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# **Co-occurrence of Photochemical and Microbiological Transformation Processes in Open-Water Unit Process Wetlands**

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# Co-occurrence of Photochemical and Microbiological Transformation Processes in Open-Water Unit Process Wetlands

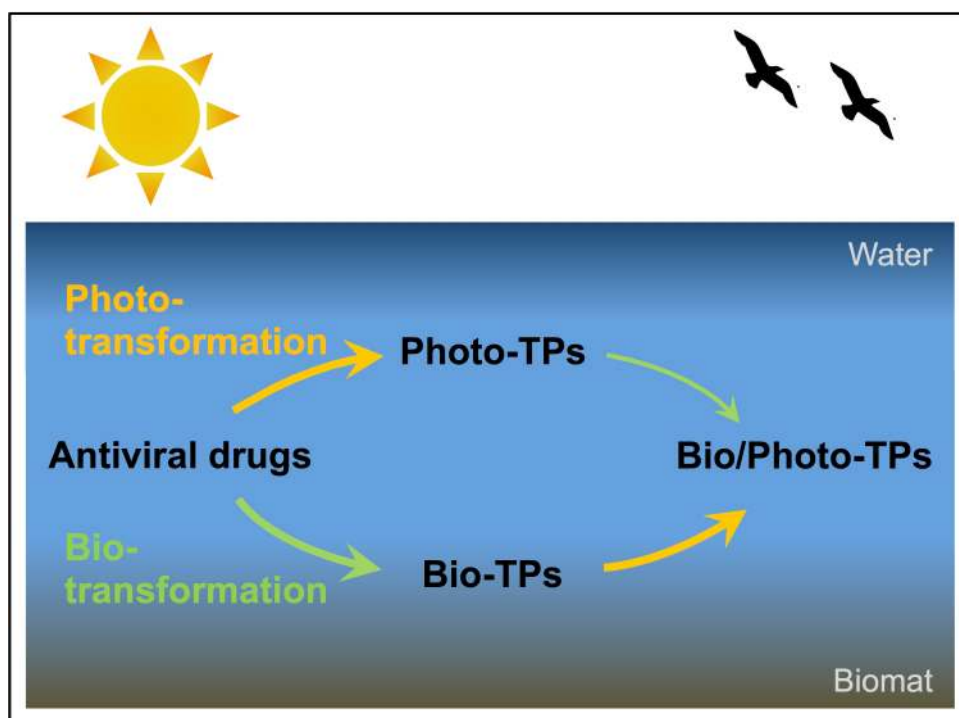
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## Abstract

The fate of anthropogenic trace organic contaminants in surface waters can be complex due to the occurrence of multiple parallel and consecutive transformation processes. In this study, the removal of five antiviral drugs (i.e., abacavir, acyclovir, emtricitabine, lamivudine and zidovudine) via both bio- and photo-transformation processes was investigated in laboratory microcosm experiments simulating an open-water unit process wetland receiving municipal wastewater effluent. Phototransformation was the main removal mechanism for abacavir, zidovudine and emtricitabine, with half-lives ( $t_{1/2, \text{photo}}$ ) in wetland water of 1.6 h, 7.6 h and 25 h, respectively. In contrast, removal of acyclovir and lamivudine was mainly attributable to slower microbial processes ( $t_{1/2, \text{bio}} = 74$  h and 120 h, respectively). Identification of transformation products revealed that bio- and photo-transformation reactions took place at different moieties. For abacavir and zidovudine, rapid transformation was attributable to the high reactivity of the cyclopropylamine and azido moiety, respectively. Despite substantial differences in kinetics of different antiviral drugs, biotransformation reactions mainly involved oxidation of hydroxyl groups to the corresponding carboxylic acids. Phototransformation rates of parent antiviral drugs and their biotransformation products were similar, indicating that prior exposure to microorganisms (e.g., in a wastewater treatment plant or a vegetated wetland) would not affect the rate of transformation of the part of the molecule that was susceptible to phototransformation. However, phototransformation strongly affected the rates of biotransformation of the hydroxyl groups, which, in some cases, resulted in greater persistence of phototransformation products.

## Introduction

The discharge of municipal wastewater effluents into surface waters can result in the presence of trace organic contaminants at concentrations that pose potential risks to aquatic ecosystems and drinking water resources. After their release, many trace organic contaminants are attenuated by biological and photochemical processes. Although these processes often occur simultaneously or sequentially in the environment, most studies have considered the occurrence of only one transformation process at a time.<sup>1-4</sup> Thus, it is difficult to predict which transformation products will be formed and whether or not transformation reactions occurring at one moiety alter the kinetics of subsequent transformation reactions. Furthermore, if partial transformation of a compound enhances the reactivity of other moieties, interaction of transformation processes could result in changes in the distribution of transformation products as well as their rates of removal. For example, carbamazepine, a compound that is particularly resistant to biotransformation is slowly transformed upon exposure to sunlight via direct photolysis and reaction with  $\cdot\text{OH}$ .<sup>5,6</sup> This leads to the formation hydroxylated derivatives,<sup>7</sup> which are more easily biodegraded than the parent compound.<sup>8</sup>

Open water unit process wetlands have been developed as a polishing treatment step for municipal wastewater effluents.<sup>9</sup> These managed natural systems utilize sunlight to remove trace organic compounds and inactivate pathogens.<sup>10-12</sup> In addition, microorganisms in the biomat formed at the bottom of these treatment basins reduce nitrate and contribute to aerobic biodegradation of trace organic contaminants.<sup>13,14</sup> To assess the importance of the co-occurrence of biological and photochemical transformation reactions to reaction kinetics and product distribution, the fate of five antiviral drugs (i.e., abacavir, emtricitabine, lamivudine, zidovudine and acyclovir, see Figure 1) was studied under conditions comparable to those encountered in open-water unit process wetlands. Antiviral drugs were chosen because they are widely used for the treatment of diseases such as herpes, hepatitis and HIV, and have been detected at concentrations above  $1\text{ }\mu\text{g L}^{-1}$  in municipal wastewater effluents.<sup>15-18</sup> No information about potential environmental effects resulting from the release of these compound into the aquatic environment is available so far. Furthermore, little is known about the effects of these compounds on

environmental viruses, a group of microorganisms that play a very important role in aquatic ecosystems.<sup>19</sup>

By investigating transformation kinetics and transformation mechanisms under conditions comparable to those encountered in open-water unit process wetlands it is possible to gain insight into how simultaneously occurring bio- and photo-transformation reactions affect the overall fate of antiviral drugs in sunlit surface waters. These compounds also serve as models for other families of compounds that contain moieties that are susceptible to bio- and phototransformation.

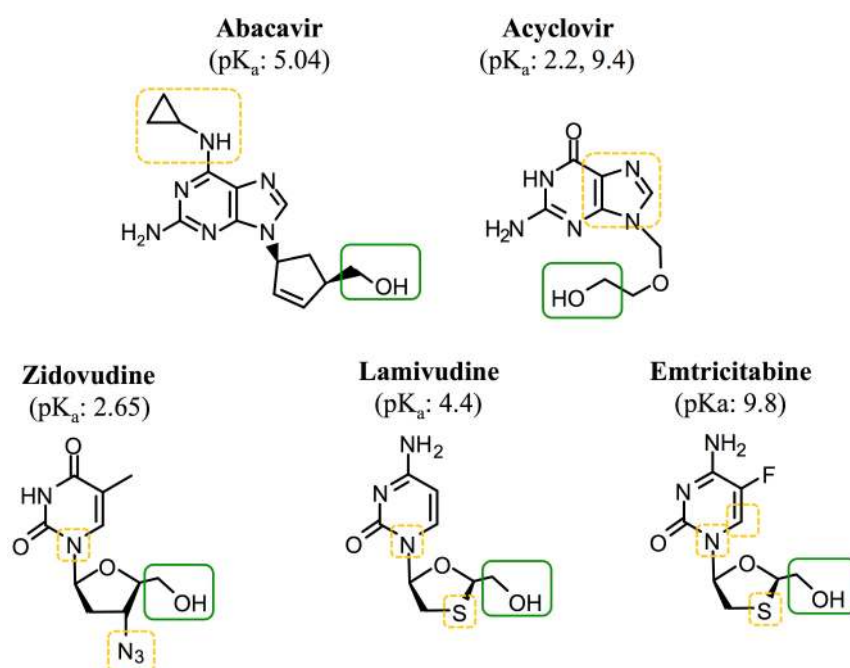


Figure 1. Antiviral drugs and their most likely sites of proposed photo- (□) and biotransformation (■) reactions.

## Materials and Methods

### Chemicals

Analytical reference standards of antiviral drugs and stable isotope-labeled analogues used as internal standards (purity > 99%) were purchased from Toronto Research Chemicals (Ontario, Canada). All other chemicals and solvents were obtained from Fisher Scientific (Fairlawn, NJ).

98

99 *Wetland water sampling conditions*

100 Phototransformation experiments were conducted in water collected from a pilot-scale  
101 open-water unit process wetland located in Discovery Bay, CA. The facility treats about  
102 10,000 gallons per day ( $4.4 \times 10^{-4} \text{ m}^3 \text{ s}^{-1}$ ) of nitrified wastewater effluent from an adjacent  
103 municipal wastewater treatment plant. Details about the open-water unit process wetland  
104 were described previously.<sup>10,13</sup> Water collected from the open-water wetland typically  
105 contained 10 - 20 mg L<sup>-1</sup> -N NO<sub>3</sub><sup>-</sup>, 5 - 10 mg L<sup>-1</sup>-C DOC, and 60 - 80 mg L<sup>-1</sup>-C dissolved  
106 inorganic carbon (HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup>). Samples for laboratory irradiation experiments were  
107 collected from the mid-point of the wetland. All samples were filtered through pre-rinsed  
108 1µm (nominal pore size) glass fiber filters (Whatman) and were stored in the dark at 4°C  
109 until analysis, which occurred within 5 days.

110

111 *Laboratory photo- and biotransformation experiments*

112 Irradiation experiments were performed using a collimated beam Oriel Solar Simulator  
113 (Spectra Physics, serial no. 91194) equipped with a 1000 W Xe lamp and either two  
114 successive atmospheric attenuation filters (Spectra Physics, serial no. 81088 & 81017) or  
115 one atmospheric and one UVB-filter (Spectra Physics, serial no. 81088 & 81050). Spectral  
116 irradiance was routinely measured with a spectroradiometer (RPS 380, International light)  
117 at different locations of the irradiated area to assess variability, which was always < 5%.  
118 Details on lamp irradiance energies and the spectra of different configurations are given in  
119 section 1.1 of the Supporting Information (SI). Irradiation experiments were carried out in  
120 100 mL black-painted glass beakers that were placed in a water bath at constant  
121 temperature (18 ± 2°C). Initial concentrations of antivirals of approximately 0.5 µM were  
122 used for all kinetics experiments. Pseudo-first order phototransformation rate constants of  
123 antivirals and photochemical probe compounds used for the quantification of  
124 concentrations of reactive intermediates were calculated from the slopes of linear  
125 regression of the natural log of concentration versus time. No degradation of antiviral  
126 drugs was observed in control experiments in the dark indicating that their transformation  
127 in filtered wetland water was only attributable to photochemical processes.

For the elucidation of biotransformation kinetics, beakers were additionally supplemented with 10 mL of the biomat taken from the bottom of a pilot-scale open-water wetland and kept in the dark (see Jasper et al.<sup>13</sup> for further details). Biodegradation of compounds followed pseudo-first order degradation kinetics, indicating stable conditions throughout the experiments. In addition, observed transformation rates in good agreement with results from a preliminary study used to design the more detailed experiments.

*Direct and indirect phototransformation.* Experiments to assess direct phototransformation of antiviral drugs were conducted in buffered ultrapure water at pH-values ranging from 6 to 10 (pH 6 - 8: 5 mM phosphate buffer; pH 9 - 10: 5 mM borate buffer). Samples (1 mL) were collected at regular time intervals and stored at 4°C in the dark until analysis. Electronic absorption spectra of antiviral drugs at different pH values (see Fig. S2) were recorded with a UV-2600 UV-Vis Spectrophotometer (Shimadzu) using quartz-glass cuvettes (Hellma, Germany). Further details on determination of quantum yields using the *p*-nitroanisole (PNA)/pyridine(PYR) method<sup>20</sup> and related calculations are provided in section 1.6 of the SI.

Indirect phototransformation of antiviral drugs was investigated by the addition of specific quenchers to wetland water: *N,N*-dimethylaniline (DMA; 10 µM) was used to scavenge CO<sub>3</sub>-radicals<sup>10</sup>, sorbic acid (2.5 mM) was used to scavenge excited triplet states of the dissolved organic matter (<sup>3</sup>DOM\*)<sup>21</sup>, histidine (20 mM) was used to scavenge singlet oxygen (<sup>1</sup>O<sub>2</sub>)<sup>22</sup> and isopropyl alcohol (IPA; 26 mM) was used to scavenge •OH-radicals.<sup>23</sup> In addition, experiments with specific photosensitizers were conducted in ultrapure buffered water to determine reaction rate constants of antiviral drugs with individual reactive intermediates: For CO<sub>3</sub><sup>•-</sup>, either NaNO<sub>3</sub>/NaHCO<sub>3</sub> or duroquinone/NaHCO<sub>3</sub> photosensitizer methods were used.<sup>24,25</sup> The excited triplet state photosensitizers 3-methoxyacetophenone (3MAP) and anthraquinone-2-sulfonate (AQ2S) served as proxies for <sup>3</sup>DOM\*.<sup>26</sup> Hydroxyl-radicals were generated by the irradiation of NaNO<sub>3</sub> solutions.<sup>27</sup> For <sup>1</sup>O<sub>2</sub> production, Rose Bengal was used as a photosensitizer.<sup>28</sup> To further verify the role of <sup>1</sup>O<sub>2</sub>, some experiments were performed in D<sub>2</sub>O. Reaction rate constants were either determined by competition kinetics or by comparing reaction rates of antiviral drugs with those of established photochemical probe compounds (experimental details and calculations are provided in section 1.5 and



1.7). For all indirect phototransformation experiments, the concentration changes of photochemical probe compounds and antiviral drugs during irradiations were determined by HPLC-UV. Experimental and analytical details, including comprehensive results are provided in the SI section 1.2.

Given the structural similarities of antivirals with DNA bases, additional irradiation experiments were performed with adenine, 2-amino adenosine, cytosine, cytidine, guanine, thymidine and thymine (SI section 2.1.1) to obtain further information about the photoreactive moieties in the molecules to aid the identification of transformation products.

*Identification of photo- and biotransformation products.* High resolution mass spectrometry (HR-MS; LTQ Orbitrap Velos, Thermo Scientific, Bremen, Germany) was used to conduct accurate MS and MS/MS analysis of transformation products of antiviral drugs. To this end, experiments at elevated concentrations (40  $\mu$ M) were used. The LTQ Orbitrap Velos was coupled to a Thermo Scientific Accela liquid chromatography system (Accela pump and autosampler). HR-MS was conducted in the positive electrospray ionization (ESI) mode. To obtain information on the chemical structure of the TPs, MS<sup>n</sup> fragmentation experiments were conducted using data-dependent acquisition. Further information on the applied setup and data dependent acquisition parameters can be found in the SI (section 1.3). Product formation of antiviral drugs in laboratory experiments was determined by liquid chromatography tandem mass spectrometry (LC/MS/MS). Details are provided in the SI (section 1.4).

*Combined bio- and photodegradation experiments.* The fate of antiviral drugs in the presence of sunlight and microorganisms was investigated over a 72 h period in the laboratory. Black-painted glass beakers (250mL) were filled with 180 mL of wetland water and 20 mL of freshly collected biomat material from the bottom of the Discovery Bay open-water unit process wetland. The experimental setup was the same as described above for photochemical experiments, but with three day/night cycles to simulate field conditions (8 h of daily irradiation followed by 16 h in the darkness; 72 h total). Antiviral drugs were added individually at concentrations of 0.5  $\mu$ M to ensure detection of both parent antiviral

compounds and their transformation products. Samples were collected at regular time intervals and stored at 4°C in the dark prior to LC/MS/MS analysis, which occurred within 24 h. Further details about the analytical method can be found in the SI.

## Results and Discussion

### *Phototransformation in wetland water*

Phototransformation of the five investigated antiviral drugs in wetland water followed first-order kinetics ( $r^2 \geq 0.98$ ; Figure S4-S8). In native wetland water (pH 8.9), the fastest phototransformation was observed for abacavir ( $k_{\text{obs}} = 0.52 \pm 0.06 \text{ h}^{-1}$ ), zidovudine ( $k_{\text{obs}} = 0.09 \pm 0.002 \text{ h}^{-1}$ ) and emtricitabine ( $k_{\text{obs}} = 0.03 \pm 0.002 \text{ h}^{-1}$ ) whereas the transformation of acyclovir and lamivudine were significantly slower ( $k_{\text{obs}} = 0.012 \pm 0.001 \text{ h}^{-1}$  and  $0.011 \pm 0.001 \text{ h}^{-1}$ , respectively) (Figure 2). No degradation of antiviral drugs in wetland water occurred in the dark indicating that their removal was solely attributable to photochemical processes. Photosynthetic activity leads to significant diurnal fluctuations of pH in open-surface wetlands.<sup>10</sup> Therefore, phototransformation kinetics of antiviral drugs in wetland water were also determined at pH 6.5 and pH 10 (Figure 2).

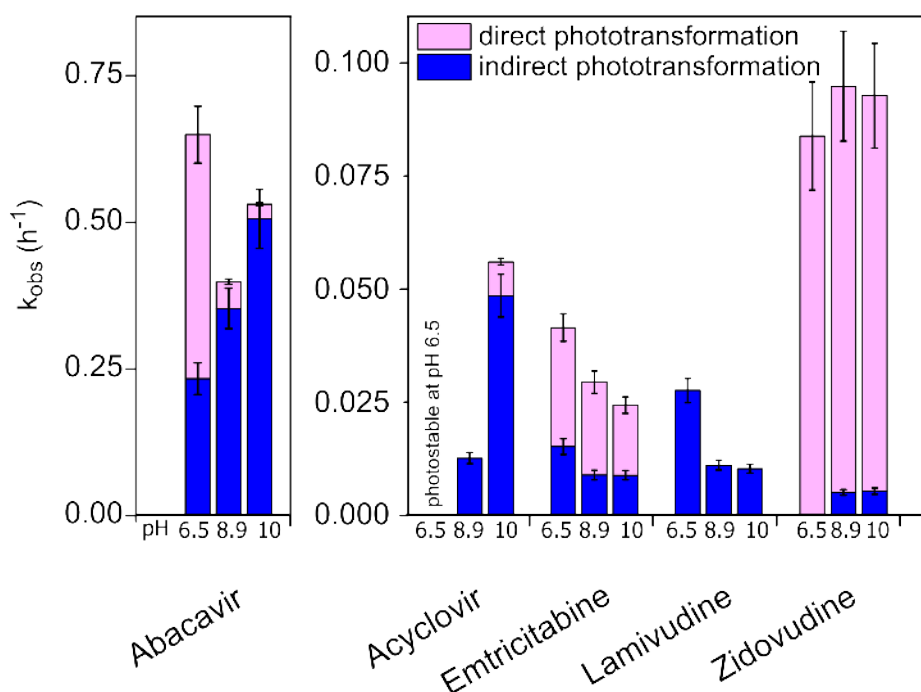


Figure 2. Phototransformation kinetics of antiviral drugs in experiments with

wetland water at different pH values and contribution of direct and indirect photolysis processes by comparison with results obtained in ultrapure water. Data for wetland water are corrected for light-absorption. Error bars show 95% confidence intervals.

207

208 Phototransformation of abacavir in wetland water increased when the pH value was  
209 adjusted to 6.5 or 10. This can be attributed to a higher contribution of direct photolysis  
210 due to higher quantum yields at lower pH values (i.e.,  $\Phi_{app}$  is 4.2 to 11.4 times higher  
211 between pH 6 – 8, compared to pH 9 and 10, SI Table S5) and faster indirect photolysis at  
212 higher pH values. Comparison of transformation kinetics with results obtained in ultrapure  
213 water revealed the dominance of indirect photodegradation processes at pH 8.9 and 10,  
214 whereas direct photolysis was more important at pH 6.5. The addition of sorbic acid and  
215 histidine significantly reduced phototransformation rates of abacavir in wetland water  
216 (Fig. S4). Although interpretation of results from experiments with scavengers requires  
217 caution,<sup>29</sup> these results suggest the involvement of  $^3DOM^*$  and  $^1O_2$  in the photochemical fate  
218 of this compound. This was also supported by experiments with specific singlet oxygen and  
219 excited triplet state sensitizers (see below). Negligible removal of the structural analogues  
220 adenine and 2-amino-adenosine further indicated that the photolability of abacavir can be  
221 attributed to the cyclopropyl-moiety (see SI section 2.1.1).

222 Rates of phototransformation of zidovudine were not affected by changes in pH.  
223 Comparison with reaction rates in both ultrapure water and wetland water in the presence  
224 of scavengers revealed the dominance of direct photolysis (Fig. S5). Similar to abacavir,  
225 comparison with the depletion of structural analogues thymine and thymidine indicated  
226 that the azide moiety was responsible for the observed photoreactivity of zidovudine as  
227 both analogues showed no removal when exposed to light (see SI section 2.1.1).

228 Phototransformation rates of acyclovir in wetland water increased with increasing pH.  
229 Comparison with results from ultrapure water revealed that removal at pH 8.9 was solely  
230 due to indirect photolysis, whereas at pH 10 direct photolysis was also important.  
231 Significantly reduced rates of acyclovir phototransformation in the presence of histidine  
232 and sorbic acid indicated the importance of  $^1O_2$  and  $^3DOM^*$  to indirect photolysis (Fig. S6).  
233 In contrast to abacavir and zidovudine, phototransformation kinetics were similar to those

observed for the structural analogue guanine (SI Fig. S15). Thus, phototransformation of acyclovir can be attributed primarily to the guanine moiety.

For lamivudine and emtricitabine, phototransformation kinetics in wetland water decreased with increasing pH. No removal of lamivudine was observed in ultrapure water indicating that its removal was entirely attributable to indirect photolysis. Higher phototransformation rates of emtricitabine relative to lamivudine further indicated the strong influence of the fluorine atom for emtricitabine's photolability. The presence of the fluorine substituent led to greater light absorption at 300-320 nm (SI Fig. S2). Even though the absorption spectrum of emtricitabine did not change with pH, the quantum yield steadily decreased with increasing pH (Table S5). Phototransformation of lamivudine in wetland water was fully inhibited by sorbic acid, histidine and IPA but was unaltered in the presence of DMA (Fig. S7). This indicates the importance of  $^3\text{DOM}^*$ ,  $^1\text{O}_2$  and OH-radicals for its indirect phototransformation. For emtricitabine, phototransformation rates in wetland water were only affected by IPA and sorbic acid (Fig. S8), suggesting that reactions with  $^1\text{O}_2$  are less important for this compound. The high photostability of its associated DNA base cytosine and nucleotide cytidine revealed the importance of structural modifications (thiol group (both compounds) and fluorine (emtricitabine) to the observed photodegradation.

Additional experiments with individual reactive species revealed second-order reaction rates with  $\cdot\text{OH}$  at or above (abacavir, zidovudine) diffusion controlled rates ranging from  $5 \cdot 10^9$  to  $1.1 \cdot 10^{11} \text{ M}^{-1}\text{s}^{-1}$  (Table 1). Antiviral compounds were reactive with  $\text{CO}_3^{\cdot-}$ , at rates between  $1.2 \cdot 10^6$  and  $1.2 \cdot 10^9 \text{ M}^{-1}\text{s}^{-1}$ , while only abacavir ( $1.2 \cdot 10^9 \text{ M}^{-1}\text{s}^{-1}$ ) and acyclovir ( $1.2 \cdot 10^7 \text{ M}^{-1}\text{s}^{-1}$ ) reacted with  $^1\text{O}_2$ . With the exception of abacavir, no depletion of antiviral compounds was observed in the presence of the model triplet photosensitizer 3MAP. However depletion of all compounds was observed in the presence of AQ2S at rates similar to or higher than the reference probe compound TMP, indicating selective reactivity with excited triplet states. Comparison of measured and predicted rate constant for antivirals under wetland conditions (obtained by multiplication of steady-state concentrations of reactive species measured in wetland water with measured second-order reaction rate constants of antivirals with  $^1\text{O}_2$ ,  $\cdot\text{OH}$  and  $\cdot\text{CO}_3^-$ ) were in good agreement, indicating reasonable results.

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266  
267

Table 1. Quantum yields (pH 9) and apparent second-order reaction rate constants of indirect phototransformation of antiviral drugs via reaction with  $^1\text{O}_2$ ,  $\cdot\text{OH}$ ,  $\cdot\text{CO}_3^-$  and excited triplet states (given relative to the degradation of the  $^3\text{Sens}^*$  probe compound TMP). Quantum yields of antiviral drugs at pH 6-8 and pH 10 can be found in SI Table S5.

	[M Es <sup>-1</sup> ]	[M <sup>-1</sup> s <sup>-1</sup> ]				[-]	
	$\Phi_{\text{app}}(300-400\text{nm})$ (pH 9)	$^1\text{O}_2$	$\cdot\text{OH}$	$\cdot\text{CO}_3^-$ ( $\text{NO}_3^- + \text{HCO}_3^-/\text{CO}_3^{2-}$ )	$\cdot\text{CO}_3^-$ (DQ)	$^3\text{SENS}^*$ (AQ2S)	$^3\text{SENS}^*$ (MAP)
Abacavir	0.014 (±0.003)	$1.2 \times 10^9$ (± 18%)	$1.1 \times 10^{11}$ (± 3%)	$1.2 \times 10^9$ (± 4%)	- <sup>a</sup>	4.88	13.5
Zidovudine	0.45 (±0.15)	n.d.	$1.3 \times 10^{10}$ (± 2%)	$2.4 \times 10^6$ (± 5%)	$1.3 \times 10^6$ (± 4%)	0.62	n.d.
Acyclovir	0.01 (±0.005)	$1.2 \times 10^7$ (± 25%)	$5.0 \times 10^9$ (± 2%)	$1.2 \times 10^8$ (± 2%)	$6.3 \times 10^7$ (± 4%)	0.08	n.d.
Emtricitabine	0.016 (±0.005)	n.d.	$9.3 \times 10^9$ (± 2%)	$3.0 \times 10^6$ (± 4%)	$4.3 \times 10^6$ (± 12%)	2.03	n.d.
Lamivudine	n.d.	n.d.	$9.2 \times 10^9$ (± 1%)	$1.2 \times 10^6$ (± 3%)	$1.7 \times 10^6$ (± 3%)	1.86	n.d.

n.d.: not detected above level of uncertainty; <sup>a</sup> not applicable due to reaction of abacavir with DQ in the dark

269

## 270 Comparison of photo- vs biotransformation rates

271 Dark experiments conducted with wetland water in the presence of biomat material  
272 indicated that biotransformation rates varied considerably among antiviral drugs.  
273 Biotransformation half-life times ( $t_{1/2,\text{bio}}$ ) ranged from 74 h for acyclovir to 500 h (21 d) for  
274 emtricitabine (Fig. 3; Fig. S13). Under typical wetland treatment conditions (i.e., hydraulic  
275 retention times of 2-3 days), significant biological attenuation of acyclovir and abacavir is  
276 expected whereas removal of the other antiviral drugs via microbial processes is unlikely  
277 to be important. Comparison of transformation rates of antiviral drugs in the dark to those  
278 observed in irradiated wetland water indicated that phototransformation processes were  
279 dominant for abacavir, zidovudine and emtricitabine, while for acyclovir and lamivudine  
280 biotransformation was similar or more important than photolysis during typical  
281 summertime conditions (Fig. 3).

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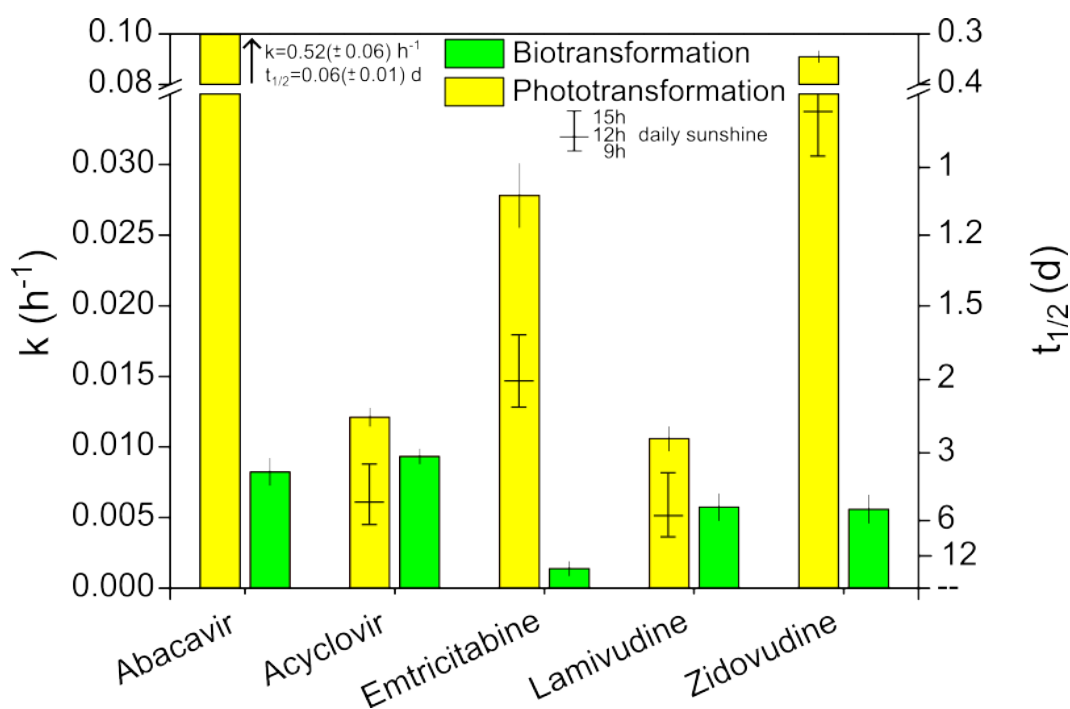


Figure 3. Photo- and biotransformation rate constants  $k$  ( $\text{h}^{-1}$ ) and associated half-life time  $t_{1/2}$  (d) of antiviral drugs in laboratory experiments. Small bars within phototransformation columns indicate half-life times based on daily sunshine hours (9-15 hours). For the determination of biodegradation half-life times, experiments were conducted in the presence of the biomat in the dark. Error bars represent 95% confidence intervals obtained from linear regressions.

#### Transformation of abacavir

HRMS analysis indicated that four primary transformation products (TP318, TP288, TP284 and TP246) were formed during photolysis of abacavir in wetland water (SI section 2.2; Table S7). In agreement with results obtained for the structural analogues 2-amino-adenosine and adenine, fragmentation patterns of TP318, TP288 and TP246 revealed that the cyclopropylamine moiety was the main site of reaction, leaving the 2-amino-adenine (fragments:  $m/z$  151.073, 134.046 and 109.051) and the 2-cyclopenten-1-methanyl moieties (fragments:  $m/z$  95.353 and 79.054) unaltered.

Exact mass calculations of TP318 showed addition of two oxygen atoms to the cyclopropyl moiety ( $\Delta m$  +31.9898 Da). Results from MS<sup>2</sup> experiments were consistent with the scission of the cyclopropyl ring and the presence of a terminal hydroxyl group, as indicated by the cleavage of H<sub>2</sub>O and CH<sub>2</sub>O.

For TP288, MS data suggested modification of the cyclopropyl moiety via loss of one carbon atom and the addition of one oxygen atom, leading to the formation of an acetamide,

298 whereas TP246 was formed via cleavage of the cyclopropyl ring. The chemical structure of  
299 TP246 was confirmed by comparison with a commercially available reference standard.  
300 The exact mass and fragmentation pattern of TP284 was consistent with loss of two  
301 protons from either the cyclopropylamine or the 2-amino-adenosine moiety (fragments  
302  $m/z$  149.069 and 189.088 instead of  $m/z$  151.073 and 191.104 compared to abacavir and  
303 the other TPs). Considering the high photolability of the cyclopropyl moiety, these  
304 structural changes were most likely due to the formation of a cyclopropylimine.  
305 To assess the relative importance of direct and different indirect photolysis processes for  
306 formation of the observed abacavir transformation products, their formation was  
307 investigated in buffered water (direct photolysis only), wetland water (direct and indirect  
308 photolysis), and wetland water in the presence of different reactive intermediate  
309 scavengers. The results revealed that both direct and indirect photolysis of abacavir  
310 produced the same suite of TPs at similar relative concentrations, despite the fact that the  
311 disappearance of the parent compound was significantly accelerated in the presence of  
312 DOM and individual reactive intermediates (Fig. S17 & S18). Similar results have been  
313 reported for irgarol, an algaecide that is structurally similar to abacavir, suggesting that the  
314 cyclopropylamine moiety is the main site of reaction under all conditions.<sup>30</sup>  
315 Photodegradation experiments in buffered ultrapure water with different optical filters  
316 indicated that wavelengths below 320 nm preferentially led to cleavage of the cyclopropyl  
317 moiety (TP246), whereas wavelengths above 320 nm (UV-A & visible light) led to scission  
318 of the cyclopropyl ring followed by partial oxidation (TP318) (Fig. S19).  
319 These findings suggest that phototransformation of abacavir is initiated by a one-electron  
320 oxidation of the cyclopropylamine moiety, leading to the formation of a  
321 cyclopropylaminium radical cation,<sup>31,32</sup> followed by subsequent reactions resulting in the  
322 formation of various products. Interestingly, this phenomenon has also been utilized for  
323 the investigation of electron-hopping in DNA by modifying guanine and adenine with  
324 cyclopropyl moieties.<sup>33,34</sup> Due to the instability of the initially formed closed ring radical  
325 cation, the modification results in rapid cyclopropyl ring-opening as well as 1,2-hydrogen  
326 migration, leading to the formation of an ionized allylamine.<sup>31,35</sup> Scission of the ring is  
327 followed either by a complete cleavage of the cyclopropyl moiety (TP246) or reaction of the  
328 ring opened radical cation with  $H_2O/O_2$ .<sup>33,35</sup> In the latter case, electron release from the

carbon centered radical followed by hydrolysis leads to the formation of a 3-hydroxypropanaminium cation<sup>36</sup> and subsequent addition of water results in the formation of the 3-hydroxypropanamide (TP318). In our system, TP288 is formed by photolytic cleavage of the hydroxymethyl group which leads to the formation of the acetamide product.<sup>36,37</sup> TP284 was most likely formed via H-atom abstraction, resulting in the formation of a neutral cyclopropyl radical followed by an electron transfer reaction and/or hydrolysis and elimination of water even though this reaction has only been shown to be catalyzed by enzymes so far.<sup>38,39</sup>

Experiments with biomat material in the dark to determine the relative importance of biotransformation reactions indicated that microbial transformation of abacavir mainly occurred via oxidation of the primary alcohol group of the 2-cyclopenten-1-hydroxymethanyl side chain to produce the corresponding carboxylic acid (abacavir carboxylate, Fig. S13). This was consistent with previous experiments conducted with mixed liquor suspended solids from an activated sludge treatment plant.<sup>40</sup>

When abacavir was exposed simultaneously to light and microorganisms (Fig. 4), a rapid loss of the compound was observed during the first 8-hour light period (i.e., the initial concentration decreased by approximately 90 %). For the next 16 hours (i.e., the dark period) abacavir removal was significantly slower. When the light was turned back on, nearly all remaining abacavir disappeared. As expected, the light-induced transformation of abacavir gave rise to the four photo-TPs described above (middle panel of Fig. 4). The concentrations of these photo-TPs decreased by approximately 25% over the next 2.5 days, indicating that further transformation took place, either via photolytic or microbial processes.

Additional biodegradation experiments with the four photo-TPs of abacavir revealed that biotransformation occurs at the same moiety as observed for the parent compound, leading to the corresponding carboxylates (TP246 carboxylate, TP284 carboxylate, TP288 carboxylate and TP318 carboxylate; Fig. S20). Exact mass data and fragmentation patterns of bio-photo TPs determined by HRMS analysis are included in section 2.2 of the SI. Consequently, the observed decrease in concentration of photo-TPs shown in the middle



panel of Fig. 4 was mainly attributable to biotransformation, leading to a steady formation of carboxylate photo-TPs (bottom panel of Fig. 4). Faster transformation rates of abacavir photo-TPs observed during irradiation periods may have been attributable to enhanced biotransformation due to elevated oxygen concentrations or elevated pH values that occurred when photosynthetic microbes in the biomat were active. Differences in biotransformation rates of TP246, TP284, TP288 and TP318, compared to abacavir (Fig. S14), indicate that alteration of chemical structure influences biotransformation kinetics, e.g. by affecting enzyme binding affinities or steric properties. Light-exposure of abacavir carboxylate formed in the dark led to its phototransformation, ultimately yielding the same photo-TPs as abacavir (bottom panel of Fig. 4). Considering that abacavir is already transformed extensively to abacavir carboxylate in activated sludge treatment,<sup>40</sup> a rapid elimination of both compounds can be expected in open-water unit process wetlands. In contrast to biotransformation reactions, similar phototransformation kinetics were observed for abacavir and abacavir carboxylate (Fig. S12). TP246 carboxylate was identified as the main product that accumulates over time because it is not susceptible to further reactions.

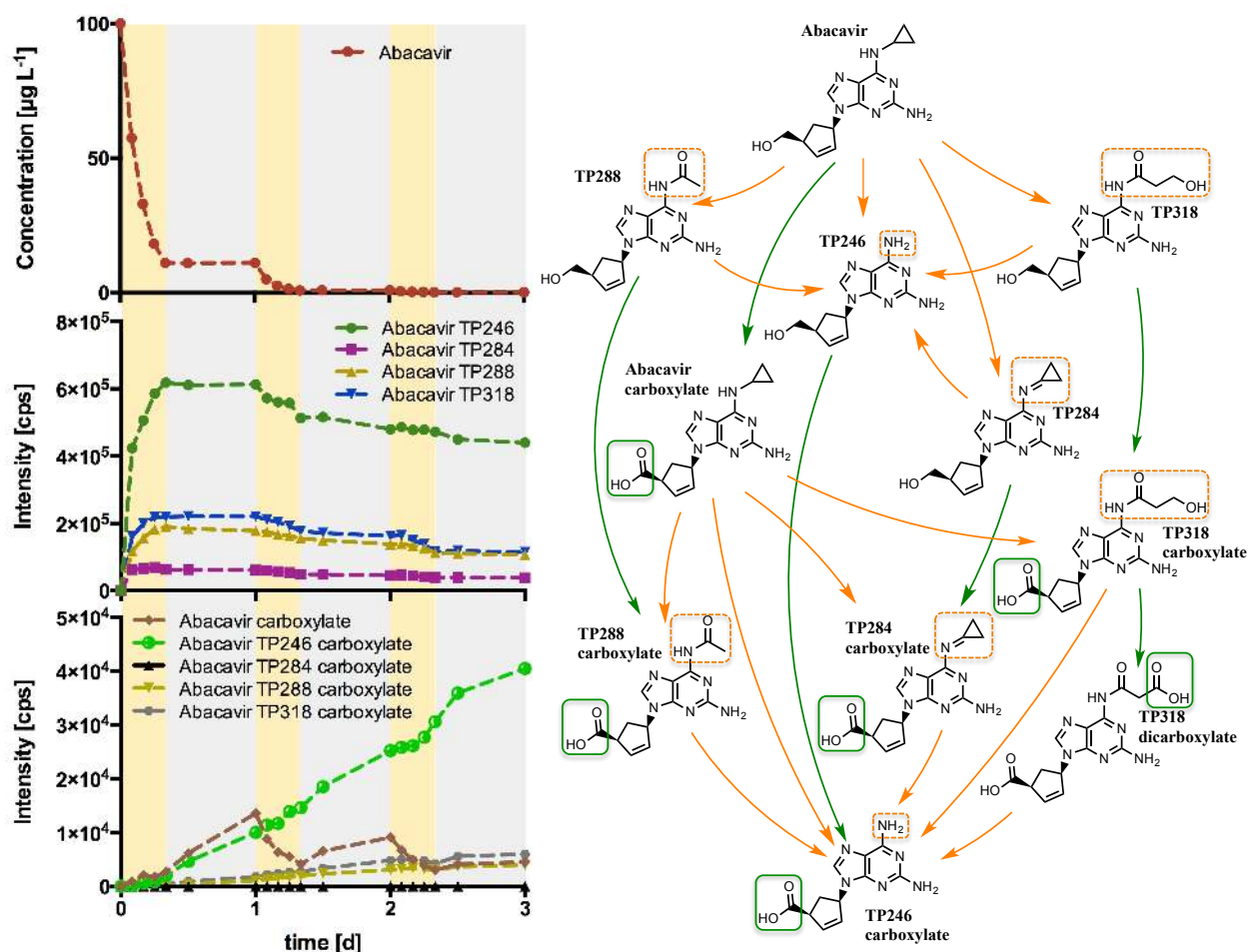


Figure 4. Transformation of abacavir (left, top) and resulting formation of photo-TPs (left, middle) and bio- / bio-photo-TPs (left, bottom)) as well as proposed transformation pathway (right) in combined in 3 day experiments in the presence of biomat with 8 hours of daily irradiation. In the transformation pathway, photo- and biotransformation reactions and structural changes in the molecules are indicated in orange and green, respectively.

### Transformation of acyclovir

In contrast to abacavir, the transformation of acyclovir was dominated by microbial processes (Fig. 5), with biotransformation resulting in the formation of acyclovir carboxylate, which was not susceptible to further microbial transformation. These results are consistent with previous biotransformation experiments conducted with acyclovir in sewage sludge.<sup>41</sup>

In the absence of biomat material, exposure of wetland water to simulated sunlight resulted in formation of two main photo-TPs (*i.e.*, TP257 and TP223). HRMS analysis indicated that TP257 contains two additional oxygen atoms on the guanine moiety, as

evidenced by the detection of fragment  $m/z$  184 instead of  $m/z$  152 (Table S8; Fig. S16). Photosensitized degradation of guanine and guanosine occurs by reaction with excited triplet states,  $^1O_2$ ,  $\cdot OH$  or  $\cdot CO_3^-$ .<sup>42,43</sup> The main product of the reaction of guanine with  $^1O_2$  has been identified as spiroiminodihydantoin.<sup>44-46</sup> To assess the role of  $^1O_2$  in the phototransformation of acyclovir in wetland water, experiments were conducted in both  $H_2O$  and  $D_2O$  in the presence of the  $^1O_2$  sensitizer Rose Bengal (Fig. 5). Lifetimes of  $^1O_2$  in  $D_2O$  are more than an order of magnitude higher than in  $H_2O$ <sup>39</sup> and faster transformation of acyclovir in  $D_2O$  confirmed the role of  $^1O_2$  in the indirect photolysis of acyclovir. In addition, the yield of TP257 increased in  $D_2O$ . Due to its photochemical properties, acyclovir is likely to undergo self-sensitization via photoexcitation and subsequent formation of  $^1O_2$  as shown for guanine and guanosine.<sup>48-50</sup> For the second acyclovir photo-TP (TP223), HRMS analysis indicated the loss of two protons, most likely from the side chain, as evidenced by the detection of fragments  $m/z$  152, 135 and 110, suggesting that the guanine moiety remained unchanged (Table S8). Additional information obtained from the fragmentation of the side chain was inconclusive but indicated oxidation of the terminal alcohol to the corresponding aldehyde via reaction with  $\cdot OH$ .<sup>51</sup>

Results from the 72h simulated sunlight experiments conducted in the presence of the biomat revealed a steady decrease of acyclovir during light and dark periods, indicating the dominance of biotransformation processes (Fig. 5b). However, biotransformation of acyclovir was significantly faster in the sunlight experiments compared to dark controls (Fig. 5a&b) suggesting that the higher oxygen concentrations and the elevated pH values that occurred when microorganisms in the biomat were undergoing photosynthesis played a role in the biotransformation processes.<sup>10</sup> In the presence of simulated sunlight, production of the two phototransformation products (i.e., TP257 and TP224) was observed. No significant removal of TP257 was detected during dark periods, suggesting limited biotransformation via oxidation of the terminal hydroxyl-group of the side chain. Although the exact reason for this is unknown, a plausible explanation is that the structural modifications of the guanine core moiety prevented enzymatic oxidation of TP257. In contrast, concentrations of TP223 decreased in the dark. For the biotransformation product (i.e., acyclovir carboxylate), increasing concentrations were only observed during dark periods, whereas its concentration decreased upon exposure to sunlight. This

indicates that the compound was transformed further by photolytic processes, most likely via the same mechanisms as acyclovir. This was confirmed by additional irradiation experiments with acyclovir carboxylate in wetland water (results not shown).

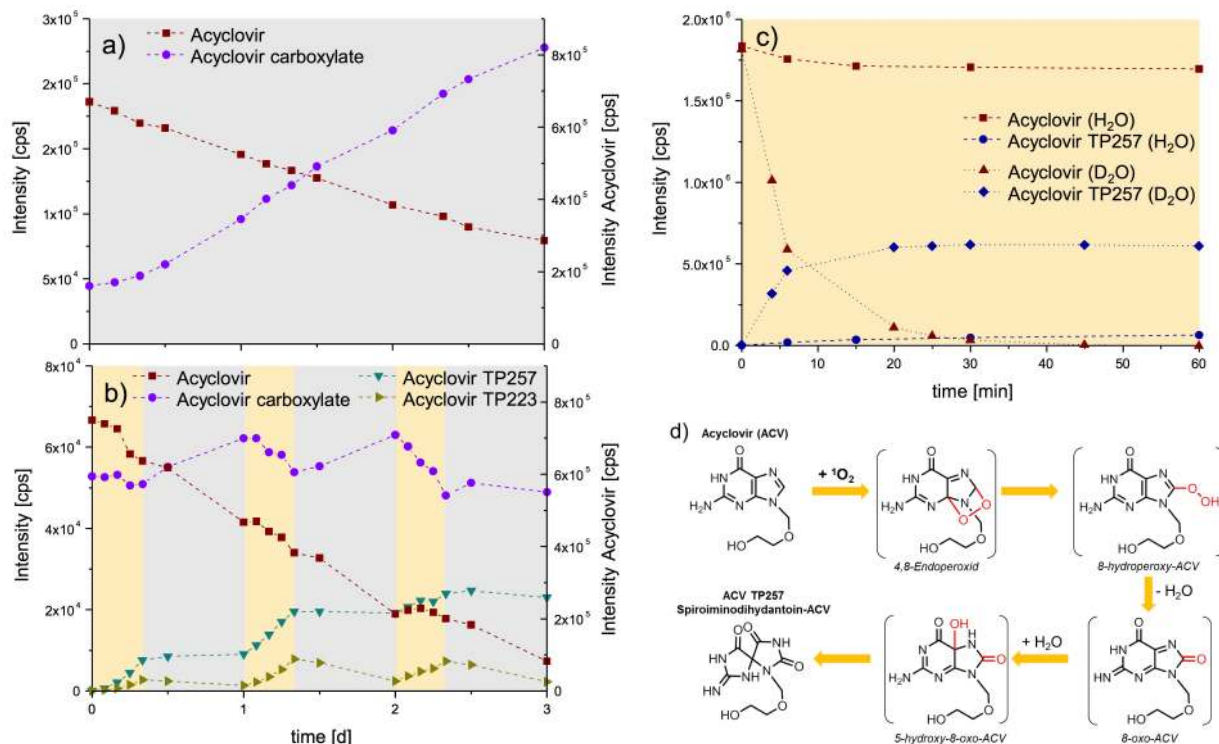


Figure 5. Transformation of acyclovir in the presence of biomat in the dark (a) in combined photo- and biotransformation experiments (b), as well as formation of TP257 via reaction of acyclovir with  $^1O_2$  in  $D_2O$  and  $H_2O$  using Rose Bengal as photosensitizer (c) and its proposed phototransformation pathway (d). The occurrence of acyclovir carboxylate at  $t_0$  in (a) and (b) is due to its emission by the WWTP that feeds the wetland.

#### Transformation of zidovudine, lamivudine and emtricitabine

MS spectra of the phototransformation products of emtricitabine, lamivudine and zidovudine indicated structural changes at different positions on the molecules (Table S9-S11). For lamivudine and emtricitabine, HRMS analysis revealed oxidation of the riboside moiety (lamivudine TP245 and emtricitabine TP263), most likely via S-oxidation. This was confirmed by comparison with commercially available reference standards. Addition of  $H_2O$  to the 5-fluoro-cytosine moiety was observed for emtricitabine (emtricitabine TP265). Experiments conducted with the fluorine-free analogue lamivudine illustrate the importance of fluorine substitution: the F-moiety increases the light absorbance at

wavelengths > 300 nm (Fig. S2) for emtricitabine and leads to faster photodegradation (Figure 1, Table S5). Emtricitabine TP265 was formed via hydration of the double bond of the 5-fluorocytosine moiety, yielding a hydroxyl-group at position C6. For zidovudine, observed phototransformations were mainly attributable to the photolability of the azido moiety. Formation of zidovudine TP239 can be explained by cleavage of N<sub>2</sub>, yielding a nitrene intermediate, which reacts further via intramolecular C-H insertion to an aziridine.<sup>52,53</sup> Subsequent nucleophilic attack of the aziridine by water leads to the hydroxylation of the C atom in  $\beta$ -position or the formation of a hydroxylamine (zidovudine TP257).<sup>52,54</sup> Results from HRMS analysis of zidovudine TP221 were inconclusive but indicated cleavage of N<sub>2</sub> and H<sub>2</sub>O from the furanosyl moiety.

In addition, photolytic cleavage of the nitrogen-carbon bond between the DNA base moieties and the riboside analogue side chains was observed for all three compounds, resulting in formation 5-fluoro-cytosine (emtricitabine TP129), cytosine (lamivudine TP111) and thymine (zidovudine TP126). None of these TPs were detected in sunlight experiments in the presence of biomat (Fig. S21-22), indicating that they were rapidly transformed, most likely via microbial processes. For zidovudine, this was confirmed by additional biodegradation experiments with the photo-TPs (i.e., thymine, TP239, TP257), showing the rapid elimination of thymine (Fig. S22). Considering the importance of both thymine and cytosine as DNA building blocks, it is likely that they were incorporated into the microbial biomass. The fate of 5-fluorocytosine remains unclear.

Similar to abacavir and acyclovir, biotransformation of emtricitabine, lamivudine and zidovudine was shown to result in the formation of carboxylated TPs via oxidation of the terminal alcohol as observed previously for abacavir and acyclovir (Fig. S13). As carboxylated TPs are expected to follow the same phototransformation mechanisms as the parent compounds, the interactions of photo- and biotransformation reactions is likely to result in their complete elimination via mineralization and/or microbial uptake (Fig. 6).

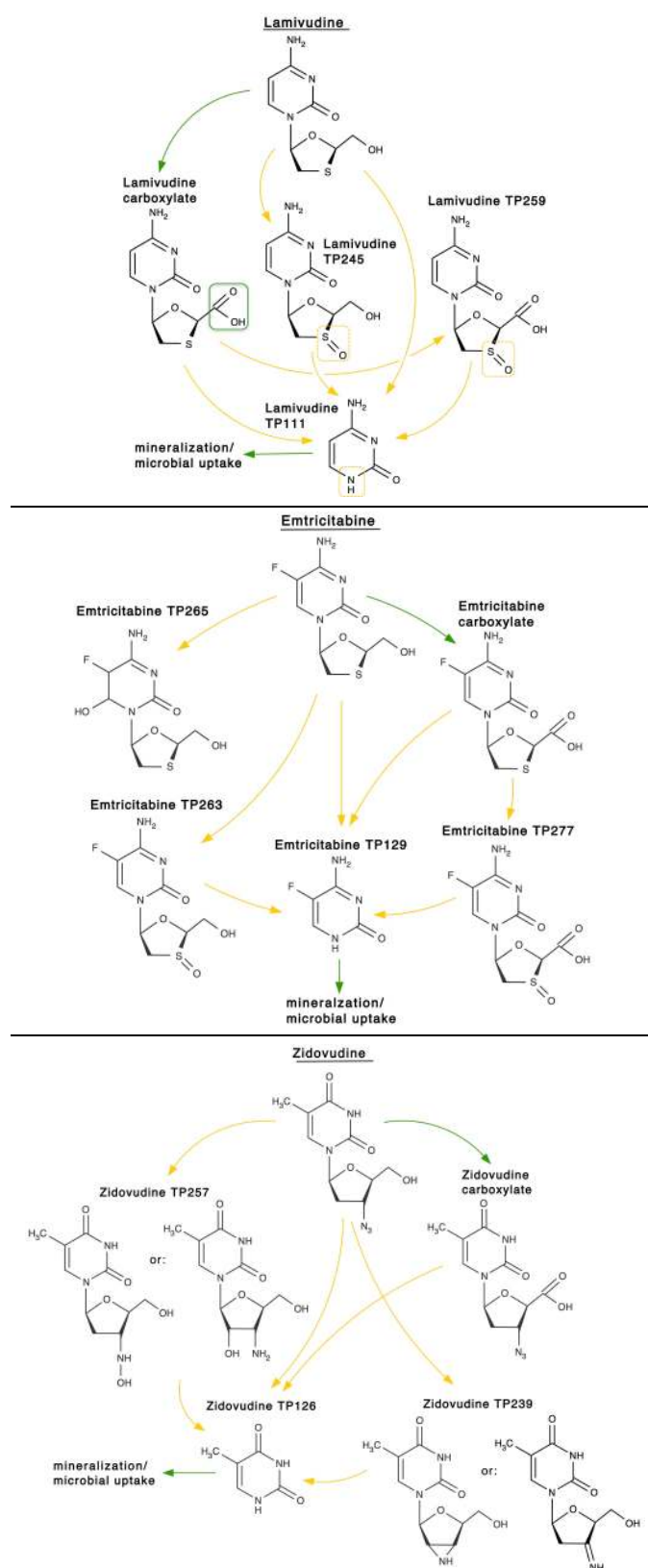


Figure 6. Proposed photo- and biodegradation pathway of lamivudine (top), emtricitabine (middle) and zidovudine (bottom) in open-water wetland cells. Orange and green

arrows indicate photo- and biotransformation reactions,  
respectively.

### *Environmental implications*

The differences between kinetics and transformation product formation in the presence and absence of the biomat highlight the complexity of transformation reactions that lead to the removal of trace organic contaminants in open water unit process wetlands and other sunlit waters. Attempts to predict the environmental fate of organic contaminants in these systems requires an understanding of both processes as well as their potential interactions. Identification of TPs showed that bio- and phototransformation reactions took place at different positions of the antiviral molecules. Phototransformation of biodegradation products was found to occur at the same location as in the parent compound. As a result, mechanisms and kinetics were similar to those observed for parent antiviral compounds. This is important because carboxylate biodegradation products are typically present in much higher concentrations in biological treated wastewater compared to parent compounds.<sup>40</sup> In contrast, biodegradation kinetics of phototransformation products of antiviral drugs differed substantially from that observed for the parent compound even though the site of enzymatic oxidation did not change. This can be explained by differences in enzyme affinities and steric hindrance. For example, phototransformation of acyclovir created a transformation product (TP257) that was not susceptible to biotransformation by microorganisms that could oxidize the parent compound in the dark. Combining kinetic studies with investigations of transformation product formation provides a better understanding of mechanisms relevant for the removal of trace organic contaminants in sunlit waters. By conducting biotransformation studies in the presence and absence of light it is possible to assess interactions between transformation processes and the likelihood that complete mineralization of trace organic contaminants will occur. These data also suggest that relative ratios of antiviral compounds and their transformation products might be useful as *in situ* probes to assess the relative importance of microbial and photochemical transformation pathways. This study highlights the need to consider the formation of different transformation products in sunlit and light-shaded systems and the possibility of using knowledge of the reactivity of specific moieties in

chemical fate assessment. Considering the variety of formed transformation products, there is a need for appropriate risk assessment tools to assess potential adverse effects of transformation products with unknown toxicities on aquatic ecosystems. Additional field studies may further confirm these laboratory microcosm results and help to assess the suitability of the approach for determining the relative importance of individual transformation processes.

### Supporting Information

Additional information on sample analysis, UV spectra of antiviral drugs, phototransformation kinetics plots, determination of indirect photolysis reaction rate constants, quantum yields, steady state concentrations of reactive intermediates in wetland water, experiments with DNA model compounds, MS<sup>n</sup> fragments of transformation products, formation and fate of abacavir photo-TPs by different reactive intermediates, results of combined bio- and phototransformation experiments with emtricitabine, lamivudine and zidovudine is available free of charge via the Internet at <http://pubs.acs.org>.

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