ACS ES&T Water

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Article

Exploring the Role of Photolysis in the Aquatic Fate of Antimicrobial Transformation Products: Implications for One Health

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Cite This: *ACS EST Water* 2025, 5, 4112–4119

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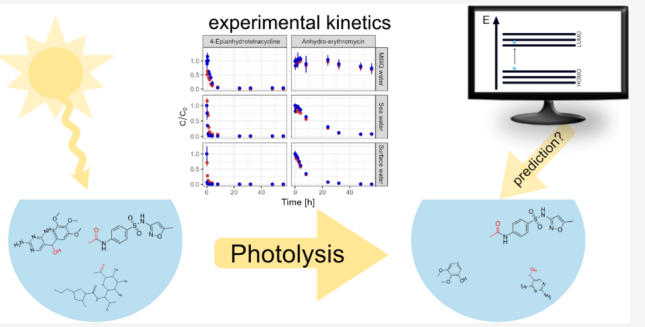
ABSTRACT: Antibiotic transformation products (TPs) are common contaminants in aquatic environments. With emerging concerns about their potential role in antimicrobial resistance (AMR), elucidating the processes governing their aquatic occurrences and behaviors is important. Yet, the environmental fate of these TPs remains largely unknown. In this pilot study, we investigate the photodegradation kinetics of antibiotic TPs across different environmental waters by exposing selected TPs, from various antibiotic families, to environmentally relevant irradiation intensities. N^4-Acetylsulfamethoxazole showed the highest tolerance to photolysis across matrices, whereas for TPs like anhydro-erythromycin, clindamycin sulfoxide, and hydroxy-trimethoprim demonstrated, water constituents clearly modulate aquatic degradation through indirect photolysis. Notably, 4-epianhydrotetracycline, erythromycin A enol ether, and hydroxy-metronidazole were highly susceptible to direct photolysis, which was further enhanced via other pathways such as indirect photolysis. Further, existing computational tools were evaluated for their predictive reliability by comparing experimentally derived half-lives with QSAR-based estimates. The results indicate poor correlation between predicted and observed estimates, highlighting the complexity of environmental photodegradation that current molecular descriptors may not fully capture. This study underscores the need for refining predictive models to improve their generalizability. Ultimately, our findings contribute to a better understanding of the antibiotic TP fate and potential role in the emergence and proliferation of AMR.



KEYWORDS: *environmental fate, antibiotic resistance, computational modeling, abiotic degradation, kinetics, half-life, solar irradiation, sulfamethoxazole*

1. INTRODUCTION

Many antibiotics are only partially removed in treatment facilities, causing both the parent compounds and related transformation products (TPs) to enter surface waters. This influx, combined with varying rates of (a)biotic degradation, can lead to pseudo-persistence—a dynamic equilibrium between environmental input and degradation.[1] While research has historically focused on parent antibiotics, the environmental presence and impact of their TPs have gained increasing recognition.[2,3] However, the fate (e.g., persistence and thus, potential imbalance of the influx-degradation equilibrium) and potential biological effects of these TPs remain largely unknown.[2] Antibiotic TPs are of particular concern due to their potential biological activity that may contribute to antimicrobial resistance (AMR) in the environment,[4] as one of the top global health threats identified by the World Health Organization.[5] Laboratory studies suggest that certain TPs retain antimicrobial properties, potentially exerting selective pressure on microbial communities at subinhibitory concentrations.[6] Moreover, some antibiotic TPs have been detected at higher environmental concentrations than their parent compounds, raising concerns about their persistence and long-term ecological impact.[7,8] Among environmental degradation processes, photodegradation plays a crucial role in the chemical fate in aquatic systems.[9] Photolysis can occur through direct absorption of sunlight or indirect pathways, where reactive oxygen species and photosensitizers—such as dissolved organic matter (DOM) and metal ions—mediate degradation.[10] While photodegradation is often considered a key removal mechanism, its impact varies across different environmental conditions, influencing the overall persistence of TPs in surface waters. Photodegradation of antibiotic TPs remains an underexplored area, with very limited experimental

Received: March 20, 2025
Revised: June 12, 2025
Accepted: June 20, 2025
Published: June 28, 2025

https://doi.org/10.1021/acsestwater.5c00327
ACS EST Water 2025, 5, 4112–4119

data available that allow us to understand their potential aquatic fate. While computational models have been developed for predicting photodegradation rates based on molecular structures and electronic properties, their performance and generalizability for TPs have yet to be systematically evaluated. Understanding how well *in silico* models capture photodegradation mechanisms is of high benefit to improve risk assessments and prioritize compounds for future studies.

In this study, we investigate the role of photolysis in the aquatic degradation of selected antibiotic TPs to address critical knowledge gaps in their environmental fate. Specifically, we aim to

1. Assess the influence of water matrix composition on photodegradation kinetics;
2. Explore the key pathways of TP degradation by comparing degradation behavior under conditions favoring direct and indirect photolysis, to infer the relative importance of each mechanism; and
3. Evaluate the comparability of TP degradation rates obtained from the experimental study with predictions from computational quantitative structure−activity relationship (QSAR) models.

By elucidating these processes, our study provides new insights into the environmental fate of antibiotic TPs and their potential contribution to AMR risks in aquatic environments. Additionally, these findings contribute to our overall knowledge of antibiotic TP degradation, helping to inform future risk assessments and mitigation strategies.

2. EXPERIMENTAL SECTION

2.1. Materials and Standards. Standards of hydroxy-metronidazole, α-hydroxy-trimethoprim, clindamycin sulfoxide, erythromycin A enol ether, and anhydro-erythromycin were purchased from TRC (Toronto Research Chemicals Inc., Toronto, Canada), and N^4-acetylsulfamethoxazole and 4-epianhydrotetracycline were purchased from Sigma-Aldrich (Madrid, Spain) with a purity higher than 95%. Stock solutions (100 μg mL^{-1}) of all compounds were prepared in methanol (Merck, Darmstadt, Germany) and stored at −80 °C. Formic acid as a mobile phase additive was purchased from Fisher Chemical (Thermo Fisher Scientific, Waltham). Mass-labeled chemicals (internal standards) were ordered from Alsachim (Graffenstaden, France; acetaminophen-^{13}C6; trimethoprim-^{13}C3,d6; sulfamethoxazole-d4), TRC (DEET-d10), CDN Isotopes (Pointe-Claire, Canada; cis-sertraline-d3), and Merck (caffeine-^{13}C3) with purity higher than 99%. Milli-Q water (LC-PAK) was generated at the laboratory from a Milli-Q IQ-7000 purification system with filters of a 0.22 μm Millipak Express membrane and an LC-PAK polishing unit by Merck Millipore (Billerica, MA).

2.2. Water Collection and Preparation. Lake and seawater were used for this study. In March 2023, surface water was collected from lake Mälaren (59°47′02.1″N, 17°38′47.9″E), near the inflow of the river Fyrisn, which flows through Uppsala while seawater was taken at Kapellskärs Ångbtsbrygga (59°43′05.4″N 19°04′16.3″E) of the Baltic Sea. Back at the laboratory, the water collected was immediately autoclaved (Tuttnauer 3850EL, Breda, the Netherlands) for 30 min at 120 °C and 250 kPa to avoid the influence of biotic degradation, homogenized, and stored at 5 °C. The physicochemical parameters measured in the collected surface and Baltic Sea water after autoclaving were consistent with values reported in regional monitoring data.[11−14] The surface water exhibited a slightly alkaline pH, along with a low ionic content, resulting in low conductivity, minimal salinity, and high resistivity (Table S1). The total dissolved solids content was also low, reflecting the low mineralization characteristic of Swedish surface waters. The seawater had a slightly alkaline pH, aligning with the expected pH range governed by atmospheric carbonate buffering. Due to the brackish nature of the Baltic Sea, the seawater displayed moderate conductivity and salinity, which are intermediate between freshwater and oceanic values. This brackish composition, enriched by a mix of salts and minerals, resulted in higher total dissolved solids levels than observed in the surface water.

2.3. Photodegradation Experiment. Antimicrobial TPs of interest were selected based on their occurrence in the surface water environments, ecological risk, potential of AMR development, and environmental hazards,[4] as well as the availability of reference standards. The chosen TPs correspond to a variety of parent antibiotic families, i.e., sulfonamides, macrolides, tetracyclines, lincosamides, and nitroimidazoles, as identified to be relevant for surface water environments.[4] For each TP, the light absorption was measured in a 5 mg L^{-1} aqueous solution by using a UV−vis spectrophotometer (Lambda 365, PerkinElmer, Waltham). The photolysis experiments were set up following OECD guideline No. 316 for phototransformation of chemicals in water[15] and performed in a Suntest XXL+FD chamber (Atlas, Linsengericht-Altenhaßlau, Germany) equipped with three xenon lamps and a daylight filter. Two irradiation intensities, 40 and 60 W m^{-2}, were applied and set over a wavelength range of 300−400 nm to ensure accurate UV intensities. According to standardized solar spectra CIE No. 20, 85, and 241, the intensities of 40 and 60 W m^{-2} correspond to a total irradiance of approximately 667 and 1000 W m^{-2}, respectively, over the total wavelength range of sunlight.[16−18] A comparison of the spectral profile used in this study with that of natural sunlight, focusing on the range between 280 and 400 nm, is presented in Figure S1, based on data provided by Atlas. With reference to Uppsala, Sweden (59°51′31.0″N, 17°38′20.0″E), an irradiation intensity of 40 W m^{-2} aligns with typical solar levels in April/May and September/October, while 60 W m^{-2} represents the peak solar irradiation observed in July (Figure S2).

Inside the Suntest chamber, the experiments with a 50 mL solution (<0.01% organic solvent content) of individual TPs at a concentration of 50 μg L^{-1} in Milli-Q water, as well as in surface and seawater (Table S1), were prepared in triplicate, alongside duplicate blanks and positive controls. The positive controls consisted of spiked water matrices covered with aluminum foil to prevent photolysis, while the blanks were nonspiked water matrices used to account for potential background concentrations. The spiking concentration falls within the upper range of TP concentrations detected in surface waters[4] and remains within the same order of magnitude as those applied in comparable photolysis studies (e.g., ref 19). The temperature was maintained at 20 °C throughout all experiments, with a tolerance of ±1 °C. Borosilicate 3.3 beakers and watch glasses (VWR, Radnor) sealed with parafilm were used to prevent evaporation. The irradiation intensity was monitored using both built-in instrumental sensors and an additional SP-110-SS sensor (SolData Instruments, Asnæs, Denmark). Samples were irradiated for 56 h. Aliquots of 180 μL were collected at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 8, 24, 32, 48, and 56 h, while aliquots

https://doi.org/10.1021/acsestwater.5c00327
ACS EST Water 2025, 5, 4112−4119

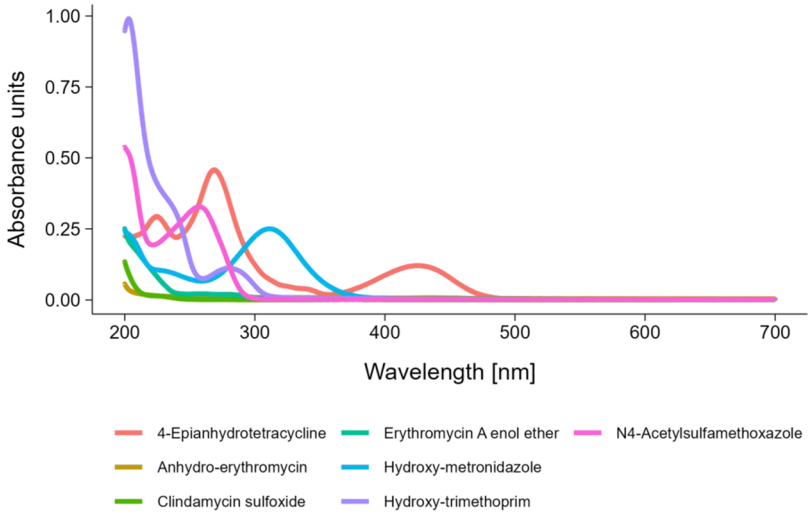


Figure 1. Absorbance spectra of TPs (5 μg mL^{-1}) in Milli-Q water.

from the blank and positive control samples were collected at 0, 8, 32, and 56 h. All the aliquots were immediately spiked with internal standards at 50 μg L^{-1} for a final volume of 200 μL and stored at −20 °C until analysis.

2.4. Instrumental Analysis. Together with 10-point calibration standards (0, 0.5, 1, 2, 5, 10, 20, 50, 100, 200 μg L^{-1}), employing a weighting of 1/x to enhance linearity across the concentration range, samples from the experiments were analyzed using direct injection onto ultrahigh-performance liquid chromatography coupled with tandem mass spectrometry (Exion LC, Sciex Triple-Quad 6500+) in positive electrospray ionization mode. The linearity of the calibration curve was at least 0.99 (Table S2), and the internal standard concentration was maintained at 50 μg L^{-1}. Chromatographic separation (Figure S3) was achieved using a Phenomenex Kinetex Biphenyl column (100 × 2.1 mm, 1.7 μm) at 40 °C. The mobile phases consisted of (A) Milli-Q water and (B) methanol, each containing 0.1% formic acid, at a flow rate of 0.5 mL min^{-1}. The injection volume was set at 3 μL. The total run time was over 14 min, following an LC-gradient: 0−1 min, 20% B; 8 min, 80% B; 8.1−11 min, 100% B; 11.1−14 min, 20% B. Data acquisition by the mass spectrometer was performed in multiple reaction monitoring (MRM), selecting two MRM transitions of highest intensity for each analyte (Table S3). The ion source was heated to 350 °C, with the following source settings: curtain gas at 35 psi, ion spray voltage at 4500, ion source gas 1 and 2 at 50 psi each.

Building on methodologies similar to those validated by the co-authors Ugolini and Lai (2024),[20] the analytical method was validated in all water matrices (Table S2 and Text S1). This included assessments of precision, as relative standard deviation, and accuracy, as percentage bias calculated by comparing measured to nominal concentrations, both within-run (n = 4) and between-run (n = 3, across three different days) performance (Table S2 and Text S1). Overall, most TPs demonstrated good precision across all matrices, with relative standard deviations ranging from 0.8 to 16.6% in within-run and between-run analyses, except for 4-epianhydrotetracycline with a slightly higher between-run variation at 30.7%. Accuracy in within-run and between-run analyses for most compounds was also found acceptable, with bias ranging from −18.7 to 22.8% in surface water and from −26.6 to 27.5% in seawater. Most TPs in Milli-Q water showed similar bias results, ranging from −23 to 21.2%, except for 4-epianhydrotetracycline with a higher bias of up to −55.8%. Method detection limits (MDLs) and method quantification limits (MQLs) for each TP in each water matrix were determined based on signal-to-noise ratios of 3 and 10, respectively. This results in MDLs at 0.01−0.63 μg L^{-1} and MQLs at 0.02−1.3 μg L^{-1} (Table S2).

2.5. Data Analysis. Data quantification for chemical concentrations was carried out using Sciex OS software (version 3.3.1.43). After exporting the result table, all further data processing and statistical analysis were conducted using *R* (version 4.3.1) with "data.table" and "dplyr" as the main libraries. Results are given as the mean ± standard deviation. Degradation rates were assessed through the general rate law (eq 1) across three integrated forms: zero-order, first-order, and second-order. The appropriate kinetic model was determined by evaluating the coefficient of determination (R^2) for the linear relationship and comparison of the Akaike Information Criterion (AIC) values derived from the linearized plots. The degradation rate was estimated from the slope of the linearized plot, and the initial concentration was back-calculated from the y-intercept for verification. The degradation half-life, $t_{1/2}$, was determined using the appropriate half-life formula based on the reaction order.

$$\frac{dC}{dt} = -kC^n \tag{1}$$

where C represents the concentration, t is the time, k is the rate constant, and n denotes the reaction order.

2.6. Computational (Photo)degradation Predictions. Experimental half-lives were compared with computational estimates from different photodegradation models in order to evaluate whether such tools can effectively capture the complexity of the environmental degradation processes. Among these models, QSAR models developed by Lyu et al. (2022) were applied, which include both a general QSAR for antibiotic photodegradation and a more targeted QSAR for specific groups of antibiotics.[21] Additionally, a QSAR for predicting the photolysis of polycyclic aromatic hydrocarbons (PAHs), developed by Chen et al. (1996), was used in the comparison.[22] For all QSARs, the energy difference between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) was calculated using the Molecular Orbital PACkage (MOPAC v23.0.3).[23]

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ACS EST Water 2025, 5, 4112−4119

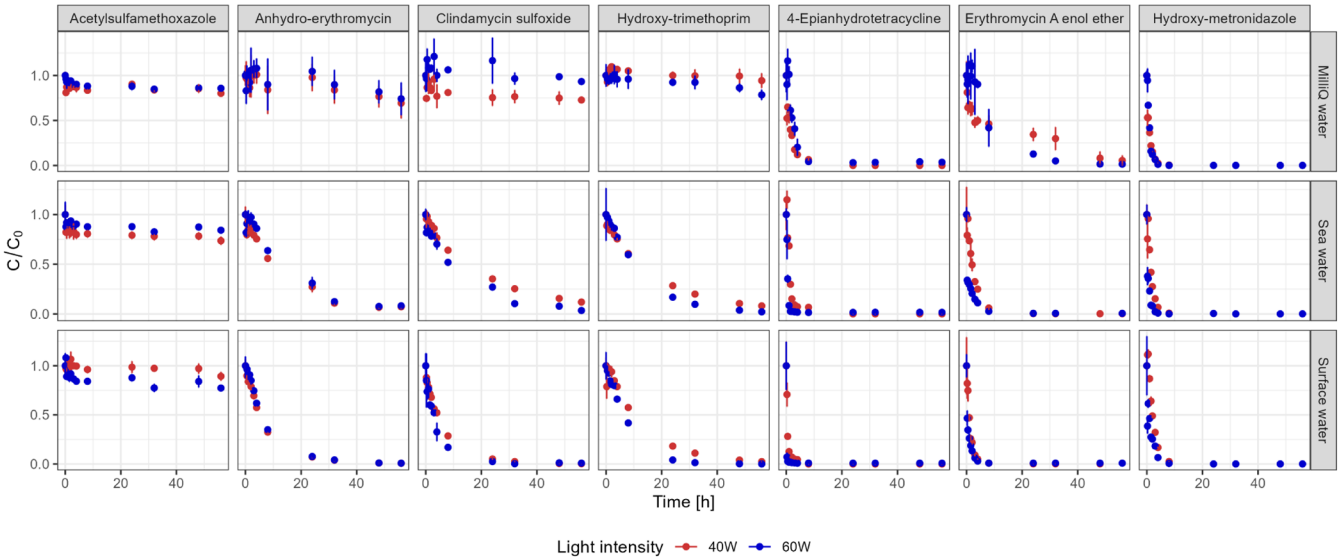

Figure 2. Degradation patterns of TPs in different water matrices and light intensities, expressed as concentrations at specific time points ($C_{t=i}$) normalized to the respective initial concentration ($C_{t=0}$) over time (56 h). Each data point represents the average (n = 3) with a standard deviation.

Semiempirical calculations used the PM7 Hamiltonian[24] with restricted Hartree−Fock wave function. Additionally, three non-photolysis-related half-lives were calculated using VEGA-HUB (version 1.1.5-b48).[25] These included the persistence in water model (IRFMN, v.1.0.1), which is based on biodegradation in water and sediments simulation test results according to the OECD guideline no. 309, the hydrolysis model (IRFMN/CORAL, v.1.0.1), which uses OECD test no. 111 end points, and the kMHalf-Life Model (Arnot/episuite, v.1.0.0), which is trained on biotransformation rates in fish. The Spearman correlation was used to assess the consistency of the relative rankings of the predictions rather than the numerical agreement.

3. RESULTS AND DISCUSSION

3.1. UV−Vis Absorbance of TPs. Most TPs absorbed UV light between 200 and 400 nm (Figure 1), but substantially varied in the intensity and wavelength of the absorption maximum. Clindamycin sulfoxide, anhydro-erythromycin, and erythromycin A enol ether showed minimal absorbance. Hydroxy-trimethoprim showed a maximum absorbance of 0.11 at 283 nm, hydroxy-metronidazole peaked at 0.25 at 311 nm, and N^4-acetylsulfamethoxazole reached 0.33 at 257 nm. 4-Epianhydrotetracycline showed a peak of 0.46 at 269 nm, in addition to its smaller peak at 425 nm, which contributes to the yellow color observed in the standard solution. The extent to which a compound absorbs light in the UV−vis range can serve as an indicator of its susceptibility to photodegradation, with higher absorbance suggesting an increased likelihood of photolytic transformation.

3.2. TP Photodegradation. The total irradiation applied during the photolysis experiments was 8100 for 40 W m^{-2} and 12300 kJ m^{-2} for 60 W m^{-2}. The TP degradation patterns were overall similar under the two light intensities applied, with no noticeable difference visually observed in the plots. This was further supported by the lack of statistically significant difference in their estimated half-lives and degradation rates, as determined using a Wilcoxon rank-sum test (DT_{50}: p = 0.97; k: p = 0.44). As expected, degradation varied between the studied TPs (Figure 2 and Table S4). Most TPs showed less degradation in Milli-Q water compared to sea or surface water (Figure 2), indicating a significant role of indirect photolysis in complex matrices through, for example, reactive oxygen species (e.g., oxygen, hydroxyl, or peroxide radicals) or other photosensitizers. For example, after 56 h, anhydro-erythromycin remained at a residual concentration of ∼70% of the initial concentration in Milli-Q water, in contrast to its nearly full degradation with <10% left in sea and surface water. Similarly, clindamycin sulfoxide showed little degradation with >70% remaining in Milli-Q water after 56 h, but degraded extensively in the other matrices, in which its residual concentration accounted for ∼10% of the initial concentration at 40 W and ∼3% at 60 W in seawater, while <1% trace amounts were quantifiable in surface water at the end of the experiments. The fact that anhydro-erythromycin and clindamycin sulfoxide only showed what appeared to be negligible light absorbance in Milli-Q water (Figure 1) underscores the role of indirect photolysis in the TP degradation in sea and surface water matrices. For hydroxy-trimethoprim, despite the strong absorption in the UV range (Figure 1), very little degradation was observed in Milli-Q water, with residual concentrations of 94% at 40 W and 79% at 60 W. This indicates a limited transferability of UV−vis absorbance as a predictor for direct photolysis. This is further highlighted by N^4-acetylsulfamethoxazole, which barely showed degradation in any of the matrices (with remaining levels between 74% and 89% of the initial concentration) (Table S4), despite showing an absorption peak at 257 nm. In contrast, the other three TPs, 4-epianhydrotetracycline, erythromycin A enol ether, and hydroxy-metronidazole, degraded almost completely in all matrices over the course of the experiment. The difference between the two erythromycin TPs underscores the importance of understanding their fate individually. This highlights that even structurally related TPs, e.g., within the same antibiotic family, may not necessarily show similar degradation patterns. For instance, while anhydro-erythromycin showed stability in Milli-Q water, erythromycin A enol ether degraded rapidly under similar conditions.

https://doi.org/10.1021/acsestwater.5c00327
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The photodegradation predominantly followed (pseudo)-first-order kinetics (Table S5), consistent with other environmental photolysis studies.[9,15,26] This included clindamycin sulfoxide, hydroxy-metronidazole, hydroxy-trimethoprim, and anhydro-erythromycin, especially in surface and seawater matrices, which showed high R^2 values (>0.9) (Table S5) for the kinetic models. As discussed earlier, matrix composition showed influence on the TP degradation (Figure 2), as surface and seawater matrices mostly exhibited faster degradation rates and better fits to the (pseudo)first-order model(s). This was likely due to the presence of reactive oxygen species, DOM, and metal ions in these water matrices enhancing photodegradation. Interestingly, no tested kinetic model fit well (R^2 < 0.9) for N^4-acetylsulfamethoxazole, which showed high stability across matrices (Figure 2). This observation contrasts with previous findings of Peria et al. (2013)'s study, which reported degradation of four acetylated TPs of sulfonamides and that acetylsulfamethoxazole demonstrated the longest half-life in their study (6.7 h at pH 8). This is still 1.5−2 orders of magnitude shorter than any half-life that could be estimated from our data, which, under the assumption of first-order kinetics, range from 227 h in seawater to 439 h in surface water under 60 W light intensity. If the degradation follows higher-order kinetics, this could be explained by the difference in analyte spiking concentration (200x lower in the present study). While light intensity and pH are comparable between the studies, the difference in the half-lives could also be due to other factors, such as organic solvent content and also matrix components. Potentially, high experimental concentrations could promote enhanced reaction rates through self-sensitized photolysis or aggregation effects.[27,28] Variability in degradation kinetics has also been observed in related environmental fate studies, such as biodegradation of sulfonamides, where, for example, acetylsulfapyridine was fully degraded after 32 days, while acetylsulfamethazine showed no detectable change in concentration over a 90-day experiment.[29]

As the topic of photodegradation of antibiotic TPs remains largely underexplored, it is challenging to directly compare our new findings with any related literature. While such studies for their respective parent antibiotics are more readily available, it should be noted that, even among the studies on parent compounds, substantial variability in their degradation rates is noticed, therefore complicating the comparisons. Periša et al. (2013) reported differences in photodegradation rates of up to 2 orders of magnitude between parent sulfonamides and their TPs.[19] Similarly, large variations can also be observed across studies investigating the same parent antibiotic under different conditions.[19] For instance, Chabilan et al. (2023) and Patrolecco et al. (2018) reported a persistent nature of sulfamethoxazole with half-lives of 40 and 25 days, respectively, investigating several degradation pathways.[1,30] This is in contrast to other photodegradation studies, such as those by Batchu et al. and Baena-Nogueras et al., which estimated much shorter half-lives of only a few hours.[31,32] The variations in half-lives across different studies underscore the challenges in comparison to our data on the respective TPs. This is also often due to limited reporting of key information such as matrix components, normalized light intensities (e.g., W m^{-2}), and sometimes, even basic experimental details such as exposure time. Furthermore, Baena-Nogueras et al. (2017) observed no degradation for trimethoprim during a 24 h experiment with an irradiation intensity of 500 W m^{-2}, while Chabilan et al. (2023) reported a half-life of 17 days.[30,32] For anhydro-erythromycin, rapid degradation was observed by Voigt and Jaeger (2017) across varying pH levels (pH 3: $t_{1/2}$ = 6.7 min, pH 7: $t_{1/2}$= 1.2 min, pH 10: $t_{1/2}$ = 3.7 min) during a brief 10 min study using a 15 W light source, aligning with our rather short half-life of 4−7 h in surface waters for the erythromycin TPs.[33] In contrast, the parent compound erythromycin was observed to be rather persistent, with half-lives up to 10 days or even no significant degradation.[30,31] These examples highlight the critical need for detailed methodological transparency to facilitate reliable comparisons and interpretations of photodegradation research.

The influence of other abiotic degradation pathways such as hydrolysis or thermal degradation was investigated by using samples of all water matrices covered with aluminum foil to shield them from irradiation. Based on the study of Ugolini and Lai (2024),[20] an effect of sorption to glass in our experiments can be excluded for N^4-acetylsulfamethoxazole and hydroxy-metronidazole. The effect can also be reasonably considered minimal for clindamycin sulfoxide, erythromycin A enol ether, anhydro-erythromycin, and hydroxy-trimethoprim, given the low sorption of their respective parent compounds[20] which share very similar molecular structures. Conversely, sorption might influence the rapid degradation of 4-epianhydrotetracycline, as tetracycline has been shown to generally adhere to glassware. The fact that 4-epianhydrotetracycline absorbs light in the UV and visible ranges might further contribute to the fast degradation. This TP also demonstrated the highest nonphotochemical degradation among the studied TPs (Table S6). After 56 h, only about 20% of the initial concentration in Milli-Q, 33% in seawater, and 3% in surface water remained in the samples covered with aluminum foil, highlighting the substantial contribution of other abiotic pathways to 4-epianhydrotetracycline degradation (Table S6). Similar results were observed for erythromycin A enol ether with more than 50% of its removal attributed to other abiotic degradation pathways. For all other TPs, photolysis was the dominant abiotic pathway, contributing more than 50% to their removal (Table S6). The contribution of other abiotic pathways to the total removal varied considerably, ranging from 12% (maximum) for hydroxy-trimethoprim to 44% for hydroxy-metronidazole, 37% for clindamycin sulfoxide, 19% for N^4-acetylsulfamethoxazole, and 28% for anhydro-erythromycin (Table S6).

3.3. Computational (Photo)Degradation Predictions and Comparison. Based on the estimated HOMO−LUMO energies for each TP, the *in silico* degradation rates and the respective half-lives are predicted via different QSAR models (Table S7). The QSARs for antibiotics developed by Lyu et al. (2022) apply stepwise multiple linear regression using electronic and molecular descriptors to predict photodegradation rates. These models are either general, covering multiple antibiotic classes, or class-specific, tailored for individual antibiotic categories. Notably, the final models primarily include the energy gap between HOMO and LUMO and the fluorine atom count as key predictors. In contrast, the QSAR model for PAHs by Chen et al. (1996) is based on a parabolic function of the HOMO−LUMO gap, reflecting a different mechanistic approach to photodegradation prediction.

Generally, for all the studied TPs, the photolysis QSARs for specific antibiotics[21] estimated the highest persistence with half-lives up to 7794 years (≈2.84 × 10^6 d for 4-epianhydrotetracycline) (Table S7). Both antibiotic photolysis

https://doi.org/10.1021/acsestwater.5c00327
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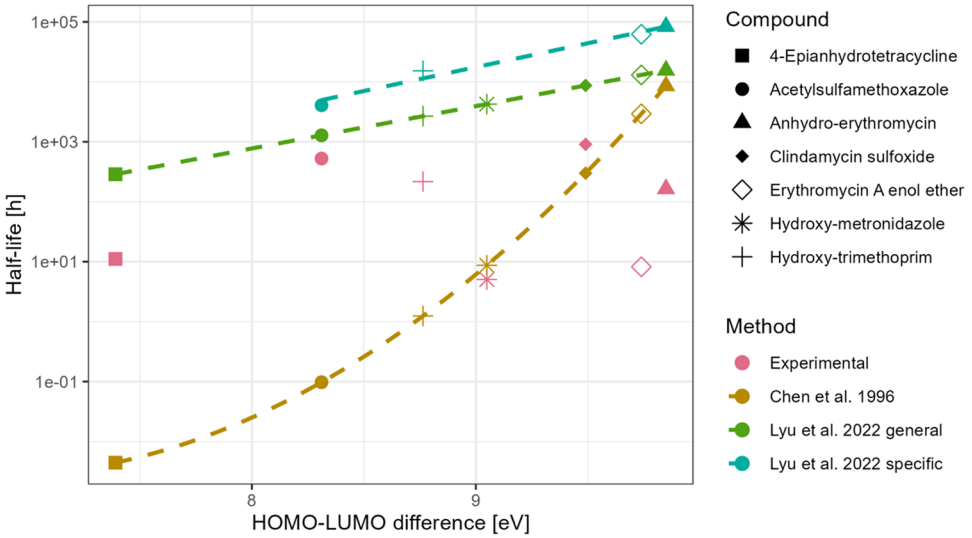

Figure 3. Experimental (Milli-Q water) and QSAR-derived half-lives as a function of the HOMO−LUMO gap.

QSARs (general and class-specific) resulted in the highest half-life estimations, with most being between 10^3 and 10^4 h, which is at least 1 order of magnitude above the experimentally derived values (Figures 3 and S4). The QSAR for photolysis of PAHs[22] predicted half-life estimates in the same order of magnitude as the ones determined in this study for most TPs. Only N^4-acetylsulfamethoxazole and 4-epianhydrotetracycline were underestimated by several orders of magnitude. Comparing the photodegradation models for PAHs and general antibiotics with experimental results in Milli-Q water revealed weak positive correlations (Spearman rank correlation = 0.0714), while the antibiotic group-specific QSAR showed a negative correlation (Spearman rank-based correlation = −0.429), highlighting substantial discrepancies. Given that these correlations are based on limited numbers of data points, the reliability of these trends remains uncertain. These results suggest that the current computational models may not fully capture the key mechanisms governing photodegradation for the studied TPs. To further explore potential improvements in predictive modeling, we assessed the relationship between degradation half-lives and HOMO−LUMO gaps (Figure 3), by plotting the half-lives as a function of the HOMO−LUMO gaps to determine whether a direct relationship exists, as implied by the QSAR models. Only a weak linear relationship (R^2=0.083) and very low Spearman rank correlation (0.029) were found, suggesting that the HOMO−LUMO gap alone does not strongly predict photodegradation half-lives. These findings indicate that additional descriptors, such as excited-state properties or charge distribution, or presence of potentially photosensitive functional groups, may be necessary to improve predictive accuracy. Potentially, photosensitive functional groups could be speculated to be the polycyclic ether/acetal structures in the erythromycin derivatives, and the nitro group in hydroxy-metronidazole. For these three compounds, we in fact obtained photodegradation rates that were outside the range estimated by the different QSAR models of Chen et al. and Lyu et al., whereas the degradation rates obtained for all other compounds in this study fall within that range.

Comparing the experimental half-lives with other non-photolysis degradation models, we observed that for both erythromycin TPs, the kMHalf-Life model obtained the closest estimates, followed by the hydrolysis model. In contrast, the persistence model and all photodegradation QSARs overestimated the degradation times for these two TPs by more than 82 days. The pattern where either the hydrolysis model or the kMHalf-Life model produced estimates closest to experimental values was consistently observed across all studied TPs. However, this does not indicate that photolysis follows hydrolysis or biodegradation mechanisms but rather supports that the currently available photodegradation QSARs are not adequately capturing the key factors governing phototransformation. The trend is further supported by the lowest overall mean deviation in the half-lives, with the hydrolysis model (3.75 days) and the kMHalf-Life model (5.5 days) showing the best agreement with experimental values (Table S8). The persistence model, on the other hand, had an average error of approximately 40 days, while all photodegradation QSAR models exhibited even larger deviations, with mean errors exceeding 75 days (Table S8).

4. IMPLICATIONS

The occurrence of antibiotic TPs in surface waters can pose a risk for resistance development.[4] Understanding the fate of antibiotics as well as their TPs is important for an enhanced and realistic risk assessment. The TPs tested in this study degraded to various extents across different water matrices (Figure 2, Tables S4 and S5). Most TPs are susceptible to photodegradation in environmental waters, mainly via indirect photolysis. In other words, the type of water matrix can substantially influence the photodegradation rates, emphasizing the critical role of indirect photolysis facilitated by water constituents in determining the environmental fate of the TPs. Among all the tested TPs, N^4-acetylsulfamethoxazole showed the highest persistence under all experimental conditions (Figure 2), suggesting that its environmental degradation likely relies on biotic pathways. To the best of our knowledge, studies on the biodegradation of acetylsulfamethoxazole in environmental waters remain very limited. However, the two structurally similar TPs, N^4-acetylsulfapyridine and N^4-acetylsulfamethazine, showed contrasting results.[29] N^4-Acetylsulfapyridine was fully degraded after 32 days compared to the sulfamethazine TP showing no degradation over 90 days.[29] This variability in biodegradation behavior among structurally

https://doi.org/10.1021/acsestwater.5c00327
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similar compounds implies the potential difficulty in estimating the TP persistence in aquatic environments. While sulfamethoxazole has been shown to undergo rapid biodegradation,[34] N^4-acetylsulfamethoxazole persisted with little to no biodegradation in other environmental compartments, including digestate-amended soil and river sediment.[34,35] In river sediments, sorption to sediment particles was excluded as a major removal pathway, emphasizing the importance of water-sediment interactions for its biodegradation.[35] Furthermore, this TP has been shown to enhance the risk of conjugative transfer at environmentally relevant concentrations in controlled bacterial systems, even more so than the parent compound sulfamethoxazole, highlighting its potential to promote ARG mobilization.[36] However, the extent to which this occurs in complex environmental settings remains largely unknown, underscoring a critical gap in our understanding of TP-mediated resistance dissemination. Together, the findings from the previous and our studies highlight N^4-acetylsulfamethoxazole as a TP of high concern for the environment.

Our results show no significant difference in degradation patterns of TPs (Figure 2) between the two tested light intensities, which correspond to, for example, seasonal variations in Uppsala, Sweden (Figure S2). The absence of variation in degradation rates may reflect a saturation of the photolysis reaction, where maximum efficiency is already reached at the lower light intensity, or suggest that other factors, such as availability of reactive intermediates, play a dominant role in degradation rather than light intensity alone. Given that temperature influences indirect photodegradation yet was controlled in our studies, future research should investigate these dynamics to enhance predictions of photodegradation under diverse environmental conditions. However, the consistency in photolysis response suggests that similar outcomes might be anticipated in regions with comparable solar irradiation profiles, potentially expanding the applicability of our findings globally.

Studying the degradation of antibiotic TPs in aquatic environments helps understand their long-term impacts on water quality and public health, aligning with the One Heath approach. TPs with long half-lives can contribute to prolonged environmental exposure, where they may either revert to the parent antibiotic, as observed for N^4-acetylsulfamethoxazole,[35] or potentially exert selective pressure on their own. Their persistence, most likely at sublethal concentrations, may thus contribute to the development of AMR in pathogens in the environment. The findings of our study offer a selection of antimicrobial TPs that should be investigated in the future for their effects on microbial communities to assess potential resistance development. While several TPs were shown to have rather short half-lives, their continuous discharge into aquatic systems from wastewater treatment plants can lead to "pseudo-persistence", where constant input maintains environmental concentrations. To better assess environmental risks, it would be beneficial to comprehensively elucidate the photodegradation pathways of antibiotic TPs in future studies, including identification and characterization of secondary TPs and their potential persistence and toxicity. High-resolution mass spectrometry is essential to detecting their intermediate TPs and to determining whether mineralization is achieved. Such mechanistic insights are critical for evaluating the environmental fate and risk potential of both primary and subsequent TPs under realistic conditions. This underscores the importance of considering TPs in environmental monitoring and risk assessment strategies, as their presence and potential interactions may play a role in the emergence of AMR.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.5c00327.

Details on water chemistry parameters and light exposure; analytical method validation and retention time data; measured concentrations and degradation kinetics of transformation products (TPs) across environmental waters; abiotic removal results under light-shielded conditions; computational predictions including HOMO/LUMO energies and QSAR-estimated half-lives; spectral comparison of irradiation sources; and chromatograms of native and internal standards; and full method validation procedure (XLSX)

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CRediT: **Paul Löffler** conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing - original draft; **Henning Henschel** formal analysis, methodology, writing - review & editing; **Valentina Ugolini** methodology, writing - review & editing; **Harold Flores Quintana** methodology, writing - review & editing; **Karin Wiberg** conceptualization, writing - review & editing; **Foon Yin Lai** conceptualization, funding acquisition, project administration, supervision, writing - review & editing.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was funded by the Swedish Research Council (project number: 2020-03675). The authors would like to thank Marcus Korvela for his prompt and comprehensive

support with the laboratory equipment. Further, the authors would like to thank Atlas for kindly providing the data to compare irradiation intensities.

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