothalonil, Diuron, and ZnPT have harmful effects on non-target organisms (Martins *et al.*, 2018). In addition, studies regarding their toxicity to benthic species and environmental monitoring in marine sediments are scarce, representing an important gap in environmental hazard and risk assessments of these compounds.

Sediment compartment represents an important route of contaminant exposure to marine organisms and consequently affects the entire ecosystem (Chapman *et al*., 2002; Maranho *et al.*, 2009). Benthic organisms contribute to the conversion of energy, mass, and nutrients between benthic and pelagic zones, a process also known as benthic-pelagic coupling (Marcus & Marcus, 1998). In addition, sediment bioturbators such as polychaetes, amphipods, and copepods are considered ecosystem engineers and contribute to the maintenance of marine biodiversity and ecosystem functioning (Caliman *et al.,* 2013).

The laboratory toxicity assessment of xenobiotics towards benthic species is usually carried out through the artificial contamination of pristine sediment in a process known as sediment spiking, followed by ecotoxicological tests in which the test organisms are exposed to the contaminated sediment. There are three main guidelines for sediment spiking proposed by the USEPA (2001), Environment Canada (1995), and ASTM (2008) respectively. These guidelines recommend an equilibrium phase, where the newly contaminated sediment is kept refrigerated and in the dark for a certain period, allowing the system to reach chemical equilibrium or a steady-state phase. The equilibrium phase duration may vary according to the degradation rate and physicochemical nature of the contaminant; and it may range from hours (low K_{ow} values) to months for organic contaminants with high K_{ow} values ($K_{ow} > 6$).

Antifouling biocides are not yet included in sediment quality guidelines and have not been considered in sediment spiking protocols, hampering the proper assessment of their ecological risks. Most of these biocides have short half-lives in water and little (or no) information regarding their fate and behavior in sediments (Campos *et al.*, 2022). Therefore, during a sediment spiking procedure, the appropriate period for the equilibrium phase remains unknown, and this uncertainty can drastically influence the determination of the effective concentration during toxicity tests. The objective of this study was to evaluate the half-life and degradation kinetics of DCOIT, Irgarol, Diuron, and Dichlofluanid in spiked sediments during the equilibrium phase of 24 h. Our findings may guide future sediment toxicity tests of these compounds.

MATERIAL AND METHODS

Chemicals

The DCOIT (4,5-dichloro-2-n-octyl-4-isothiazolin-3-one), Irgarol 2(-metiltio-4-terbutilamino-6-ciclopropilamino-s-triazina), Diuron (1-dimethylurea), and Dichlofluanid (N-[dichloro(fluoro)methyl]sulfanyl-N-(dimethylsulfamoyl) aniline) were purchased from Sigma Aldrich (Brazil) with purity > 99%. Stock solutions were prepared using ultrapure water (20 ml) and 800 µl of HPLC-grade acetone (Sigma Aldrich, Brazil) as a co-solvent. Stock solutions were further used to spike the sediment.

Sediment Spiking

Sediment spiking with antifouling biocides was performed in natural estuarine sediments (water content = 18.91%, organic matter = 3.99%, $CaCO_3$ = 1.24%, sand = 94.76% (very coarse sand = 0.02%, coarse sand = 0.03%, medium sand = 1.93%, fine sand = 46.23%, very fine sand = 41.02%, silt and clay = 5.5%) sampled in the South of the Lagamar Protected Area located in Cananéia, in the State of São Paulo, Brazil. This region is considered a reference site because of its high biodiversity, low contamination status, and absence of relevant anthropogenic impacts (Campos *et al*., 2016; Cruz *et al.*, 2014).

The sediment spiking technique was based on the slurry method (USEPA, 2001) adapted by Perina *et al.* (2023). Three aliquots of 300 g of wet sediment were contaminated with DCOIT, Irgarol, Diuron, and Dichlofluanid from stock solutions, and diluted in acetone, at the following concentrations: 10 ng g^{-1}, 100 ng g^{-1}, and 1000 ng g^{-1} (e.g., 10 ng g^{-1} sediment aliquot contained all four biocides in the corresponding concentration). The sediment was thoroughly homogenized for 30 min using a glass rod. To reach equilibrium, the sediment was kept in the dark at 4 °C for 24 h. The control sediment (no biocide added) also underwent all processes.

Degradation Kinetics

During the spike equilibrium phase, three aliquots (surface, middle, and bottom) of 2 g of sediment column were taken at three distinct time intervals: T0, just after the contamination (with no equilibrium); T6, 6 h from contamination; and T24, 24 h from contamination; these sediments were used for quantification of the respective biocide concentrations. The choice for these time intervals considered the existing literature stating that DCOIT rapidly degrades in the environment (Jacobson & Willingham, 2000; Sakkas *et al*., 2002; Willingham and Jacobson, 1997). Steen *et al*. (2004) showed that in field DCOIT degraded with a rate constant in the order of 1 h^{-1}. The three aliquots representing different areas of the sediment containers were taken to test the homogeneity of the spike method and evaluate biocide

degradation over time. Before quantification, each aliquot was cooled at -80°C for 30 min and lyophilized for subsequent extraction and analyses.

Extraction and Liquid Chromatography Analysis

DCOIT, Irgarol, Diuron, and Dichlofluanid were extracted according to the procedures described by Abreu *et al*. (2020), in which 1 g of dry sediment was weighed in 25 mL glass vials. The samples were spiked with 100 µL of Atrazine D5 (100 ng L^{-1}, surrogate standard), after which 10 mL of acetonitrile was added. Next, the samples were then mixed for 1 min, sonicated (50 ºC for 30 min), and centrifuged (4000 rpm for 7 min). This step was repeated thrice. The resulting supernatants were combined and evaporated (Syncore®) to a volume of 1 mL. The extracts were cleaned by solid-phase extraction (SPE) using C18 cartridges previously activated with 4 mL ethyl acetate and ultrapure water (Milli-Q®). The extracts were diluted with 50 ml of ultrapure water, passed through SPE cartridges, dried for 1 h, and then eluted 2 times using 2 mL of ethyl acetate. The eluates were decreased in volume under nitrogen flow to 1 mL before being transferred to a new vial with methanol. All obtained sediment extracts were analyzed in triplicate. The samples were analyzed by gas chromatography using liquid chromatography with mass spectrometry (LC-ESI –MS/MS; Alliance Separations, Waters).

Quality assurance and quality control (QA/QC) procedures were based on regular analysis of blanks, spiked matrices, and certified reference material (CRM – PACS-3, National Research Council of Canada, Ottawa, Canada). Limits of detection (LOD) and quantification (LOQ) were calculated by the signal to noise ratio; for Diuron and Irgarol the LD and LQ were 0.5 and 1 ng g^{-1} respectively, for DCOIT these values were 1 and 3 ng g^{-1}, and for they were Dichlofluanid 1 and 5 ng g^{-1}.

Statistical analyses and degradation kinetics modeling

To verify the success of the homogenization process, the coefficient of variance (CV) between the surface, middle, and bottom of the sediments collected from the spiking container was calculated for each biocide, as shown in Eq. 1.

$$CV = \left(\frac{SD}{Mean\ concentration\ (surface, middle, bottom)}\right) * 100\% \qquad \textbf{(Eq. 1)}$$

CV = coefficient of variance; SD = standard deviation

The time taken for a 50% decline in mass or concentration (DT_{50} or half-life) of each biocide was calculated through degradation kinetics models using the "xxDeg" R package, based on NAFTA (2015) and USEPA (2015) guidelines for the degradation kinetics of pesticides in environmental media. For DCOIT and Dichlofluanid, the degradation kinetics were calculated for the 1000 ng g^{-1} treatment, and the following models were evaluated: bi-exponential model (DFOP) and First-Order Multi-Compartment model (FOMC). For Irgarol and Diuron, all three concentration treatments (10, 100, and 1000 ng g^{-1}) were assessed using DFOP, FOMC, Single first-order kinetics (SFO), and Hockey-stick model (HS).

Due to the limited time intervals (3) the model fitting could not be assessed statistically, just by the comparison of the residuals and visual inspection (both are also considered according to the guidelines). The presented models were chosen based on fitting. We acknowledge that the absence of statistical fitting validation may be a limitation; however, our results and discussion provide a first glance at the degradation of such compounds in sediment, providing important data that can be used in further and more detailed studies.

RESULTS AND DISCUSSION

The concentrations of antifouling biocides at each time, as well as the CV for homogenization in each treatment, are presented in Table 1. Dichlofluanid presented the worst homogenization with a CV > 30%, indicating heterogeneity of the data. The results of DCOIT, Irgarol, and Diuron, were similar, with better homogenization at 1000 and 100 ng g^{-1}. The worst CVs were found at 10 ng g^{-1}, with values >30% at T0 and T24 for all biocides. Overall, these results indicate that spiking procedures with higher concentrations are easier to achieve and homogenize (Figure 1). The USEPA (2001) guidelines suggest the use of a 4 h continuous homogenization or 60 s homogenization twice a day for one week, but these two procedures are impractical for some of the antifouling biocides studied herein due to their short half-lives.

Table 1: Concentrations of antifouling biocides in T0, 6 and 14 h after spiking under equilibrium condition. Letters a, b, and c indicate the coefficient of variance between the aliquots from surface, middle and bottom.

Antifouling Biocides	Nominal concentration (ng.g^{-1})	Time		
		T0	**6 h**	**24 h**
DCOIT (LD = 1; LQ = 3)	Blank	<LQ	<LQ	<LQ
	10	3.5 ± 1.1^{b}	<LQ	<LQ
	100	4.88	3.5 ± 0.3^{a}	3.5 ± 0.8^{b}
	1000	7616.8 ± 1226.2^{a}	3480.7 ± 776.6^{b}	2558.9 ± 337.6^{a}
Diuron (LD = 0.5; LQ = 1)	Blank	<LQ	<LQ	<LD
	10	22.6 ± 7.7^{c}	15.2 ± 1.1^{a}	13.8 ± 4.7^{c}
	100	214.6 ± 61^{b}	194.2 ± 14.6^{a}	174.5 ± 29.2^{a}
	1000	4694.3 ± 241.4^{a}	1977.6 ± 337^{b}	1712.6 ± 177.0^{a}
Irgarol (LD = 0.5; LQ = 1)	Blank	<LQ	<LQ	<LQ
	10	26.2 ± 7^{c}	18. ± 2.8^{a}	16.8 ± 5.4^{c}
	100	180.9 ± 14.6^{a}	166.3 ± 35.7^{a}	159.2 ± 30.8^{c}
	1000	4882.5 ± 268.8^{a}	2369.2 ± 584.4^{c}	2029.4 ± 244.7^{a}
Dichlofluanid (LD = 1; LQ = 5)	Blank	<LQ	<LD	<LQ
	10	<LD	<LQ	<LD
	100	<LQ	<LQ	<LQ
	1000	1189.5 ± 460.4^{c}	157.7 ± 36^{c}	16.9 ± 7.4^{c}

a = CV ≤ 15% = homogeneous data; b = 30% ≤CV> 15%; = average dispersion; c= CV> 30% = heterogeneous data.

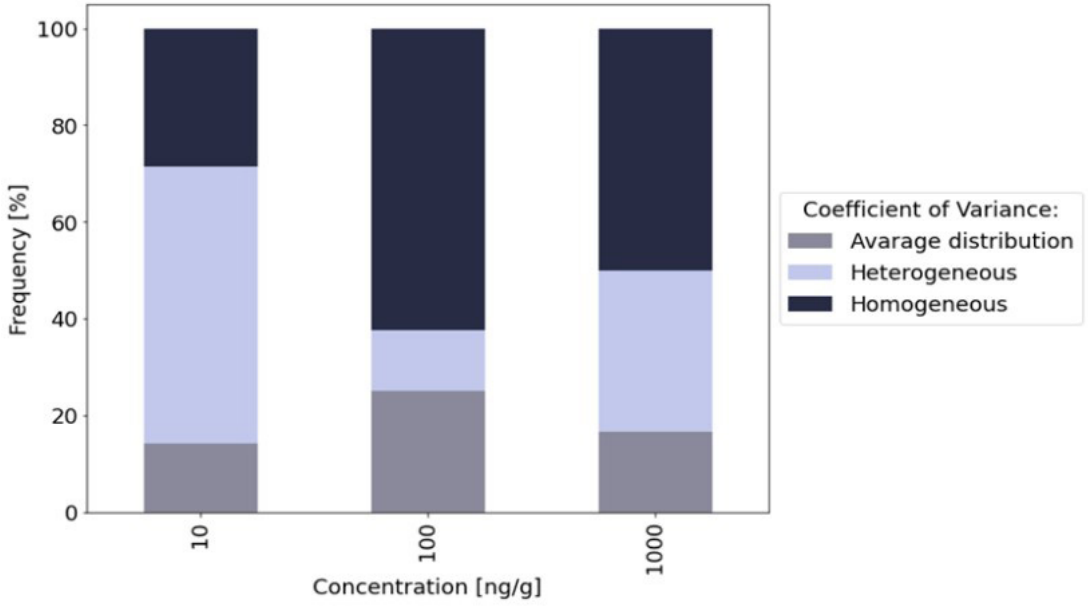

Figure 1. Coefficient of variance (CV) between surface, middle, and bottom of the spiking container for each tested concentration (10 ng g^{-1}, 100 ng g^{-1}, and 100 ng g^{-1}). CV represents the success of the homogenization process, where CV ≤ 15% = homogeneous data; 30% ≤ CV > 15% = average dispersion; CV > 30% = heterogeneous data.

The antifouling biocides presented a fast initial degradation phase followed by a slower one, which is a classic pattern of biphasic degradation kinetics. In the present study, single first-order kinetics (SFO) and three biphasic degradation models (HS, DFO, and FOMC) were evaluated to describe the degradation of the tested substances. Data were presented only for models that could run the available dataset.

The HS model consists of two sequential first-order curves; it assumes that the biocide concentration initially declines according to first-order kinetics with at a constant rate k1. At a certain time, the constant rate changes to a different value (k2). The FOMC model considers the sediment matrix to be heterogeneous, and this is accounted for the model by dividing the soil into many sub-compartments each with a different first-order k1. The DFOP model addresses nonlinear degradation by calculating two degradation coefficients that are later integrated (FOCUS, 2006).

The tested models calculated inconsistent half-life values for biocides that did not achieve ≥50% degradation. This pattern occurred mainly at the lowest concentrations (10 – 100 ng g^{-1}), where the observed degradation over 24 h ranged from 0% to 36% (far below the 50% threshold). For these cases, we considered that the model's results were unreliable and that new analyses would require more sampling points and longer experiments. Even so, sharing this data with the scientific community is essential, as it provides important information and insights for future work. On the other hand, for all biocides at 1000 ng g^{-1}, the degradation was greater than 50% during the experiment, and the models were consistent, so we considered these data to be much more accurate and reliable.

DCOIT was not detected at 10 ng g^{-1} and showed no degradation at 100 ng g^{-1} for 24 h. At 1000 ng g^{-1}, T6 and T24 presented degradation rates of 54% and 66%, respectively. Figure 2 shows the degradation kinetics of the DCOIT. The half-life ranged from 2.38 h in the DFOP model to 4.87 h in the HS model.

Thomas et al. (2003) corroborate our results by calculating a half-life <0.5 days for DCOIT. Because of the high log Koc of DCOIT (4.19), it tends to bind and be partitioned into the sediment, which may act as a reservoir for DCOIT (Chen & Lam, 2017). DCOIT has been detected in marine sediments worldwide (Campos et al., 2022) at concentrations up to 281 ng g^{-1} in some harbors, as observed in Korea by Lee *et al.* (2015).

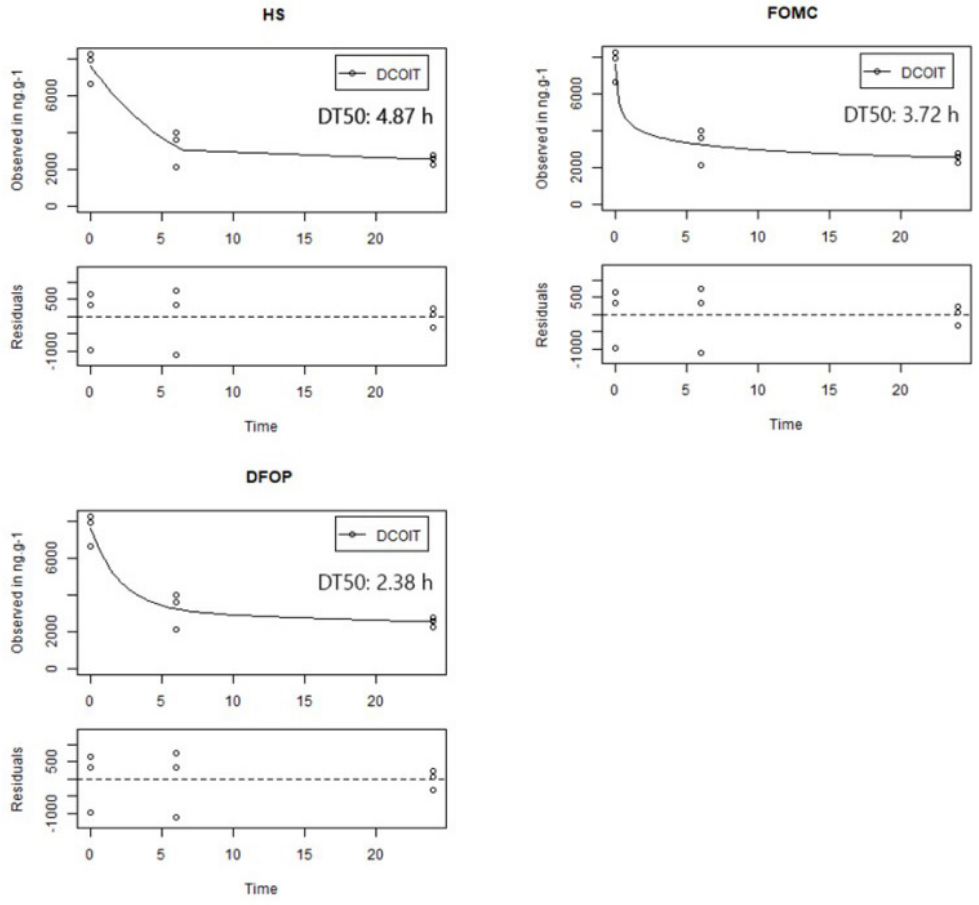

Figure 2. Degradation kinetics of DCOIT (1000 ng g^{-1}), under Hockey-stick model (HS), Bi-exponential model (DFOP) and the Gustafson and Holden model (FOMC) with their respective time taken for a 50% decline in concentration (half-life = DT_{50}).

Similar to DCOIT, Diuron presented biphasic degradation kinetics (figure 3). At 10 ng g^{-1}, the degradation rates at T6 and T24, compared with T0, were 33% and 39%, respectively. At 100 ng g^{-1}, these rates were 10 % and 19%, respectively, while at 1000 ng g^{-1}, they were 58% and 64%. The degradation kinetics model for 10 ng g^{-1} indicated a half-life of 493 h by the FOMC model, 70.8 h by the DFOP model, and 39.9 h by the SFO model, owing to the high residue (difference between measured and estimated concentration). At 100 ng g^{-1}, only the SFO model could calculate the half-life (84.7 h, cf. Figure 4), which we considered unreliable because of the observed degradation, indicating that this model was not suitable for the dataset. For the 1000 ng g^{-1} treatment, the FOMC, DFOP, and HS models presented more similar half-lives, determined as 1.15, 2.97, and 4 h, respectively (Figure 5).

Diuron has a well-documented persistence in the marine environment (Faÿ *et al.*, 2018), with a half-life in seawater ranging from 1 month to 1 year, depending on environmental conditions (Dafforn *et al*., 2011). Biodegradation is the primary route of its degradation. A Diuron high Koc of 4.85 indicates a high sediment adsorption capacity and, therefore, a heterogeneous partition in soil (Giacomazzi & Cochet, 2004). Thomas *et al.* (2003) determined, through a pseudo-first order kinetic model, that Diuron has an anaerobic half-life of 14 days in marine sediments. The highest Diuron concentration in coastal sediments (0.14 µg g^{-1}) was found in Korean harbor areas (Lam et al., 2017).

Irgarol at 10 ng g^{-1} presented degradation rates of 31% and 39% at T6 and T24, respectively, compared with T0. These data indicate that after 6h the degradation rate decreased, and the degradation rate reached a “steady” state (Figure 6). The FOMC and DFOP models indicate half-lives of 5379 h (7.3 months) and 109 h (4.5 days), respectively. For 100 ng g^{-1}, Irgarol degraded 9% and 19% at T6 and T24, respectively, compared to T0, and exhibited the same equilibrium pattern after 6 h. The FOMC and SFO models indicated half-lives of $6.9x10^{8}$ h and 145 h, respectively (Figure 7). The observed concentrations did not reach the 50% degradation threshold during the experiment, and because of the limited time points, the model could not properly estimate the half-life, resulting in inconsistent half-life values. Considering that the SFO model for 100 ng g^{-1} gave a half-life value similar to that of the DFOP model for 10 ng g^{-1}, we considered that 145 h from the SFO was more reliable than the value produced by the DFOP. At 1000 ng g^{-1}, T6 and T24 degraded 51% and 58%, respectively, compared to T0. The half-lives for the three tested models were consistent, and the results indicated respective values of 4.5 h (FOMC model), 5.2h (DFOP model), and 5.5 h (HS model) (Figure 8).

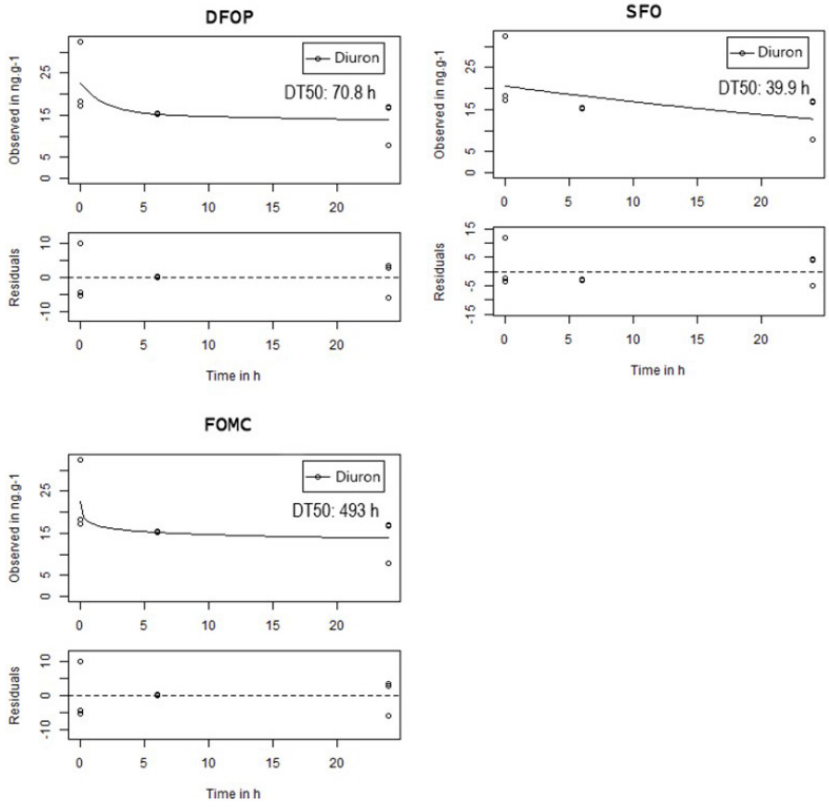

Figure 3. Degradation kinetics of Diuron for 10 ng g^{-1} Spiking, under Single first-order kinetics (SFO), Bi-exponential model (DFOP) and the Gustafson and Holden model (FOMC) with their respective time taken for a 50% decline in mass or concentration (half-life = DT_{50}).

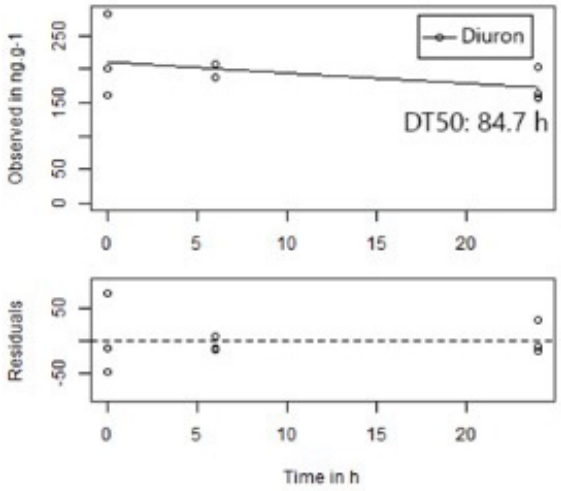

Figure 4. Degradation kinetics of Diuron for 100 ng g^{-1} Spiking, under Single first-order kinetics (SFO) with their respective time taken for a 50% decline in mass or concentration (half-life = DT_{50}).

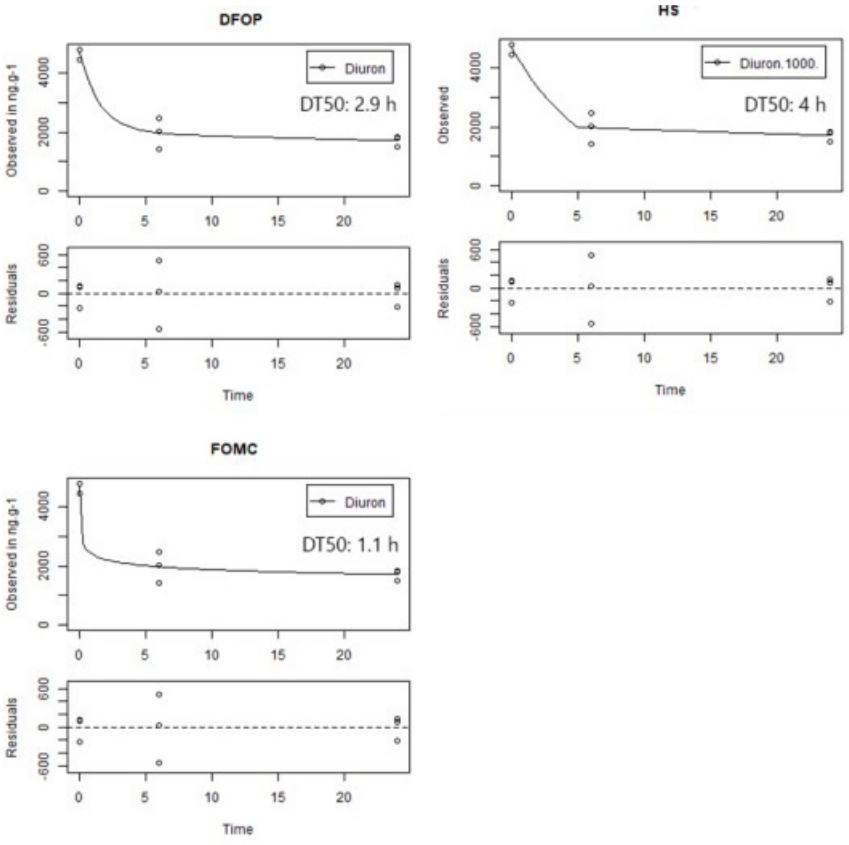

Figure 5. Degradation kinetics of Diuron for 1000 ng g^{-1} Spiking, under Hockey-stick model (HS), Bi-exponential model (DFOP) and the Gustafson and Holden model (FOMC) with their respective time taken for a 50% decline in mass or concentration (half-life = DT_{50}).

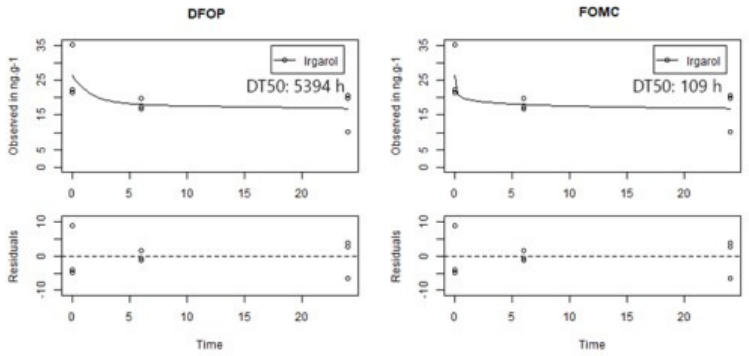

Figure 6. Degradation kinetics of Irgarol (10 ng g^{-1}), Bi-exponential model (DFOP) and the Gustafson and Holden model (FOMC) with their respective time taken for a 50% decline in mass or concentration (half-life = DT_{50}).

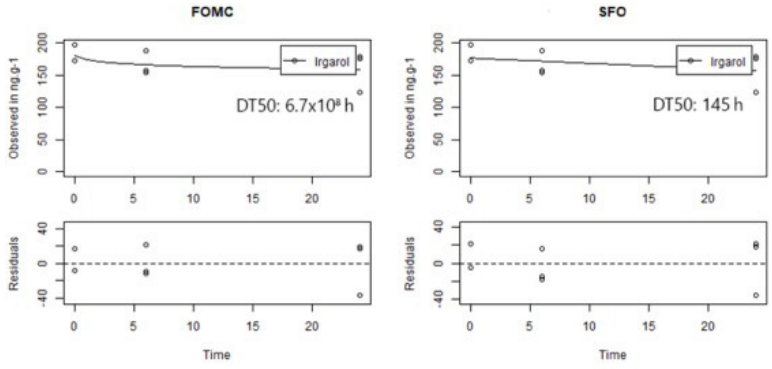

Figure 7. Degradation kinetics of Irgarol (100 ng g^{-1}), under first-order kinetics (SFO) and and the Gustafson and Holden model (FOMC) with their respective time taken for a 50% decline in mass or concentration (half-life = DT_{50}).

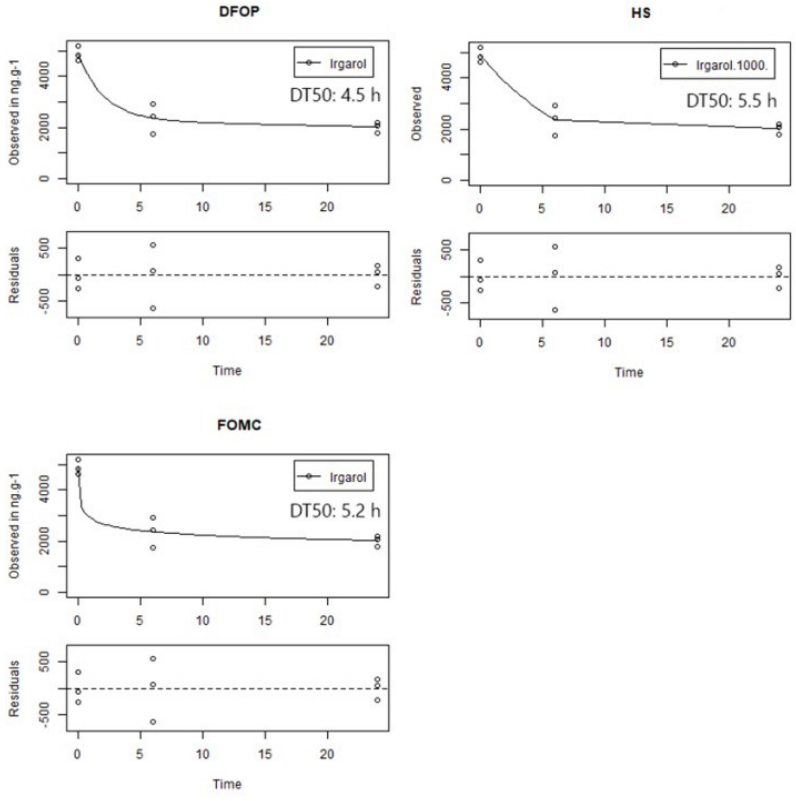

Figure 8. Degradation kinetics of Irgarol (1000 ng g^{-1}), under Hockey-stick model (HS), Bi-exponential model (DFOP) and the Gustafson and Holden model (FOMC) with their respective time taken for a 50% decline in mass or concentration (half-life = DT_{50}).

Irgarol has a relatively long half-life of up to 350 days in seawater (Omae, 2003). Thomas *et al.* (2003) observed no degradation of Irgarol during 12 days in marine sediment. In addition, Irgarol has been detected in sediments worldwide at concentrations of up to 89 μg kg^{-1}, as found in a Brazilian harbor (Viana *et al.* 2019). Our data indicate that the half-life of Irgarol varies significantly depending on the spiked concentration, even with the same sediment and equilibrium parameters (temperature and light). Nevertheless, the results obtained to the 1000 ng g^{-1} treatment were more consistent. In addition, all treatments indicated that after 6 h, Irgarol degradation slowed down, and the concentration stabilized (at least up to 24 h analyzed). Considering such stabilization, an equilibrium phase period of 24h should be adequate for sediment spiking procedures. Yet, biocides degradation is highly susceptible to biodegradation and dependent on the physicochemical properties of the media (Thomas 2001; Koning *et al,* 2021). Fine sediments with high organic matter content have high adsorbing ability and tend to be anoxic, with an anaerobic microbial community. Coarse sediments tend to have lower organic matter content and adsorption capacity, which may result in xenobiotics (or their degradation products) partitioned into the interstitial water (Campos *et al.* 2016; Viana *et al*, 2019). Accordingly, Gatidou *et al.* (2007) observed a strong correlation between antifouling biocide concentrations, such as Irgarol, and sediment physicochemical properties, namely a positive correlation with particles <63 μm, and a negative correlation with pH and organic carbon. In the present study, the sediment tested was sandy, with low organic matter content. Thus, we highlight that the behavior of such biocides in muddy and/or organically rich sediments may differ from that reported in the present study.

Dichlofluanid was only detected at 1000 ng g^{-1}, with degradation rates of 87% and 99% at T6 and T24, respectively. The FOMC and DFOP presented similar half-lives of 1.436 and 1.92 h, respectively. Dichlofluanid was the biocide with the lowest half-life. This compound has low solubility in water (< 2 mg L^{-1}) and Log Koc of 3.7 (Wezel & Vlaardingen, 2004). Despite its low solubility, accumulation in sediment is unlikely to occur due to its rapid degradation in water (half-life of 1.2 h in seawater). Hamwijk *et al.* (2005) observed half-lives ranging between 1.2 and 3 h in water-sediment systems at 20°C and pH 7.5 - 8.1. Thomas *et al.* (2003) corroborate our results, by the determination of half-lives of <0.5 days for Dichlofluanid. Despite its rapid degradation, the highest observed environmental concentrations of Dichlofluanid in coastal sediments range from 0.016 μg g^{-1} in Brazil (Abreu *et al.*, 2020) to 0.8 μg g^{-1} in Malaysia (Mukhtar *et al*., 2019). Additionally, Koning *et al.* (2021) observed that in water/sediment systems, Dichlofluanid rapidly hydrolyses into N,N-dimethyl-N′-phenylsulfamide (DMSA) which, in turn, degrades quantitatively to form N,N-dimethylsulfamide (N,N-DMS) in marine systems; according to these authors, N,N-DMS is persistent in marine systems. Thus, the formation and toxicity of by-products and their partitioning into porewater need to be considered in studies regarding sediment contamination and toxicity due to antifouling biocides.

The tested antifouling biocides showed bi-phasic degradation kinetics with two distinct degradation rates (K1 and K2) divided by a breakpoint (observed to be within 6 h). The K1 corresponded to a sharp and exponential degradation rate; after the breakpoint, the degradation rate (K2) reduced drastically and assumed a linear pattern. Therefore, we considered 24 h a viable time for the spike equilibration phase since after 6 h the concentrations tend to stabilize and be more reliable for ecotoxicological testing (apart from Dichlofluanid, which completely degraded within 24h).

CONCLUSION

Our data indicate that the 24 h equilibrium phase during the spiking procedure is adequate for DCOIT, Irgarol, and Diuron, which presented a significant reduction in the degradation after 6h (breaking point). However, studies regarding the fate and effects of these compounds should consider their respective initial concentrations and the degradation rates the compound will undergo during the process. In our study, at 1000 ng g^{-1}, the half-lives of DCOIT and Diuron were < 5 h, while the half-lives of Dichlofluanid were < 2 h and for Irgarol, 6h. Regarding Dichlofluanid, in 6h it presented a degradation of >90%, thus indicating that the 24 h period of equilibrium is not suitable for this compound. However, because the degradation of these biocides forms stable by-products, such compounds should also be considered when sediments spiked with these biocides are being assessed for chemical composition and toxicity.

ACKNOWLEDGEMENTS

The authors thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES (CIMAR No 1988/2014, Process No 1111/13–01.14.0141.00 and Grant #88881.156405/2017–01), Financiadora de Estudos e Projetos - FINEP (Process 1111/13–01.14.0141.00), and Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (Grant #456372/2013–0), for the financial support, and Rede Nacional de Estudos de Anti-incrustantes (RNEA) for the technical and loggistic support. B.G.C. (Grant #2017/10211–0) and D.M.S.A. (Grant #2020/03004–0) thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the financial support. D.M.S.A. (PQ #308533/2018–6) and G.F. (PQ #314202/2018–8 and #311133/2022-3) are research fellows of CNPq. F.C.P. thanks CAPES for the fellowship (Grant # 88887.144657/2017-00). Thanks are also due to the financial support to CESAM by FCT/MCTES (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020) through national funds.

CREDIT AUTHOR STATEMENT

BGC: Conceptualization, Methodology, Investigation, Formal Analysis, Writing - Original Draft; **FCP**: Conceptualization, Methodology, Investigation, Writing - Review & Editing; **FELA**: Methodology, Investigation, Formal Analysis, Validation; **GF**: Conceptualization, Resources, Project administration, Funding acquisition, Writing - Review & Editing; **DA**: Conceptualization, Supervision, Project administration, Funding acquisition, Writing - Review & Editing.

COMPETING INTERESTS

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.

REFERENCES

Abreu, F. E. L., Natália, J., Braga, Í., & Fillmann, G. (2020). Are antifouling residues a matter of concern in the largest South American port ? *Journal of Hazardous Materials*, *398*, 5 November 2020, 122937, https://doi.org/10.1016/j.jhazmat.2020.122937

ASTM [American Society for Testing and Materials] (2008). Standard Guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing and for Selection of Samplers Used to Collect Benthic Invertebrates (E 1391 – 03 (2008).

Caliman, A., Carneiro, L. S., Leal, J. J. F., Farjalla, V. F., Bozelli, R. L., & Esteves, F. de A. (2013). Biodiversity effects of benthic ecosystem engineers on the spatial patterns of sediment CH4 concentration in an urban Neotropical coastal lagoon. *Acta Limnologica Brasiliensis*, *25*(3), 302–314. https://doi.org/10.1590/s2179-975x2013000300009

Campos, B. G., Cruz, A. C. F., Buruaem, L. M., Rodrigues, A. P. C., Machado, W. T. V., & Abessa, D. M. S. (2016). Using a tiered approach based on ecotoxicological techniques to assess the ecological risks of contamination in a subtropical estuarine protected area. *Science of the Total Environment*, *544*, 564–573. https://doi.org/10.1016/j.scitotenv.2015.11.124

Campos, B. G., Figueiredo, J., Perina, F., Abessa, D. M. S., Loureiro, S., & Martins, R. (2022). Occurrence, effects and environmental risk of antifouling biocides (EU PT21): Are marine ecosystems threatened? *Critical Reviews in Environmental Science and Technology*, *52*(18), 3179-3210 https://doi.org/10.1080/10643389.2021.1910003

Chapman, P. M., Ho, K. T., Munns, W. R., Solomon, K., & Weinstein, M. P. (2002). Issues in sediment toxicity and ecological risk assessment. *Marine Pollution Bulletin*, *44*(4), 271–278. https://doi.org/10.1016/S0025-326X(01)00329-0

Chen, L., & Lam, J. C. W. (2017). SeaNine 211 as antifouling biocide: A coastal pollutant of emerging concern. *Journal of Environmental Sciences*, *61*, 68–79. https://doi.org/10.1016/j.jes.2017.03.040

Cruz, A. C. F., Davanso, M. B., Araujo, G. S., Buruaem, L. M., Santaella, S. T., Morais, R. D., & Abessa, D. M. S. (2014). Cumulative influences of a small city and former mining activities on the sediment quality of a subtropical estuarine protected area. *Environmental Monitoring and Assessment*, *186*(11), 7035–7046. https://doi.org/10.1007/s10661-014-3908-1

Dafforn, K. A., Lewis, J. A., & Johnston, E. L. (2011). Antifouling strategies: History and regulation, ecological impacts and mitigation. *Marine Pollution Bulletin*, *62*(3), 453–465. https://doi.org/10.1016/j.marpolbul.2011.01.012

Environment Canada. (1995). Guidance document on measurement of toxicity test precision using control sediments spiked with a reference toxicant. Report EPS/1/RM/30. September 1995. Environment Canada. https://publications.gc.ca/collections/collection_2014/ec/En49-24-1-30-eng.pdf (accessed on March 08, 2024.

Faÿ, F., Horel, G., Linossier, I., & Vallée-réhel, K. (2018). Effect of biocidal coatings on microfouling: In vitro and in situ results. *Progress in Organic Coatings*, *114*, 162–172. https://doi.org/10.1016/j.porgcoat.2017.10.017

Fernandes, J. A., Santos, L., Vance, T., Fileman, T., Smith, D., Bishop, J. D. D., Viard, F., Queirós, A. M., Merino, G., Buisman, E. & Austen, M. C. (2016). Costs and benefits to European shipping of ballast-water and hull-fouling treatment: Impacts of native and non-indigenous species. *Marine Policy*, 64, 148–155. http://doi.org/10.1016/j.marpol.2015.11.015

FOCUS. (2006). Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0 Fate Studies.

Gatidou, G., Thomaidis, N. S., & Zhou, J. L. (2007). Fate of Irgarol 1051, diuron and their main metabolites in two UK marine systems after restrictions in antifouling paints. *Environment International*, *33*(1), 70–77. https://doi.org/10.1016/j.envint.2006.07.002

Giacomazzi, S., & Cochet, N. (2004). Environmental impact of diuron transformation: A review. *Chemosphere*, *56*(11), 1021–1032). https://doi.org/10.1016/j.chemosphere.2004.04.061

Hamwijk, C., Schouten, A., Foekema, E. M., Ravensberg, J. C., Collombon, M. T., Schmidt, K., & Kugler, M. (2005). Monitoring of the booster biocide dichlofluanid in water and marine sediment of Greek marinas. *Chemosphere*, *60*(9), 1316–1324. https://doi.org/10.1016/j.chemosphere.2005.01.072

Koning, J. T., Bollmann, U. E., & Bester, K. (2021). Biodegradation of third-generation organic antifouling biocides and their hydrolysis products in marine model systems. *Journal of Hazardous Materials*, *406*, 124755. https://doi.org/10.1016/j.jhazmat.2020.124755

Jacobson, A. H., & Willingham, G. L. (2000). Sea-nine antifoulant: An environmentally acceptable alternative to organotin antifoulants. *Science of the Total Environment*, 258(1–2), 103–110. https://doi.org/10.1016/S0048-9697(00)00511-8

Lam, N. H., Jeong, H. ho, Kang, S. dong, Kim, D. J., Ju, M. J., Horiguchi, T., & Cho, H. S. (2017). Organotins and new antifouling biocides in water and sediments from three Korean Special Management Sea Areas following ten years of tributyltin regulation: Contamination profiles and risk assessment. *Marine Pollution Bulletin*, *121*(1–2), 302–312. https://doi.org/10.1016/j.marpolbul.2017.06.026

Lee, M. R. N., Kim, U. J., Lee, I. S., Choi, M., & Oh, J. E. (2015). Assessment of organotin and tin-free antifouling paints contamination in the Korean coastal area. *Marine Pollution Bulletin*, *99*(1–2), 157–165. https://doi.org/10.1016/j.marpolbul.2015.07.038

Maki, J. S., & Mitchell, R. (2003). Biofouling in the Marine Environment. *Encyclopedia of Environmental Microbiology*. https://doi.org/doi:10.1002/0471263397.env275

Maranho, L. A., Abreu, I., Santelli, R., Cordeiro, R. C., Soares-gomes, A., & Moreira, L. B. (2009). Sediment toxicity assessment of Guanabara Bay, Rio de Janeiro, Brazil. *Journal of Coastal Research*, *56*, 851–855. http://spa.sites.uff.br/wp-content/uploads/sites/290/2017/11/JCR2009.pdf

Marcus, N. H. & Marcus, N. H. (1998). The importance of benthic-pelagic coupling and the forgotten role of life cycles in coastal aquatic systems. *Limnology and Oceanography, 43*(5), 763–768. https://doi.org/10.4319/lo.1998.43.5.0763

Martins, S. E., Fillmann, G., Lillicrap, A. & Thomas, K. V. (2018). Review: Ecotoxicity of organic and organo-metallic antifouling co-biocides and implications for environmental hazard and risk assessments in aquatic ecosystems. *Biofouling*, *34*(1), 34–52. https://doi.org/10.1080/08927014.2017.1404036

Mukhtar, A., Mohamat-Yusuff, F., Zulkifli, S., Harino, H., Ismail, A., & Inoue, K. (2019). Concentration of Organotin and Booster Biocides in Sediments of Seagrass Area from Sungai Pulai Estuary, South of Johor, Malaysia. *Environments*, *6*(2), 26. https://doi.org/10.3390/environments6020026

NAFTA. (2015). Guidance for Evaluating and Calculating Degradation Kinetics in Environmental Media. https://www.epa.gov/sites/production/files/2015-09/documents/degradation-kin.pdf

Omae, I. (2003). Organotin antifouling paints and their alternatives. *Applied Organometallic Chemistry*, *17*(2), 81–105. https://doi.org/10.1002/aoc.396

Perina, F. C., Abessa, D. M. S., Pinho, G. L. L., Castro, I. B. & Fillmann, G. (2023). Toxicity of antifouling biocides on planktonic and benthic neotropical species. *Environmental Science and Pollution Research*, 30, 61888–61903. https://doi.org/10.1007/s11356-023-26368-9

Sakkas, V. A., Konstantinou, I. K., & Albanis, T. A. (2002). Aquatic phototransformation study of the antifouling agent Sea-Nine 211: Identification of byproducts and the reaction pathway by gas chromatography-mass spectroscopy. *Journal of Chromatography A*, 959(1–2), 215–227. https://doi.org/10.1016/S0021-9673(02)00430-2

Steen, R. J. C. A., Ariese, F., van Hattum, B., Jacobsen, J., & Jacobson, A. (2004). Monitoring and evaluation of the environmental dissipation of the marine antifoulant 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT) in a Danish Harbor. Chemosphere, 57(6), 513-521. https://doi.org/10.1016/j.chemosphere.2004.06.043

Thomas, K. (2001). The environmental fate and behaviour of antifouling paint booster biocides: A review. *Biofouling*, *17*(1), 73–86. https://doi.org/10.1080/08927010109378466

Thomas, K. V., McHugh, M., Hilton, M., & Waldock, M. (2003). Increased persistence of antifouling paint biocides when associated with paint particles. *Environmental Pollution, 123*(1), 153–161. https://doi.org/10.1016/S0269-7491(02)00343-3

USEPA. (2001). Methods for Collection, Storage and Manipulation of Sediments for Chemical and Toxicological Analyses: Technical Manual (EPA-823-B-01-002).

USEPA. (2015). Standard Operating Procedure for Using the NAFTA Guidance to Calculate Representative Half-life Values and Characterizing Pesticide Degradation. https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/standard-operating-procedure-using-nafta-guidance

Viana, J. L. M., Santos, S. R.V., Franco, T. C. R. S., & Almeida, M. A. P. (2019). Occurrence and partitioning of antifouling booster biocides in sediments and porewaters from Brazilian Northeast. *Environmental Pollution*, *255*(Part 1), 112988. https://doi.org/10.1016/j.envpol.2019.112988

Voulvoulis, N. (2006). Antifouling paint booster biocides: occurrence and partitioning in waterand sediments. *Antifouling Paint Biocides*, 155–170. https://doi.org/10.1007/698_5_053

Wezel, A. P. V., & Vlaardingen, P. V. (2004). Environmental risk limits for antifouling substances. *Aquatic Toxicology*, *66*(4), 427–444. https://doi.org/10.1016/j.aquatox.2003.11.003

Willingham, G. L., & Jacobson, A. H. (1996). Designing an environmentally safe marine antifoulant. *ACS Symposium Series,* 640, 224–233. https://doi.org/10.1021/bk-1996-0640.ch011

Yebra, D. M., Kiil, S., & Dam-Johansen, K. (2004). Antifouling technology - Past, present and future steps towards efficient and environmentally friendly antifouling coatings. *Progress in Organic Coatings,* 50(2), 75–104. https://doi.org/10.1016/j.porgcoat.2003.06.001