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# 1 Occurrence of carbamazepine, diclofenac, and their related 2 metabolites and transformation products in a French aquatic 3 environment and preliminary risk assessment

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## 8 Abstract

9 With questions emerging on the presence and risks associated with metabolites and  
10 transformation products (TPs) of organic contaminants in the aquatic environment, progress  
11 has been made in terms of monitoring and regulation of pesticide metabolites. However, less  
12 interest is shown for pharmaceutical residues, although their pseudo-persistence and adverse  
13 effects on non-target organisms are proven. This study provides original knowledge about the  
14 contamination of ten sites located along three French rivers (water, sediments, biofilms,  
15 clams) by pharmaceutical metabolites and TPs, as well as a preliminary environmental risk  
16 assessment. Studied compounds included carbamazepine with five metabolites and TPs, and  
17 diclofenac with three metabolites and TPs. Results show that metabolites and TPs are present  
18 in all studied compartments, with mean concentrations up to 0.52  $\mu\text{g L}^{-1}$  in water, 229  $\text{ng g}^{-1}$   
19 in sediments, 2153  $\text{ng g}^{-1}$  in biofilms, and 1149  $\text{ng g}^{-1}$  in clams. QSAR estimations (OECD  
20 toolbox) were involved to predict the studied compounds ecotoxicities. QSAR models showed  
21 that diclofenac and its metabolites and TPs could be more toxic than carbamazepine and its  
22 metabolites and TPs to three aquatic species representing green algae, invertebrates, and fish.  
23 However, real ecotoxicological effects are still to be determined. The environmental risk  
24 assessment showed that hydroxydiclofenac, 2-[(2-chlorophenyl)-amino]-benzaldehyde and

25 dibenzazepine could present a greater risk than other studied compounds for aquatic  
26 organisms. In addition, the risk associated with a mixture of diclofenac and its related  
27 metabolites and TPs has been found to be greater than that of the compounds considered  
28 individually.

## 29 **Keywords**

30 Pharmaceuticals, metabolites and transformation products, ecotoxicity, risk assessment, rivers

## 31 **Highlights**

- 32 • Pharmaceutical degradation products are found in several aquatic compartments.
- 33 • Levels of degradation products are comparable with those of their parent compounds.
- 34 • By-products have similar or greater risk than pharmaceuticals in water and sediment.
- 35 • Some mixtures have a greater risk than pharmaceuticals considered individually.

36

## 37 **1. Introduction**

38 Pharmaceuticals enter into rivers mainly through wastewater discharge (wastewater treatment  
39 plants – WWTPs or septic tanks) and, to a lesser extent, from livestock (Daughton & Ternes,  
40 1999; Fent *et al.*, 2006). These compounds are biologically active and are selected for their  
41 specific actions on the human or animal organism. Therefore, an exposure outside the medical  
42 or veterinary setting may lead to unwanted effects (toxic or disruptive effects) on living  
43 organisms. Many studies have already reported developmental disorders in frog tadpoles  
44 (Foster *et al.*, 2010), fish feminization (Petrovic *et al.*, 2002), changes in diversity and  
45 abundance of microbial communities (Liebig *et al.*, 2010), *etc.* related to the exposure to  
46 pharmaceutical residues (at concentrations ranging from  $\text{ng L}^{-1}$  to  $\mu\text{g L}^{-1}$ ) *via* the aquatic

47 environment. In the long term, all these effects could have major consequences on  
48 biodiversity and water use (bathing, drinking water resources) in relation to human health:  
49 e.g. exposure to endocrine disruptors or resistant pathogenic microorganisms (Amarasiri *et*  
50 *al.*, 2020; Gonsioroski *et al.*, 2020).

51 In the environment, pharmaceuticals are found not only as active molecules (i.e. parent  
52 compounds), but also as metabolites and biotic or abiotic transformation products (TPs) (Patel  
53 *et al.*, 2019). Pharmaceuticals are first transformed in the human or animal body after uptake.  
54 They are partially metabolized into more polar and soluble forms (pharmacologically active  
55 or not) by a variety of oxidative and conjugative enzymes (glucuronide conjugation,  
56 sulfoconjugation, acetylation, amino acid conjugation, glutathione conjugation and  
57 methylation) (Jančová & Šiller, 2012). These transformations facilitate their elimination  
58 through urine and faeces. Other transformation pathways occur in wastewaters during their  
59 treatment in WWTPs. Pharmaceutical compounds and their metabolites are transformed by  
60 biodegradation and physicochemical reactions (e.g. hydrolytic cleavage of amide bonds, ether  
61 cleavage, demethylation, hydroxylation...) during secondary treatment (Quintana *et al.*, 2005;  
62 Barra Caracciolo *et al.*, 2015). Once released in the environment, pharmaceutical compounds  
63 and their metabolites are further transformed by direct photodegradation initiated by sunlight,  
64 or indirect photodegradation in the presence of free radicals or singlet oxygen (generated by  
65 the action of UV rays on natural organic matter) (Andreozzi *et al.*, 2003). Chemical  
66 hydrolysis and oxydo-reduction reactions can also lead to their transformation (as a function  
67 of pH and temperature). Another main transformation route in the aquatic environment  
68 involves biodegradation by different families of microorganisms if the molecules toxicities do  
69 not inhibit microbial activity (Barra Caracciolo *et al.*, 2015).

70 While the presence of pharmaceutical compounds in natural waters has been demonstrated  
71 since the 1980s (Patel *et al.*, 2019), many studies report an accumulation of these

72 contaminants in other aquatic compartments (Kümmerer, 2004). Thus, sediments can  
73 accumulate pharmaceutical compounds or their metabolites and TPs (Li *et al.*, 2014; Tamtam  
74 *et al.*, 2011) but also favor microbial degradation processes (Kunkel & Radke, 2008). Several  
75 studies have reported significant accumulation of pharmaceuticals in benthic organisms: river  
76 biofilms (Aubertheau *et al.*, 2017; Huerta *et al.*, 2016) and invertebrates (Burket *et al.*, 2019;  
77 de Solla *et al.*, 2016; Du *et al.*, 2015; Xie *et al.*, 2019), as well as in fish (Brooks *et al.*, 2005;  
78 Huerta *et al.*, 2013; Xie *et al.*, 2019). Only aquatic plants seem not to accumulate these  
79 compounds, or only weakly (Wilkinson *et al.*, 2018). Such a widespread presence of  
80 pharmaceuticals makes it evident that metabolites and TPs of pharmaceutical residues could  
81 also be present in many of these compartments. The issue is all the more important because  
82 metabolites and TPs can exhibit toxicity to living organisms, which can be lower, similar, or  
83 even higher than the parent compounds (Bleeker *et al.*, 1999; Bort *et al.*, 1999; Bourgeois &  
84 Wad, 1984; Schulze *et al.*, 2010). However, the risks associated with these metabolites and  
85 TPs, as well as their presence in the environment, are still very little studied due to their very  
86 large number and the absence of analytical standards for their quantification.

87 Questioning relating to the presence of organic contaminants metabolites and TPs in natural  
88 waters and drinking water are emerging more and more worldwide. This is largely due to the  
89 knowledge gained on pesticide metabolites and their incidence on health. In France and other  
90 European countries, the presence of pesticide residues and their metabolites in water intended  
91 for human consumption is regulated by Council Directive 98/83/EC which sets quality limits  
92 for pesticides and their relevant metabolites of  $0.1 \mu\text{g L}^{-1}$  per individual substance and  $0.5 \mu\text{g}$   
93  $\text{L}^{-1}$  for the sum of these molecules (European Council, 1998). To date, no regulation relating  
94 to the quality of natural waters includes pharmaceutical residues. However, six molecules or  
95 families of molecules (17-alpha-ethinylestradiol, 17-beta-estradiol, estrone, macrolide  
96 antibiotics, amoxicillin, ciprofloxacin) are included on the Watch list of substances for Union-

97 wide monitoring established in 2018 by Implementing Decision (EU) 2018/840 (European  
98 Commission, 2018).

99 The issue of risk assessment is today very important because of the rise of all sorts of  
100 emerging pollutants that form an “environmental soup” of a multitude of compounds, in large  
101 majority at low concentrations. Indeed, it has been shown that organic pollutants may have  
102 unpredictable biochemical interactions when mixed together, leading to different effects of  
103 what might be observed when evaluating the toxicity of a single molecule (Filby *et al.*, 2007;  
104 Sumpter & Johnson, 2005).

105 The present work draws up a first assessment of the contamination of the aquatic environment  
106 by pharmaceutical metabolites and TPs. Thus, two “iconic” pharmaceutical compounds  
107 (carbamazepine – CBZ and diclofenac – DCF) and eight of their associated metabolites and  
108 TPs were quantified in water, sediments, biofilms, and clams (*Corbicula fluminea*) from  
109 rivers of Western France. Then, the QSAR toolbox (OECD & European Chemicals Agency,  
110 2020) was used to predict the missing toxicity data for these compounds considered  
111 individually or in mixtures. This allowed a first environmental risk assessment in French  
112 waters and sediments and then to open the discussion about the relevance of considering  
113 pharmaceutical metabolites and TPs for future guidelines and regulations.

## 114 **2. Material and methods**

### 115 **2.1. Target compounds**

116 Carbamazepine, Diclofenac, and the metabolites and TPs considered in this work are  
117 presented in Table 1. Analytical grade standards of carbamazepine (CBZ), 3-  
118 hydroxycarbamazepine (3OH-CBZ), 10,11-epoxycarbamazepine (CBZ-epox), dibenzazepine  
119 (Dibenz), acridone (Acrid), diclofenac (DCF), 4'-hydroxydiclofenac (4'OH-DCFBZ) and 5-

120 hydroxydiclofenac (5OH-DCF) were purchased from Sigma-Aldrich (Darmstadt, Germany),  
121 while standards of 10,11-dihydro-10,11-trans-dihydroxycarbamazepine (Trans-CBZ) and 2-  
122 [(2-chlorophenyl)-amino]-benzaldehyde (Benz) were purchased from Santa Cruz  
123 Biotechnology (Heidelberg, Germany) and Synchem UG & Co. KG (Altenburg, Germany),  
124 respectively. Individual stock solutions were prepared in LC-MS grade methanol (Carlo Erba  
125 Reagents, Val de Reuil, France) at 200 mg L<sup>-1</sup> and stored at -20 °C.

## 126 **2.2.Study area and sampling strategy**

127 A sampling campaign was conducted in September 2018 along three rivers (Vienne, Clain  
128 and Thouet) located in the same part of Western France (North of the Vienne and South of the  
129 Deux-Sèvres French departments) (Figure 1). Their watersheds present close rural/urban  
130 occupation, agricultural/socio-economic activities, and hydro-climatic conditions. They are  
131 typical of this part of France. The sampling took place at the end of summer, during the low-  
132 water flow period, thus facilitating accessibility to the studied compartments.

133 Ten different sampling sites were selected based on their location: upstream or downstream  
134 from urban areas (Figure 1). On each site, a surface of 20 m<sup>2</sup> was prospected to collect  
135 samples of all four compartments of interest. Thus, 2 L of water were grab sampled in a high-  
136 density polyethylene (PEHD) bottle. Biofilms were collected from ten rocks chosen randomly  
137 at a depth of 50 to 100 cm. For that, rock surface was scrapped with a clean toothbrush and  
138 ultrapure water (Milli-Q IQ 7000, Merck KGaA, Darmstadt, Germany). Bulk sample of  
139 surface sediment was collected as a composite sample, with a shovel, by pooling three  
140 samples at each location, and sieved on site to < 2 mm. Approximately 100 clams (*C.*  
141 *fluminea*) with a size > 16 mm were collected by hand. All samples were transferred to clean  
142 PEHD bottles and stored in a cool box until the end of the sampling day.

## 143 **2.3.Extraction of target compounds**

144 Target analytes were extracted from solid matrices (i.e. biofilms, sediments and clams) using  
145 pressurized liquid extraction (PLE) (ASE 350, Thermo Scientific Inc., Waltham, USA)  
146 followed by solid phase extraction (SPE) (Autotrace 150, Thermo Scientific Inc., Waltham,  
147 USA). The method developed by Aubertreau *et al.* (2017) was used for biofilms and  
148 sediments, while the method developed by Alvarez-Muñoz *et al.* (2015) was adapted for *C.*  
149 *fluminea*. Tables SI-1 and SI-2 summarize the conditions used by matrix. For each sample,  
150 two extraction cells were prepared. Each of the two obtained extracts was diluted with  
151 ultrapure water to 500 mL for biofilms and *C. fluminea*, and to 1 L for sediments, and loaded  
152 on two SPE cartridges. Then, the four obtained extracts were evaporated to dryness under a  
153 gentle stream of nitrogen at 30 °C (TurboVap LV, Biotage AB, Uppsala, Sweden). Finally,  
154 they were each recovered in methanol/water (10/90; v/v) spiked with increasing  
155 concentrations of a standards mixture (final concentrations of 0, 0.02, 0.05 and 0.1 mg L<sup>-1</sup>) for  
156 standard addition quantification.

157 Recoveries were determined by spiking each matrix with all analytes to a concentration of  
158 400 ng g<sup>-1</sup> dry weight and allowing it to dry overnight in the dark at room temperature, before  
159 performing extraction and analysis. This procedure was repeated three times. Recoveries were  
160 between 19-123 % for biofilms, 30-190 % for sediments, and 37-142 % for clams (see Table  
161 SI-3).

162 2 L of each water sample were filtered through a 0.45 µm PVDF membrane (Durapore,  
163 Merck KGaA, Darmstadt, Germany) before SPE extraction of compounds on Oasis HLB (6  
164 cc, 200 mg) cartridges (Waters Corporation, Milford, USA). Table SI-4 shows the extraction  
165 conditions. Samples were each loaded on four cartridges, and the four obtained extracts  
166 underwent evaporation and restitution with the same conditions as solid matrices.

#### 167 **2.4. Quantification of target compounds**

168 Compounds were separated using ultra high performance liquid chromatography (UHPLC)  
169 (UltiMate 3000, Thermo Scientific Inc., Waltham, USA) on an Acquity UPLC BEH C18  
170 column (2.1 x 100 mm, 1.7  $\mu\text{m}$ ; Waters Corporation, Milford, USA) kept at 30 °C. A flow  
171 rate of 0.4 mL min<sup>-1</sup> was used with a mobile phase composed of water and methanol, both  
172 acidified with 0.1 % formic acid. A gradient ranging from 10 % to 99 % of methanol in 13  
173 min was applied.

174 The UHPLC system was coupled with a hybrid quadrupole – time-of-flight (Q-ToF) mass  
175 spectrometer (Impact HD QTOF, Bruker GmbH, Champs sur Marne, France) equipped with  
176 an electrospray ion source (ESI) and operated in broadband collision-induced dissociation  
177 (bbCID) mode. The mass range was from 150 to 500 m/z, the capillary tension was set to  
178 2700 V, the dry gas flow rate was 4.0 L min<sup>-1</sup>, and the dry temperature 250 °C. Table SI-5  
179 lists the retention times and the exact ionized masses used for identifying all compounds.  
180 4'OH-DCF and 5OH-DCF were subsequently grouped together as “OH-DCF” due to poor  
181 chromatographic separation.

182 Quantification by standard addition was chosen to normalise for matrix effects. In addition,  
183 the analytical method's performance was assessed *via* linearity, limits of detection and  
184 quantification, repeatability and reproducibility, according to the French AFNOR XP T90-210  
185 standard (AFNOR, 1999) (detailed methods are presented in SI-6 and Table SI-7). All limits  
186 of detection and quantification are summarized in Table SI-8.

## 187 **2.5. Metabolites and TPs ecotoxicity**

### 188 **2.5.1. Prediction of ecotoxicity data**

189 Ecotoxicity data of pharmaceutical metabolites or transformation products are often rare or  
190 even not available at all. For this reason, the OECD Quantitative Structure Activity

191 Relationship (QSAR) Toolbox (version 4.4) was used to generate environmental toxicity  
192 endpoints. This toolbox was developed by the Laboratory of Mathematical Chemistry (OASIS  
193 LMC, Bourgas, Bulgaria) for the OECD, in collaboration with the European Chemicals  
194 Agency (ECHA). Its main objective is to use QSAR methodologies to group chemicals into  
195 categories depending on their structure and/or modes of action. This helps to fill data gaps for  
196 a given compound based on the data available for its analogues, by read-across (used for  
197 qualitative endpoints), trend analysis (used for quantitative endpoints if a high number of  
198 analogues with experimental results are identified) or QSAR models. Blázquez *et al.* (2020)  
199 determined the acute toxicity for aquatic organisms of a biocidal active substance and its  
200 metabolites to assess the suitability of available QSAR models to predict the obtained values.  
201 The advantages of the OECD QSAR Toolbox were also detailed by Schultz *et al.* (2018).

202 In this study, the trend analysis approach was used to estimate acute toxicities of individual  
203 compounds with respect to the green algae *Pseudokirchneriella subcapitata* (EC<sub>50</sub>, growth  
204 rate, 96 h), the invertebrate *Daphnia magna* (LC<sub>50</sub>, mortality, 48 h), and the fish *Pimephales*  
205 *promelas* (LC<sub>50</sub>, mortality, 96 h). This approach involves modeling the relationship between  
206 the considered toxicity endpoint and a physicochemical property (by default, the Log K<sub>OW</sub>),  
207 using the experimental data available for the compound's analogues.

208 The toxicity estimations obtained for the individual molecules were also used for the  
209 prediction of the acute toxicity of a mixture of parent molecule and its associated metabolites  
210 and TPs, respecting the molar fractions observed from the average concentrations measured in  
211 the studied waters (OECD, 2020). For the two mixtures considered (CBZ and its metabolites  
212 and TPs; DCF and its metabolites and TPs), it appears that the compounds exhibit different  
213 modes of action according to the acute aquatic toxicity classification by Verhaar (Modified),  
214 the ECOSAR (ECOSAR is the Ecological Structure Activity Relationships predictive model  
215 developed by the US-EPA) classification for aquatic toxicity, and the acute aquatic toxicity

216 modes of action classification by OASIS (OASIS is the laboratory that developed the QSAR  
217 Toolbox). Consequently, trend analysis calculations were performed considering an  
218 independent modes of action approach (OECD, 2020).

### 219 **2.5.2. Environmental risk assessment**

220 According to the ECHA guidelines (European Chemicals Agency, 2008), experimental or  
221 calculated toxicity endpoints cannot be directly used for an environmental risk assessment  
222 because natural conditions are different than laboratory test conditions. Indeed, ecosystems  
223 are more sensitive to chemical compounds than individual organisms in a laboratory  
224 environment. For this reason, these endpoints ( $LC_{50}$  or  $EC_{50}$ ) are used for the estimation of  
225 predicted no effect concentrations in water ( $PNEC_{water}$ ).  $PNEC_{water}$  are obtained by dividing  
226 the lowest ecotoxicity value by an appropriate assessment factor. The scarcer the available  
227 data, the higher is the applied assessment factor. In this study, only short-term toxicity data  
228 were available. The estimation of effects on organisms throughout their life cycle was  
229 therefore less realistic than with long-term data. Hence, a high assessment factor of 1000 was  
230 applied (European Chemicals Agency, 2008).

231 Then, the hazard quotient (HQ) was calculated by dividing the maximal environmental  
232 concentration (MEC) measured in water by the calculated  $PNEC_{water}$ . When the HQ is less  
233 than 0.1, the concerned compound does not present any risk for aquatic organisms. An HQ  
234 between 0.1 and 1 indicates a medium risk, while a value above 1 means that the studied  
235 molecule exhibits a high risk for the aquatic ecosystem (Straub, 2002).

236 For the sediment, no ecotoxicity parameter could be predicted by the QSAR Toolbox.  
237 Therefore, the equilibrium partitioning method (EPM), described in the European Chemicals  
238 Bureau technical guidance document (European Chemicals Bureau, 2003), was used to derive  
239 a  $PNEC_{sediment}$  from the  $PNEC_{water}$ . Thus,  $PNEC_{sediment} = (0.783 + 0.0217 * K_{oc}) *$

240  $PNEC_{water}$  where  $K_{OC}$  represents the organic carbon/water partition coefficient, hence  
241 providing an estimate of the ability of a compound to be adsorbed on the organic matter of the  
242 sediment. Indeed, a Log  $K_{OC}$  greater than 3 indicates potentially significant adsorption on the  
243 sediment (Tissier *et al.*, 2005). Therefore, the  $PNEC_{sediment}$  value is associated with the  
244 presence of organic matter in the sediment. The MEC is expressed in micrograms of pollutant  
245 per gram of dry sediment, without consideration of organic carbon content, which is of 1 %  
246 on average for this study's sediments. For the calculation of HQ, the corrected MEC was used  
247 to consider the part of organic carbon present in the sediment.

### 248 3. Results and discussion

#### 249 3.1. Occurrence of pharmaceutical metabolites and TPs in rivers

250 All studied metabolites and TPs and their parent compounds were found in all samples from  
251 the three rivers. This finding highlights the large occurrence of pharmaceutical metabolites  
252 and TPs in the aquatic environment. Figure 2 and Figure 3 show mean concentrations ( $\pm$   
253 standard deviation) of DCF and CBZ with their associated metabolites and TPs in waters,  
254 biofilms, sediments, and clams (N.B.: full data are presented in Table SI-9).

255 For the CBZ family, mean concentrations in water range from  $0.18 \pm 0.09 \mu\text{g L}^{-1}$  for Trans-  
256 CBZ to  $0.52 \pm 0.13 \mu\text{g L}^{-1}$  for CBZ. In biofilms, concentrations are between  $258 \pm 181 \text{ ng g}^{-1}$   
257 for Trans-CBZ and  $1735 \pm 811 \text{ ng g}^{-1}$  for Dibenz. In sediments, concentrations are between  $48$   
258  $\pm 25 \text{ ng g}^{-1}$  for 3OH-CBZ and  $128 \pm 56 \text{ ng g}^{-1}$  for Dibenz. Finally, in clams, levels range from  
259  $345 \pm 218 \text{ ng g}^{-1}$  for Trans-CBZ to  $1066 \pm 566 \text{ ng g}^{-1}$  for Acrid. These results show that mean  
260 concentrations of Dibenz are higher than those of CBZ in most matrices. Acrid is also found  
261 at levels close to those of CBZ. The other metabolites and TPs are generally less concentrated.

262 For the DCF family, the data show that metabolites and TPs are more concentrated than the  
263 parent compound. Thus, mean concentrations in waters range from  $0.19 \pm 0.27 \mu\text{g L}^{-1}$  for

264 Benz to  $0.26 \pm 0.15 \mu\text{g L}^{-1}$  for OH-DCF. In biofilms, levels vary from  $348 \pm 349 \text{ ng g}^{-1}$  for  
265 DCF to  $2153 \pm 1532 \text{ ng g}^{-1}$  for Benz. In sediments, concentrations range from  $51 \pm 55 \text{ ng g}^{-1}$   
266 for DCF to  $229 \pm 250 \text{ ng g}^{-1}$  for Benz. Finally, in clams, concentrations are between  $493 \pm$   
267  $428 \text{ ng g}^{-1}$  for DCF and  $1149 \pm 916 \text{ ng g}^{-1}$  for OH-DCF.

268 It is worth noting that CBZ and its metabolites and TPs are generally present at higher  
269 concentrations than DCF and its metabolites and TPs in water and sediments, while DCF and  
270 its metabolites and TPs are more accumulated than CBZ and its metabolites and TPs in  
271 biofilms (especially Benz) and clams.

272 As shown in Table 2, in waters, CBZ concentrations are close to the levels determined by  
273 Koba *et al.* (2018) in a Czech pond used for the tertiary treatment of wastewater effluent ( $290$   
274  $- 560 \text{ ng L}^{-1}$ ), but higher than the concentrations measured by Du *et al.* (2014) in a  
275 watercourse impacted by WWTP releases in central Texas, United States ( $370 \pm 14 \text{ ng L}^{-1}$ ).  
276 DCF levels are of the same order of magnitude as those determined by Koba *et al.* (2018) ( $22$   
277  $- 870 \text{ ng L}^{-1}$ ), or by Wilkinson *et al.* (2017) in three rivers of southern England ( $<0.96 - 253$   
278  $\text{ ng L}^{-1}$ ), but higher than those measured by Du *et al.* (2014) ( $86 \pm 55 \text{ ng L}^{-1}$ ). Then, in  
279 biofilms, the levels of CBZ are higher than those measured by Aubertreau *et al.* (2017) in the  
280 Vienne River. For example, at the downstream site of the Châtellerault WWTP, the CBZ  
281 concentration in this study is  $1095 \pm 28 \text{ ng g}^{-1}$ , while in the study by Aubertreau *et al.* (2017)  
282 it was  $583.5 \text{ ng g}^{-1}$ . Likewise for the DCF concentration, which amounts to  $64 \pm 26 \text{ ng g}^{-1}$  at  
283 the downstream site of the Châtellerault WWTP in this study, and to  $37.2 \text{ ng g}^{-1}$  in the study  
284 by Aubertreau *et al.* (2017). In addition, in sediments, Koba *et al.* (2018) determined much  
285 lower levels of CBZ ( $5.1 - 16 \text{ ng g}^{-1}$ ) and DCF ( $2.6 - 30 \text{ ng g}^{-1}$ ). Finally, CBZ was not  
286 detected in clams from Taihu Lake in China (Xie *et al.*, 2015), and DCF concentrations ( $1.41$   
287  $- 5.42 \text{ ng g}^{-1}$ ) were lower than those measured in this study ( $493 \pm 428 \text{ ng g}^{-1}$ ).

288 Metabolites and TPs are much less studied than their related parent pharmaceuticals (Table 2).  
289 Stülten *et al.* (2008) detected 4'OH-DCF and 5OH-DCF in WWTP effluents in Germany, at  
290 concentrations higher than the levels measured in this study, up to  $0.71 \mu\text{g L}^{-1}$  and  $0.45 \mu\text{g L}^{-1}$ ,  
291 respectively. In their study of a Canadian river, Miao & Metcalfe (2003) did not detect  
292 3OH-CBZ or CBZ-epox in any sample, but determined concentrations of Trans-CBZ of  $2.2 \pm$   
293  $0.3 \text{ ng L}^{-1}$ , much lower than those measured in this study. Koba *et al.* (2018) detected CBZ-  
294 epox and Trans-CBZ in a lagoon water at concentrations up to  $71 \text{ ng L}^{-1}$  (lower than in this  
295 study) and  $490 \text{ ng L}^{-1}$  (higher than in this study), respectively, and in the sediment at  
296 concentrations lower than the limit of quantification. Finally, Aubertheu *et al.* (2017)  
297 detected CBZ-epox in the Vienne River biofilms with a maximal concentration estimated at  
298  $5.3 \text{ ng g}^{-1}$  which is lower than those determined in this study.

299 The distributions observed for the different compartments can be explained by several  
300 parameters/conditions which probably interact between them. First, the contamination of  
301 biofilms, sediments, and clams depends on the compounds carried by water. However, the  
302 composition of water can change rapidly (Ort *et al.*, 2010) while that of solid matrices is  
303 constrained by sorption/desorption kinetics (Gonzalez *et al.*, 2012). Therefore, concentrations  
304 measured in water samples represent an image of the contamination over a short period of  
305 time while those determined in solid matrices are the result of accumulation over a certain  
306 exposure period. This could explain the significant difference observed in the distribution of  
307 compounds in water compared to the other matrices, especially for the DCF family for which  
308 sediments and biofilms are characterized by a larger presence of Benz in comparison with  
309 water. The differences are less significant for CBZ and its metabolites and TPs – especially  
310 between water and sediment – but some compounds like Dibenz may have significant  
311 variations in distributions.

312 Another important parameter to consider is the partitioning of molecules which is generally  
313 linked to their hydrophobicity (expressed *via* the octanol/water partition coefficient -  $K_{OW}$ ).  
314  $K_{OW}$  is frequently used to predict the adsorption of pollutants to solids and thus their  
315 bioaccumulation. Rogers (1996) provided a general rule for the application of  $K_{OW}$  to the  
316 estimation of sorption: a Log  $K_{OW}$  lower than 2.5 indicates a low sorption potential, a Log  
317  $K_{OW}$  between 2.5 and 4 indicates a medium sorption potential, and a Log  $K_{OW}$  greater than 4 a  
318 high sorption potential. Therefore, the significant presence of DCF and its metabolites and  
319 TPs in benthic organisms (biofilms, clams) is consistent with their higher Log  $K_{OW}$ . Those  
320 compounds are more hydrophobic than CBZ and its metabolites and TPs which exhibit higher  
321 levels in water and sediments. Dibenz presents an exception (Log  $K_{OW}$  = 4.06) and this is  
322 reflected in its high accumulation in biofilms.

323 Finally, the presence of metabolites and TPs in the aquatic environment is also linked to  
324 pharmaceutical compounds degradation pathways. For example, Phototransformation is the  
325 main degradation pathway for DCF in the environment (Boreen *et al.*, 2003), and it takes  
326 place rapidly ( $t_{1/2}$  =  $9.6 \pm 1.2$  h (Poirier-Larabie *et al.*, 2016)), with Benz identified as the most  
327 stable product (Eriksson *et al.*, 2010). This could explain the low proportion of DCF  
328 compared to its metabolites and TPs. Moreover, it is worth noting that Benz is more abundant  
329 in matrices exposed to sunlight (biofilms, sediments) than in clams' flesh. CBZ, on the other  
330 hand, is known to be persistent in the environment (Loos *et al.*, 2009). This compound is one  
331 of the least degraded/eliminated in wastewater treatment processes ( $32.7 \% \pm 17.9 \%$  (Luo *et*  
332 *al.*, 2014);  $< 0 - 23 \%$  (Jekel *et al.*, 2015)), but leads to the formation of Dibenz and Acrid  
333 (Kosjek *et al.*, 2009). These properties may explain the similarity between the distributions.

### 334 **3.2.Individual compounds ecotoxicities**

335 Ecotoxicity of pharmaceutical metabolites and TPs was assessed for three different aquatic  
336 trophic levels: algae (*Pseudokirchneriella subcapitata*), invertebrates (*Daphnia magna*) and

337 fish (*Pimephales promelas*) (Figure 4). Values obtained from the QSAR Toolbox highlight  
338 that DCF and its metabolites and TPs (Benz and OH-DCF) have similar toxicities towards  
339 green algae ( $EC_{50} \sim 6.01 \text{ mg L}^{-1}$ ). For Daphnia, OH-DCF ( $LC_{50} = 2.3 \text{ mg L}^{-1}$ ) is found to be  
340 about 40 times more toxic than DCF ( $LC_{50} = 80.1 \text{ mg L}^{-1}$ ), while an abnormally high  $EC_{50}$   
341 value was found for Benz ( $2.88 \cdot 10^{15} \text{ mg L}^{-1}$ ) demonstrating the actual limitations of QSAR  
342 models to predict the toxicity of all compounds for all organisms (Boxall *et al.*, 2004). Benz is  
343 4 times more toxic ( $LC_{50} = 2.71 \text{ mg L}^{-1}$ ) for *P. promelas* than its parent compound ( $LC_{50} =$   
344  $11.2 \text{ mg L}^{-1}$ ), while OH-DCF has the same toxicity than DCF ( $LC_{50} = 10.7 \text{ mg L}^{-1}$ ).

345 In the CBZ family, Dibenz stands out from all the other compounds, being the most toxic for  
346 the three studied species. The other metabolites and TPs exhibit toxicities that are slightly  
347 higher, of the same order of magnitude or lower than that of the parent compound. Hence, for  
348 green algae, 3OH-CBZ is as toxic as CBZ ( $EC_{50} \sim 19.55 \text{ mg L}^{-1}$ ), while the other compounds  
349 are 10 to 70 times less toxic. Likewise, for *P. promelas*, CBZ-epox exhibits an  $LC_{50}$  value of  
350 the same order of magnitude as the parent compound ( $\sim 44.7 \text{ mg L}^{-1}$ ), while the other  
351 metabolites and TPs are 3 to 6 times less toxic. On the other hand, for daphnia, 3OH-CBZ and  
352 CBZ-epox ( $LC_{50} \sim 36.5 \text{ mg L}^{-1}$ ) are about 3 times more toxic than CBZ, Acrid is as toxic  
353 ( $LC_{50} \sim 105.45 \text{ mg L}^{-1}$ ) and Trans-CBZ 65 times less toxic.

354 All these results are in agreement with the study of Pereira *et al.* (2020). Indeed, in their  
355 systematic review on the experimental and estimated toxicities of selected pharmaceuticals in  
356 different aquatic compartments, these authors found that DCF and its metabolite 4OH-DCF  
357 have similar toxicities to invertebrates and fish. In addition, they highlighted the higher  
358 toxicities of anti-inflammatory drugs, including DCF, as compared to antiepileptics such as  
359 CBZ. However, results differ regarding the most sensitive species. Indeed, while in our study  
360 results show that green algae is the most sensitive, followed by fish and daphnia, Pereira *et al.*  
361 (2020) observed that the most sensitive species were fish, followed by invertebrates and algae.

362 Nevertheless, their observation was explained by the fact that part of the experimental data for  
363 fish was obtained through cell line or tissue testing, making it difficult to extrapolate the  
364 values to the entire organism.

### 365 **3.3.Mixtures ecotoxicities**

366 QSAR predictions were also used to assess mixtures ecotoxicities (including parent  
367 compound and its corresponding metabolites and TPs) since field data revealed their  
368 simultaneous presence in water and benthic organisms (biofilms, clams). Figure 4 also reports  
369 the predicted toxicities of mixtures of parent compounds with their associated metabolites and  
370 TPs.

371 The results show that the toxicity values of DCF or CBZ and their metabolites and TPs  
372 mixtures are overall in the middle range of the individual values predicted for the compounds.  
373 Thus, for *Pimephales promelas*, the mixture of CBZ and metabolites and TPs ( $LC_{50} = 41.5$   
374  $mg L^{-1}$ ) is approximately 12 times less toxic than Dibenz alone ( $LC_{50} = 3.27 mg L^{-1}$ ).  
375 However, the mixture is 3 to 5 times more toxic than the other metabolites and TPs, when  
376 considered individually. According to the QSAR model, the mixture's toxicity is lower than  
377 CBZ's individual toxicity for *Pimephales promelas* ( $41.5 mg L^{-1}$  versus  $37.3 mg L^{-1}$ ) and  
378 especially for the algae *P. subcapitata* ( $37 mg L^{-1}$  versus  $10.4 mg L^{-1}$ ). On the contrary, the  
379 model predicts a higher toxicity of the mixture for daphnids. This highlights the importance of  
380 completing experimental ecotoxicological data to confirm mixture effects and hence the  
381 interest of considering metabolites and TPs along with their associated parent pharmaceutical  
382 compounds in the assessment of ecological risks. It is now known that effects resulting from  
383 the exposure to a mixture of organic contaminants can be very different from observations  
384 when evaluating the toxicity of a single compound, as it was already demonstrated for  
385 endocrine disruptors for example (Filby *et al.*, 2007; Sumpter & Johnson, 2005). Until now,  
386 few studies have been interested in this mixture effect with many pharmaceutical compounds,

387 and even less with metabolites and TPs. Cleuvers (2003) showed that a mixture of CBZ and a  
388 lipid lowering agent, clofibric acid, exhibited a higher toxicity than the single compounds at  
389 the same concentration during immobilization tests of *D. magna*. The same author (Cleuvers,  
390 2004) observed acute toxicity of a mixture of anti-inflammatories including DCF, at lower  
391 concentrations than for the individual chemicals.

392 Wang *et al.* (2020) were the first to develop a QSAR model to predict mixture ecotoxicities of  
393 fluoroquinolone antibiotics with their photodegradation products for *Escherichia coli*. Their  
394 results showed that the mixture toxicity of fluoroquinolones derivatives is a concentration  
395 addition of their individual toxicities. Qin *et al.* (2018) developed a QSAR model to predict  
396 acute mixture ecotoxicities of two antibiotics and four pesticides towards *Aliivibrio fischeri*.  
397 The 45 studied mixtures exhibited additive, synergistic, and antagonistic effects. The authors  
398 also showed that, compared to traditional concentration additive and independent action  
399 models, their QSAR model better predicted mixture toxicities.

#### 400 **3.4.Comparison with pesticide metabolites**

401 Ecotoxicity values were also predicted for a common pesticide (atrazine – ATZ) and some of  
402 its main metabolites (desethylatrazine – DEA, deisopropylatrazine – DIA,  
403 desethyldeisopropylatrazine – DEDIA, hydroxyatrazine – OH-ATZ, and aniline) to compare  
404 with CBZ, DCF and their metabolites and TPs (Figure 4). Atrazine was banned in France in  
405 2001 but is still found in waters and river sediments. Moreover, ATZ and its metabolite DEA  
406 are frequently responsible for the downgrading of surface waters or groundwater quality. The  
407 comparison with ATZ and its metabolites shows that although DCF and CBZ are less toxic to  
408 the three species of interest than this pesticide, their metabolites and TPs exhibit EC<sub>50</sub> and  
409 LC<sub>50</sub> values broadly comparable to those of ATZ metabolites.

410 As mentioned before, in France, the presence of pesticide residues and their metabolites in  
411 waters is regulated by European Directive 98/83/EC (European Council, 1998) relating to the  
412 quality of waters intended for human consumption. This text sets quality limits at  $0.1 \mu\text{g L}^{-1}$   
413 per individual substance and  $0.5 \mu\text{g L}^{-1}$  for the sum. Water quality controls are increasingly  
414 revealing situations where regulatory quality limits are exceeded for metabolites. Thus, the  
415 French Directorate General of Health approached the ANSES to define a methodology for  
416 identifying relevant metabolites (ANSES, 2019). Considering the possible effects of certain  
417 pharmaceutical metabolites and TPs, this approach should be transposed to identify the most  
418 relevant metabolites and TPs for human and/or environmental health.

### 419 **3.5.Risk assessment of target compounds in the studied rivers**

420 A methodology was developed to associate both ecotoxicity and persistence data to assess the  
421 risk related to the presence of pharmaceutical metabolites and TPs in the sampled rivers.  
422 Therefore,  $\text{PNEC}_{\text{water}}$  were calculated from the predicted ecotoxicity endpoints for each  
423 individual compound as well as for mixtures, then HQs were obtained by dividing the MEC  
424 observed in waters during this study by the  $\text{PNEC}_{\text{water}}$  (N.B.: a mixture's MEC is expressed as  
425 the parent molecule's mass and corresponds to the sum of the individual compounds MECs).

426 Persistence was evaluated with the ultimate biodegradation index obtained with the  
427 EPISUITE Biowin3 Survey Model included in the QSAR Toolbox (Environmental Protection  
428 Agency, 2012). It should be noted that in this case, only biodegradation is considered in the  
429 persistence, while other processes (hydrolysis, photolysis, *etc.*) may also transform/degrade  
430 the compounds. For mixtures, the ultimate biodegradation index was obtained by calculating  
431 the mixture's constituents' indexes weighted average. Biodegradation indexes vary between  
432 2.25 and 2.75 for the CBZ and DCF degradation compounds, which means half-life of a few  
433 weeks to a few months, with the exception of Trans-CBZ (between 2.75 and 3.25) that  
434 presents a slightly faster biodegradation than the other molecules.

435 Figure 5 provides a classification of compounds according to their associated risk (HQ) for  
436 the studied rivers and their biodegradation index. Target compounds can be divided in 4  
437 groups: (1) “compounds already worrying” regarding their persistence and their high  
438 concentrations: OH-DCF, Benz and Dibenz. These compounds have an HQ lower than 1 (~  
439 qualified as medium risk for aquatic organisms) and are therefore of concern only when  
440 compared to the other studied compounds. The same applies for the notion of persistence in  
441 water; (2) “compounds to watch out for” because of their persistence in the dissolved phase.  
442 These molecules could become of concern if their levels tend to increase: DCF, CBZ, 3OH-  
443 CBZ, CBZ-epox and Acrid. (3) “compounds to watch out for” because of how far they have  
444 exceeded the  $PNEC_{water}$ : no compound identified in this study; and (4) “compounds not of  
445 concern” including compounds with a level of contamination below  $PNEC_{water}$  ( $HQ < 0.1$ )  
446 and low persistence in dissolved phase: Trans-CBZ.

447 The same methodology was also involved to assess risk regarding the sediment compartment.  
448  $PNEC_{sediment}$  were derived from the  $PNEC_{water}$  by the equilibrium partitioning method. The  
449  $K_{OC}$  of a mixture is its constituents  $K_{OC}$  values weighted average. The data thus calculated, as  
450 well as the MEC values measured in the sediments of the three sampled rivers, were used to  
451 deduce the HQs of each compound with respect to benthic organisms. Figure 6 presents the  
452 classification of compounds according to their associated risk in the sediments of the studied  
453 rivers and their biodegradation index. Consequently, OH-DCF and Benz are classified as  
454 “compounds already worrying”; DCF, CBZ, 3OH-CBZ, CBZ-epox, and Dibenz are  
455 “compounds to watch out for – group 2”; and Trans-CBZ is considered as a “compound not of  
456 concern”. Comparison between risks in water and sediments suggests that Dibenz is of higher  
457 concern for aquatic species than for benthic organisms. The rest of the compounds present the  
458 same risk in sediment and in water. In addition, the same behaviour is observed for the

459 mixtures, in particular that of DCF and its metabolites and TPs which is of more concern than  
460 the parent compound alone.

461 The results also show the importance of considering mixtures and not only the parent  
462 compounds, as illustrated by the case of DCF. Indeed, this compound – considered  
463 individually – does not seem to represent a risk (i.e. group 2, Figures 5 and 6), while its  
464 mixture with its metabolites and TPs is already worrying for the aquatic environment because  
465 of their persistence and their high levels. However, this remark is not fully generalized since  
466 CBZ alone and its mixture with metabolites and TPs show the same level of risk. This finding  
467 suggests that it would be necessary to only identify compounds for which it is relevant.

#### 468 **4. Conclusion**

469 Unsurprisingly, pharmaceutical metabolites and TPs have been found in waters, sediments,  
470 biofilms, and clams of the studied rivers, obviously in varying concentrations, but in all  
471 samples. This widespread distribution and occurrence presumably requires special attention,  
472 at least as much as the presence of pesticide metabolites. Indeed, the use of QSAR models  
473 made it possible to address the lack of experimental ecotoxicological data and to highlight  
474 that some of CBZ or DCF metabolites and TPs could be more toxic than their parent  
475 compounds for aquatic species. Thus, OH-DCF, Benz and Dibenz could represent a risk for  
476 aquatic wildlife because of their relatively high hazard quotients and their relatively slow  
477 biodegradation. The large number of possible metabolites and TPs and the possibility of  
478 individual toxicity also raises questions about a “cocktail effect”. Indeed, compounds may  
479 have unpredictable biochemical interactions when considered in a mixture, resulting in  
480 different (cumulative or not) effects than individual molecules. As shown by QSAR  
481 predictions of mixtures, it appears that the risk for the aquatic environment is higher when one  
482 considers DCF along with its metabolites and TPs. Nevertheless, our results also show that

483 this is not generalizable for all parent compounds, e.g. CBZ. Consequently, it is urgent to  
484 identify the most relevant metabolites and TPs – as is done for pesticides – to better prioritize  
485 risk assessment.

486 For future works, it is important to complete the collected data by conducting sampling  
487 campaigns at different times of the year and not only in summer. This would help better  
488 understand the impact of seasonal variations on pharmaceutical molecules degradation  
489 pathways and the presence and distribution of their metabolites and TPs in the aquatic  
490 environment. Furthermore, it is important to acknowledge that QSAR modelling constitutes a  
491 first estimation of pharmaceutical metabolites and TPs ecotoxicities as well as mixture effects.  
492 Experimental data still need to be generated to confirm these calculations. Our findings  
493 constitute a starting point for further research aimed to determine the risks coming from the  
494 presence of pharmaceutical compounds along with their metabolites and TPs in the aquatic  
495 environment, with the purpose of potentially including them in water management policies.

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500 **References**

- 501 AFNOR. (1999). XP T90-210 Water quality – Protocol for the evaluation of an alternative method of  
502 quantitative physicochemical analysis compared to a reference method. (French)
- 503 Alvarez-Muñoz, D., Huerta, B., Fernandez-Tejedor, M., Rodríguez-Mozaz, S., & Barceló, D. (2015).  
504 Multi-residue method for the analysis of pharmaceuticals and some of their metabolites in bivalves.  
505 *Talanta*, 136, 174- 182. <https://doi.org/10.1016/j.talanta.2014.12.035>
- 506 Amarasiri, M., Sano, D., & Suzuki, S. (2020). Understanding human health risks caused by antibiotic  
507 resistant bacteria (ARB) and antibiotic resistance genes (ARG) in water environments: Current  
508 knowledge and questions to be answered. *Critical Reviews in Environmental Science and Technology*,  
509 50(19), 2016- 2059. <https://doi.org/10.1080/10643389.2019.1692611>
- 510 Andreozzi, R., Raffaele, M., & Nicklas, P. (2003). Pharmaceuticals in STP effluents and their solar  
511 photodegradation in aquatic environment. *Chemosphere*, 50(10), 1319- 1330.  
512 [https://doi.org/10.1016/S0045-6535\(02\)00769-5](https://doi.org/10.1016/S0045-6535(02)00769-5)
- 513 ANSES. (2019). Recommendation of the National Agency for Food, Environmental and Occupational  
514 Health Safety on the assessment of the relevance of pesticide metabolites in water intended for human  
515 consumption. (French)
- 516 Aubertheau, E., Stalder, T., Mondamert, L., Ploy, M.-C., Dagot, C., & Labanowski, J. (2017). Impact  
517 of wastewater treatment plant discharge on the contamination of river biofilms by pharmaceuticals and  
518 antibiotic resistance. *Science of The Total Environment*, 579, 1387- 1398.  
519 <https://doi.org/10.1016/j.scitotenv.2016.11.136>
- 520 Barra Caracciolo, A., Topp, E., & Grenni, P. (2015). Pharmaceuticals in the environment:  
521 Biodegradation and effects on natural microbial communities. A review. *Journal of Pharmaceutical  
522 and Biomedical Analysis*, 106, 25- 36. <https://doi.org/10.1016/j.jpba.2014.11.040>
- 523 Blázquez, M., Andreu-Sánchez, O., Ranero, I., Fernández-Cruz, M. L., & Benfenati, E. (2020).  
524 Comparing *in vivo* data and *in silico* predictions for acute effects assessment of biocidal active  
525 substances and metabolites for aquatic organisms. *Ecotoxicology and Environmental Safety*, 205,  
526 111291. <https://doi.org/10.1016/j.ecoenv.2020.111291>
- 527 Bleeker, E. A. J., Van Der Geest, H. G., Klamer, H. J. C., De Voogt, P., Wind, E., & Kraak, M. H. S.  
528 (1999). Toxic and Genotoxic Effects of Azaarenes: Isomers and Metabolites. *Polycyclic Aromatic  
529 Compounds*, 13(3), 191- 203. <https://doi.org/10.1080/10406639908020563>
- 530 Boreen, A. L., Arnold, W. A., & McNeill, K. (2003). Photodegradation of pharmaceuticals in the  
531 aquatic environment: A review. *Aquatic Sciences*, 65, 320- 341. <https://doi.org/10.1007/s00027-003-0672-7>
- 533 Bort, R., Ponsoda, X., Jover, R., Gómez-Lechón, M. J., & Castell, J. V. (1999). Diclofenac toxicity to  
534 hepatocytes: A role for drug metabolism in cell toxicity. *The Journal of Pharmacology and  
535 Experimental Therapeutics*, 288(1), 65- 72.
- 536 Bourgeois, B. F. D., & Wad, N. (1984). Individual and combined antiepileptic and neurotoxic activity  
537 of carbamazepine and carbamazepine-10,11-epoxide in mice. *The Journal of Pharmacology and  
538 Experimental Therapeutics*, 231(2), 411- 415.

539 Boxall, A. B. A., Sinclair, C. J., Fenner, K., Kolpin, D., & Maund, S. J. (2004). Peer Reviewed: When  
540 Synthetic Chemicals Degrade in the Environment. *Environmental Science & Technology*, 38(19),  
541 368A-375A. <https://doi.org/10.1021/es040624v>

542 Brooks, B. W., Chambliss, C. K., Stanley, J. K., Ramirez, A., Banks, K. E., Johnson, R. D., & Lewis,  
543 R. J. (2005). Determination of select antidepressants in fish from an effluent-dominated stream.  
544 *Environmental Toxicology and Chemistry*, 24(2), 464- 469. <https://doi.org/10.1897/04-081R.1>

545 Burket, S. R., White, M., Ramirez, A. J., Stanley, J. K., Banks, K. E., Waller, W. T., Chambliss C. K.,  
546 & Brooks, B. W. (2019). *Corbicula fluminea* rapidly accumulate pharmaceuticals from an effluent  
547 dependent urban stream. *Chemosphere*, 224, 873- 883.  
548 <https://doi.org/10.1016/j.chemosphere.2019.03.014>

549 Cleuvers, M. (2003). Aquatic ecotoxicity of pharmaceuticals including the assessment of combination  
550 effects. *Toxicology Letters*, 142(3), 185- 194. [https://doi.org/10.1016/S0378-4274\(03\)00068-7](https://doi.org/10.1016/S0378-4274(03)00068-7)

551 Cleuvers, M. (2004). Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen,  
552 and acetylsalicylic acid. *Ecotoxicology and Environmental Safety*, 59(3), 309- 315.  
553 [https://doi.org/10.1016/S0147-6513\(03\)00141-6](https://doi.org/10.1016/S0147-6513(03)00141-6)

554 Daughton C. G., & Ternes T. A. (1999). Pharmaceuticals and personal care products in the  
555 environment: Agents of subtle change? *Environmental Health Perspectives*, 107(suppl 6), 907- 938.  
556 <https://doi.org/10.1289/ehp.99107s6907>

557 de Solla, S. R., Gilroy, È. A. M., Klinck, J. S., King, L. E., McInnis, R., Struger, J., Backus, S. M., &  
558 Gillis, P. L. (2016). Bioaccumulation of pharmaceuticals and personal care products in the unionid  
559 mussel *Lasnigona costata* in a river receiving wastewater effluent. *Chemosphere*, 146, 486- 496.  
560 <https://doi.org/10.1016/j.chemosphere.2015.12.022>

561 Du, B., Haddad, S. P., Luek, A., Scott, W. C., Saari, G. N., Kristofco, L. A., Connors, K. A., Rash, C.,  
562 Rasmussen, J. B., Chambliss, C. K., & Brooks, B. W. (2014). Bioaccumulation and trophic dilution of  
563 human pharmaceuticals across trophic positions of an effluent-dependent wadeable stream.  
564 *Philosophical Transactions of the Royal Society B: Biological Sciences*, 369(1656),  
565 20140058- 20140058. <https://doi.org/10.1098/rstb.2014.0058>

566 Du, B., Haddad, S. P., Scott, W. C., Chambliss, C. K., & Brooks, B. W. (2015). Pharmaceutical  
567 bioaccumulation by periphyton and snails in an effluent-dependent stream during an extreme drought.  
568 *Chemosphere*, 119, 927- 934. <https://doi.org/10.1016/j.chemosphere.2014.08.044>

569 Environmental Protection Agency. (2012). Sustainable Futures / P2 Framework Manual 2012 EPA-  
570 748-B12-001 5. Estimating Physical / Chemical and Environmental Fate Properties with EPI Suite™.  
571 Retrieved on February 10, 2021 from: <https://www.epa.gov/sites/production/files/2015-05/documents/05.pdf>

573 Eriksson, J., Svanfelt, J., & Kronberg, L. (2010). A Photochemical Study of Diclofenac and Its Major  
574 Transformation Products. *Photochemistry and Photobiology*, 86(3), 528- 532.  
575 <https://doi.org/10.1111/j.1751-1097.2009.00703.x>

576 Escher, B. I., & Fenner, K. (2011). Recent Advances in Environmental Risk Assessment of  
577 Transformation Products. *Environmental Science & Technology*, 45(9), 3835- 3847.  
578 <https://doi.org/10.1021/es1030799>

579 European Chemicals Agency. (2008). Guidance on information requirements and chemical safety  
580 assessment. Chapter R.10: Characterisation of dose [concentration]-response for environment.  
581 Retrieved on February 10, 2021 from:  
582 [https://echa.europa.eu/documents/10162/13632/information\\_requirements\\_r10\\_en.pdf/bb902be7-  
583 a503-4ab7-9036-d866b8ddce69](https://echa.europa.eu/documents/10162/13632/information_requirements_r10_en.pdf/bb902be7-<br/>583 a503-4ab7-9036-d866b8ddce69)

584 European Chemicals Bureau. (2003). Technical guidance document on risk assessment in support of  
585 the commission directive 93/67/EEC on risk assessment for new notified substances Commission  
586 Regulation (EC) No 1488/94 on risk assessment for existing substances directive 98/8/EC of the  
587 European Parliament and of the council concerning the placing of biocidal products on the market.  
588 Retrieved on February 10, 2021 from: [https://op.europa.eu/en/publication-detail/-  
589 /publication/212940b8-3e55-43f8-8448-ba258d0374bb](https://op.europa.eu/en/publication-detail/-<br/>589 /publication/212940b8-3e55-43f8-8448-ba258d0374bb)

590 European Commission. Commission Implementing Decision (EU) 2018/840 of 5 June 2018  
591 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant  
592 to Directive 2008/105/EC of the European Parliament and of the Council and repealing Commission  
593 Implementing Decision (EU) 2015/495 (notified under document C(2018) 3362). , Pub. L. No.  
594 32018D0840, 141 OJ L (2018).

595 European Council. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended  
596 for human consumption., Pub. L. No. 31998L0083, OJ L 330 (1998).

597 Fent, K., Weston, A. A., & Caminada, D. (2006). Ecotoxicology of human pharmaceuticals. *Aquatic*  
598 *Toxicology*, 76(2), 122- 159. <https://doi.org/10.1016/j.aquatox.2005.09.009>

599 Filby, A. L., Neuparth, T., Thorpe, K. L., Owen, R., Galloway, T. S., & Tyler, C. R. (2007). Health  
600 Impacts of Estrogens in the Environment, Considering Complex Mixture Effects. *Environmental*  
601 *Health Perspectives*, 115(12), 1704- 1710. <https://doi.org/10.1289/ehp.10443>

602 Foster, H. R., Burton, G. A., Basu, N., & Werner, E. E. (2010). Chronic Exposure to Fluoxetine  
603 (Prozac) Causes Developmental Delays in *Rana pipiens* Larvae. *Environmental Toxicology and*  
604 *Chemistry*, 29(12), 2845- 2850. <https://doi.org/10.1002/etc.345>

605 Gonsioroski, A., Mourikes, V. E., & Flaws, J. A. (2020). Endocrine Disruptors in Water and Their  
606 Effects on the Reproductive System. *International Journal of Molecular Sciences*, 21(6).  
607 <https://doi.org/10.3390/ijms21061929>

608 Gonzalez, J.-L., Bouchoucha, M., Chiffolleau, J.-F., & Andral, B. (2012). RINBIO 2011 experiment.  
609 Monitoring of the kinetics of chemical contamination of mussels (*Mytillus galloprovincialis*).  
610 (French). Retrieved on February 10, 2021 from:  
611 <https://archimer.ifremer.fr/doc/00357/46855/46743.pdf>

612 Huerta, B., Jakimska, A., Gros, M., Rodríguez-Mozaz, S., & Barceló, D. (2013). Analysis of multi-  
613 class pharmaceuticals in fish tissues by ultra-high-performance liquid chromatography tandem mass  
614 spectrometry. *Journal of Chromatography A*, 1288, 63- 72.  
615 <https://doi.org/10.1016/j.chroma.2013.03.001>

616 Huerta, B., Rodríguez-Mozaz, S., Nannou, C., Nakis, L., Ruhí, A., Acuña, V., Sabater, S., & Barcelo,  
617 D. (2016). Determination of a broad spectrum of pharmaceuticals and endocrine disruptors in biofilm  
618 from a wastewater treatment plant-impacted river. *Science of The Total Environment*, 540, 241- 249.  
619 <https://doi.org/10.1016/j.scitotenv.2015.05.049>

620 Jančová, P., & Šiller, M. (2012). Phase II Drug Metabolism. In Topics on Drug Metabolism (J.  
621 Paxton). Retrieved on February 10, 2021 from: [https://www.intechopen.com/books/topics-on-drug-](https://www.intechopen.com/books/topics-on-drug-metabolism/phase-ii-drug-metabolism)  
622 [metabolism/phase-ii-drug-metabolism](https://www.intechopen.com/books/topics-on-drug-metabolism/phase-ii-drug-metabolism)

623 Jekel, M., Dott, W., Bergmann, A., Dünnbier, U., Gnirß, R., Haist-Gulde, B., Hamscher, G., Letzel,  
624 M., Licha, T., Lyko, S., Miehe, U., Sacher, F., Scheurer, M., Schmidt, C. K., Reemtsma, T., & Ruhl,  
625 A. S. (2015). Selection of organic process and source indicator substances for the anthropogenically  
626 influenced water cycle. *Chemosphere*, 125, 155- 167.  
627 <https://doi.org/10.1016/j.chemosphere.2014.12.025>

628 Koba, O., Grabicova, K., Cervený, D., Turek, J., Kolarova, J., Randak, T., Zlabek, V., & Grabic, R.  
629 (2018). Transport of pharmaceuticals and their metabolites between water and sediments as a further  
630 potential exposure for aquatic organisms. *Journal of Hazardous Materials*, 342, 401- 407.  
631 <https://doi.org/10.1016/j.jhazmat.2017.08.039>

632 Kosjek, T., Andersen, H. R., Kompore, B., Ledin, A., & Heath, E. (2009). Fate of Carbamazepine  
633 during Water Treatment. *Environmental Science & Technology*, 43(16), 6256- 6261.  
634 <https://doi.org/10.1021/es900070h>

635 Kümmerer, K. (2004). *Pharmaceuticals in the Environment: Sources, Fate, Effects and Risks* (Second  
636 Edition). Springer.

637 Kunkel, U., & Radke, M. (2008). Biodegradation of Acidic Pharmaceuticals in Bed Sediments: Insight  
638 from a Laboratory Experiment. *Environmental Science & Technology*, 42(19), 7273- 7279.  
639 <https://doi.org/10.1021/es801562j>

640 Li, Z., Maier, M. P., & Radke, M. (2014). Screening for pharmaceutical transformation products  
641 formed in river sediment by combining ultrahigh performance liquid chromatography/high resolution  
642 mass spectrometry with a rapid data-processing method. *Analytica Chimica Acta*, 810, 61- 70.  
643 <https://doi.org/10.1016/j.aca.2013.12.012>

644 Liebig, M., Fernandez, Á. A., Blübaum- Gronau, E., Boxall, A., Brinke, M., Carbonell, G., Egeler, P.,  
645 Fenner, K., Fernandez, C., Fink, G., Garric, J., Halling- Sørensen, B., Knacker, T., Krogh, K. A.,  
646 Küster, A., Löffler, D., Cots, M. A. P., Pope, L., Prasse, C., Römbke, J., Rönnefahrt, I., Schneider, M.  
647 K., Schweitzer, N., Tarazona, J. V., Ternes, T. A., Traunspurger, W., Wehrhan, A., & Duis, K. (2010).  
648 Environmental risk assessment of ivermectin: A case study. *Integrated Environmental Assessment and*  
649 *Management*, 6(S1), 567- 587. <https://doi.org/10.1002/ieam.96>

650 Loos, R., Gawlik, B. M., Locoro, G., Rimaviciute, E., Contini, S., & Bidoglio, G. (2009). EU-wide  
651 survey of polar organic persistent pollutants in European river waters. *Environmental Pollution*,  
652 157(2), 561- 568. <https://doi.org/10.1016/j.envpol.2008.09.020>

653 Luo, Y., Guo, W., Ngo, H. H., Nghiem, L. D., Hai, F. I., Zhang, J., Liang, S., & Wang, X. C. (2014).  
654 A review on the occurrence of micropollutants in the aquatic environment and their fate and removal  
655 during wastewater treatment. *Science of The Total Environment*, 473-474, 619- 641.  
656 <https://doi.org/10.1016/j.scitotenv.2013.12.065>

657 Margot, J., Magnet, A., Thonney, D., Chèvre, N., Alencastro, D., Felipe, L., & Rossi, L. (2011).  
658 Treatment of micropollutants in wastewater – Final report on the pilot tests at the Vidy WWTP  
659 (Lausanne). (French). Retrieved on February 10, 2021 from:  
660 <https://infoscience.epfl.ch/record/163236?ln=en>

661 Martin Ruel, S., Esperanza, M., Choubert, J.-M., Valor, I., Budzinski, H., & Coquery, M. (2010). On-  
662 site evaluation of the efficiency of conventional and advanced secondary processes for the removal of  
663 60 organic micropollutants. *Water Science and Technology*, 62(12), 2970- 2978.  
664 <https://doi.org/10.2166/wst.2010.989>

665 Mazellier, P., Fuster, L., Budzinski, H., Garric, J., Couteau, J., & Aït-Aïssa, S. (2018). Seine-Aval  
666 project 5 CRAPPSE "Contamination and Reactivity of Pesticides and Pharmaceuticals in the estuary  
667 of Seine". (French). Retrieved on February 10, 2021 from: [https://www.seine-aval.fr/wp-  
668 content/uploads/2017/02/RR-SA5-CRAPPSE.pdf](https://www.seine-aval.fr/wp-content/uploads/2017/02/RR-SA5-CRAPPSE.pdf)

669 Miao, X.-S., & Metcalfe, C. D. (2003). Determination of Carbamazepine and Its Metabolites in  
670 Aqueous Samples Using Liquid Chromatography–Electrospray Tandem Mass Spectrometry.  
671 *Analytical Chemistry*, 75(15), 3731- 3738. <https://doi.org/10.1021/ac030082k>

672 OECD. (2020). OECD (Q)SAR Toolbox v.4.4.1. Example for predicting Skin Sensitization of a  
673 mixture with known components. Retrieved on February 10, 2021 from:  
674 [https://qsartoolbox.org/file/2020/04/Tutorial\\_10\\_Skin-Sensitization-of-mixture.pdf](https://qsartoolbox.org/file/2020/04/Tutorial_10_Skin-Sensitization-of-mixture.pdf)

675 OECD, & European Chemicals Agency. (2020). QSAR Toolbox. Retrieved on February 10, 2021  
676 from: <https://qsartoolbox.org/>

677 Ort, C., Lawrence, M. G., Reungoat, J., & Mueller, J. F. (2010). Sampling for PPCPs in Wastewater  
678 Systems: Comparison of Different Sampling Modes and Optimization Strategies. *Environmental  
679 Science & Technology*, 44(16), 6289- 6296. <https://doi.org/10.1021/es100778d>

680 Patel, M., Kumar, R., Kishor, K., Mlsna, T., Pittman, C. U., & Mohan, D. (2019). Pharmaceuticals of  
681 Emerging Concern in Aquatic Systems: Chemistry, Occurrence, Effects, and Removal Methods.  
682 *Chemical Reviews*, 119(6), 3510- 3673. <https://doi.org/10.1021/acs.chemrev.8b00299>

683 Pereira, A., Silva, L., Laranjeiro, C., Lino, C., & Pena, A. (2020). Selected Pharmaceuticals in  
684 Different Aquatic Compartments: Part II—Toxicity and Environmental Risk Assessment. *Molecules*,  
685 25(8), 1796. <https://doi.org/10.3390/molecules25081796>

686 Petrovic, M., Solé, M., Alda, M. J. L. D., & Barceló, D. (2002). Endocrine disruptors in sewage  
687 treatment plants, receiving river waters, and sediments: Integration of chemical analysis and biological  
688 effects on feral carp. *Environmental Toxicology and Chemistry*, 21(10), 2146- 2156.  
689 <https://doi.org/10.1002/etc.5620211018>

690 Poirier-Larabie, S., Segura, P. A., & Gagnon, C. (2016). Degradation of the pharmaceuticals  
691 diclofenac and sulfamethoxazole and their transformation products under controlled environmental  
692 conditions. *Science of The Total Environment*, 557- 558, 257- 267.  
693 <https://doi.org/10.1016/j.scitotenv.2016.03.057>

694 Qin, L.-T., Chen, Y.-H., Zhang, X., Mo, L.-Y., Zeng, H.-H., & Liang, Y.-P. (2018). QSAR prediction  
695 of additive and non-additive mixture toxicities of antibiotics and pesticide. *Chemosphere*, 198,  
696 122- 129. <https://doi.org/10.1016/j.chemosphere.2018.01.142>

697 Quintana, J. B., Weiss, S., & Reemtsma, T. (2005). Pathways and metabolites of microbial  
698 degradation of selected acidic pharmaceutical and their occurrence in municipal wastewater treated by  
699 a membrane bioreactor. *Water Research*, 39(12), 2654- 2664.  
700 <https://doi.org/10.1016/j.watres.2005.04.068>

701 Rogers, H. R. (1996). Sources, behaviour and fate of organic contaminants during sewage treatment  
702 and in sewage sludges. *Science of The Total Environment*, 185(1), 3- 26.  
703 [https://doi.org/10.1016/0048-9697\(96\)05039-5](https://doi.org/10.1016/0048-9697(96)05039-5)

704 Schultz, T. W., Diderich, R., Kuseva, C. D., & Mekenyan, O. G. (2018). The OECD QSAR Toolbox  
705 Starts Its Second Decade. In *Computational Toxicology: Methods and Protocols* (O. Nicolotti) (p.  
706 55- 77). [https://doi.org/10.1007/978-1-4939-7899-1\\_2](https://doi.org/10.1007/978-1-4939-7899-1_2)

707 Schulze, T., Weiss, S., Schymanski, E., von der Ohe, P. C., Schmitt-Jansen, M., Altenburger, R.,  
708 Streck, G., & Brack, W. (2010). Identification of a phytotoxic photo-transformation product of  
709 diclofenac using effect-directed analysis. *Environmental Pollution*, 158(5), 1461- 1466.  
710 <https://doi.org/10.1016/j.envpol.2009.12.032>

711 Straub, J. O. (2002). Environmental risk assessment for new human pharmaceuticals in the European  
712 Union according to the draft guideline/discussion paper of January 2001. *Toxicology Letters*, 131(1),  
713 137- 143. [https://doi.org/10.1016/S0378-4274\(02\)00049-8](https://doi.org/10.1016/S0378-4274(02)00049-8)

714 Stülten, D., Zühlke, S., Lamshöft, M., & Spittler, M. (2008). Occurrence of diclofenac and selected  
715 metabolites in sewage effluents. *Science of The Total Environment*, 405(1), 310- 316.  
716 <https://doi.org/10.1016/j.scitotenv.2008.05.036>

717 Sumpter, J. P., & Johnson, A. C. (2005). Lessons from Endocrine Disruption and Their Application to  
718 Other Issues Concerning Trace Organics in the Aquatic Environment. *Environmental Science &  
719 Technology*, 39(12), 4321- 4332. <https://doi.org/10.1021/es048504a>

720 Tamtam, F., Le Bot, B., Dinh, T., Mompelat, S., Eurin, J., Chevreuil, M., Bonté, P., Mouchel, J.-M., &  
721 Ayrault, S. (2011). A 50-year record of quinolone and sulphonamide antimicrobial agents in Seine  
722 River sediments. *Journal of Soils and Sediments*, 11(5), 852- 859. [https://doi.org/10.1007/s11368-  
723 011-0364-1](https://doi.org/10.1007/s11368-011-0364-1)

724 Tissier, C., Morvan, C., Bocquené, G., Gossel, H., James, A., & Marchand, M. (2005). Priority  
725 substances of the Water Framework Directive (WFD). Summary sheets. (French). Retrieved on  
726 February 10, 2021 from:  
727 [https://www.ifremer.fr/dce/content/download/35329/file/SubstancesDCE\\_ARC\\_2005.pdf](https://www.ifremer.fr/dce/content/download/35329/file/SubstancesDCE_ARC_2005.pdf)

728 Wang, D., Ning, Q., Dong, J., Brooks, B. W., & You, J. (2020). Predicting mixture toxicity and  
729 antibiotic resistance of fluoroquinolones and their photodegradation products in *Escherichia coli*.  
730 *Environmental Pollution*, 262, 114275. <https://doi.org/10.1016/j.envpol.2020.114275>

731 Wilkinson, J. L., Hooda, P. S., Swinden, J., Barker, J., & Barton, S. (2017). Spatial distribution of  
732 organic contaminants in three rivers of Southern England bound to suspended particulate material and  
733 dissolved in water. *Science of The Total Environment*, 593- 594, 487- 497.  
734 <https://doi.org/10.1016/j.scitotenv.2017.03.167>

735 Wilkinson, J. L., Hooda, P. S., Swinden, J., Barker, J., & Barton, S. (2018). Spatial (bio)accumulation  
736 of pharmaceuticals, illicit drugs, plasticisers, perfluorinated compounds and metabolites in river  
737 sediment, aquatic plants and benthic organisms. *Environmental Pollution*, 234, 864- 875.  
738 <https://doi.org/10.1016/j.envpol.2017.11.090>

739 Xie, H., Hao, H., Xu, N., Liang, X., Gao, D., Xu, Y., Gao, Y., Tao, H., & Wong, M. (2019).  
740 Pharmaceuticals and personal care products in water, sediments, aquatic organisms, and fish feeds in  
741 the Pearl River Delta: Occurrence, distribution, potential sources, and health risk assessment. *Science  
742 of The Total Environment*, 659, 230- 239. <https://doi.org/10.1016/j.scitotenv.2018.12.222>

743 Xie, Z., Lu, G., Liu, J., Yan, Z., Ma, B., Zhang, Z., & Chen, W. (2015). Occurrence, bioaccumulation,  
744 and trophic magnification of pharmaceutically active compounds in Taihu Lake, China. *Chemosphere*,  
745 138, 140- 147. <https://doi.org/10.1016/j.chemosphere.2015.05.086>

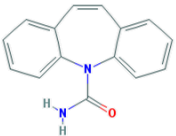
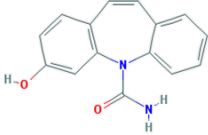
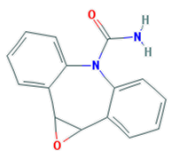
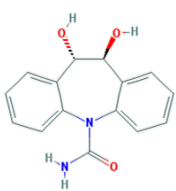
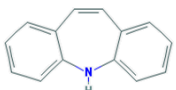
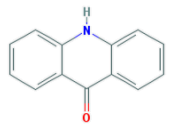
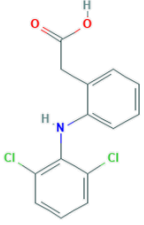
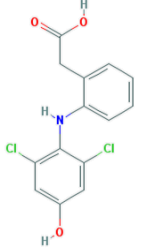
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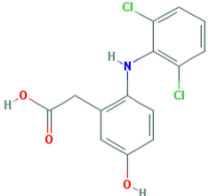
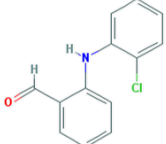
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750 **Table 1: Properties of selected compounds**

Compound	Class	CAS number	pKa*	Log K <sub>ow</sub> *	Chemical structure
<b>Carbamazepine (CBZ)</b>	Parent compound	298-46-4	16.00	2.45	
3-Hydroxycarbamazepine (3OH-CBZ)	Metabolite	68011-67-6	9.10	1.42	
10,11-Epoxycarbamazepine (CBZ-epox)	Metabolite	36507-30-9	16.00	0.95	
10,11-dihydro-10,11-trans-dihydroxycarbamazepine (Trans-CBZ)	Metabolite	58955-93-4	12.20	-0.21	
Dibenzazepine (Dibenz)	Metabolite + WWTP degradation product	256-96-2	19.50	4.06	
Acridone (Acrid)	Metabolite + WWTP degradation product	578-95-0	0.32	1.69	
<b>Diclofenac (DCF)</b>	Parent compound	15307-86-5	4.05	4.51	
4'-Hydroxydiclofenac (4'OH-DCF)	Metabolite	64118-84-9	3.76	3.70	

5-Hydroxydiclofenac (5OH-DCF)	Metabolite	69002-84-2	3.81	3.18	
2-[(2-chlorophenyl)-amino]- benzaldehyde (Benz)	Photodegradation product	71758-44-6	8.18	3.65	

751 \*Values predicted by the QSAR Toolbox (version 4.4)

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754 **Table 2: Mean concentrations and comparison with literature.**

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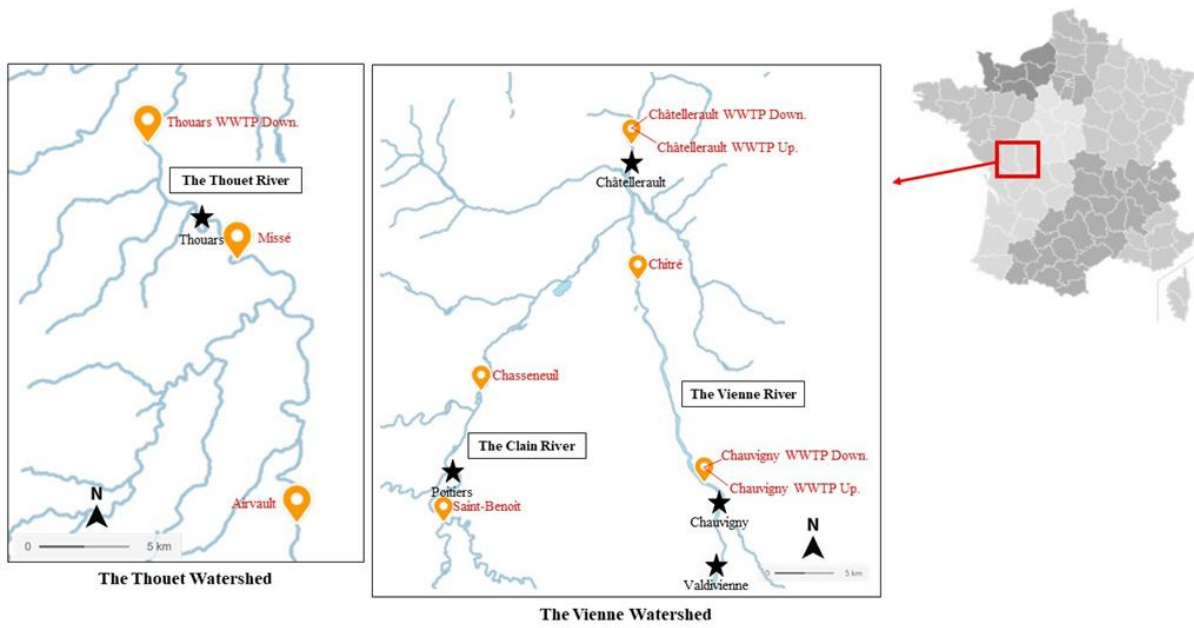
	Compound	Measured concentration	Literature (ng L <sup>-1</sup> )
<b>Water</b> (µg L <sup>-1</sup> )	CBZ	0.52 ± 0.13	290 – 560 ng L <sup>-1</sup> [tertiary treatment pond] (Koba <i>et al.</i> , 2018) 370 ± 14 ng L <sup>-1</sup> [stream impacted by WWTP] (Du <i>et al.</i> , 2014)
	DCF	0.20 ± 0.14	22 – 870 ng L <sup>-1</sup> [tertiary treatment pond] (Koba <i>et al.</i> , 2018) <0.96 – 253 ng L <sup>-1</sup> [surface water] (Wilkinson <i>et al.</i> , 2017) 86 ± 55 ng L <sup>-1</sup> [stream impacted by WWTP] (Du <i>et al.</i> , 2014)
	4'OH-DCF		710 ng L <sup>-1</sup> [WWTP effluents] (Stülten <i>et al.</i> , 2008)
	5OH-DCF	0.26 ± 0.15	450 ng L <sup>-1</sup> [WWTP effluents] (Stülten <i>et al.</i> , 2008)
	3OH-CBZ	0.23 ± 0.08	n.d. [surface water] (Miao & Metcalfe, 2003)
	CBZ-epox	0.30 ± 0.07	n.d. [surface water] (Miao & Metcalfe, 2003) 71 ng L <sup>-1</sup> [tertiary treatment pond] (Koba <i>et al.</i> , 2018)
	Trans-CBZ	0.18 ± 0.09	2.2 ± 0.3 ng L <sup>-1</sup> [surface water] (Miao & Metcalfe, 2003) 490 ng L <sup>-1</sup> [tertiary treatment pond] (Koba <i>et al.</i> , 2018)
<b>Biofilm</b> (ng g <sup>-1</sup> )	CBZ	722 ± 219	583.5 [Châtellerault WWTP downstream] (Aubertheau <i>et al.</i> , 2017)
	DCF	348 ± 349	37.2 [Châtellerault WWTP downstream] (Aubertheau <i>et al.</i> , 2017)
	CBZ-epox	329 ± 133	5.3 [Vienne River] (Aubertheau <i>et al.</i> , 2017)
<b>Sediment</b> (ng g <sup>-1</sup> )	CBZ	119 ± 54	5.1 – 16 [tertiary treatment pond] (Koba <i>et al.</i> , 2018)
	DCF	51 ± 55	

	CBZ-epox	100 ± 47	2.6 – 30 [tertiary treatment pond] (Koba <i>et al.</i> , 2018)
	Trans-CBZ	57 ± 39	<LoQ [tertiary treatment pond] (Koba <i>et al.</i> , 2018)
<b>Clams (ng g<sup>-1</sup>)</b>	CBZ	962 ± 381	<LoD [lake] (Xie <i>et al.</i> , 2015)
	DCF	493 ± 428	1.41 – 5.42 [lake] (Xie <i>et al.</i> , 2015)

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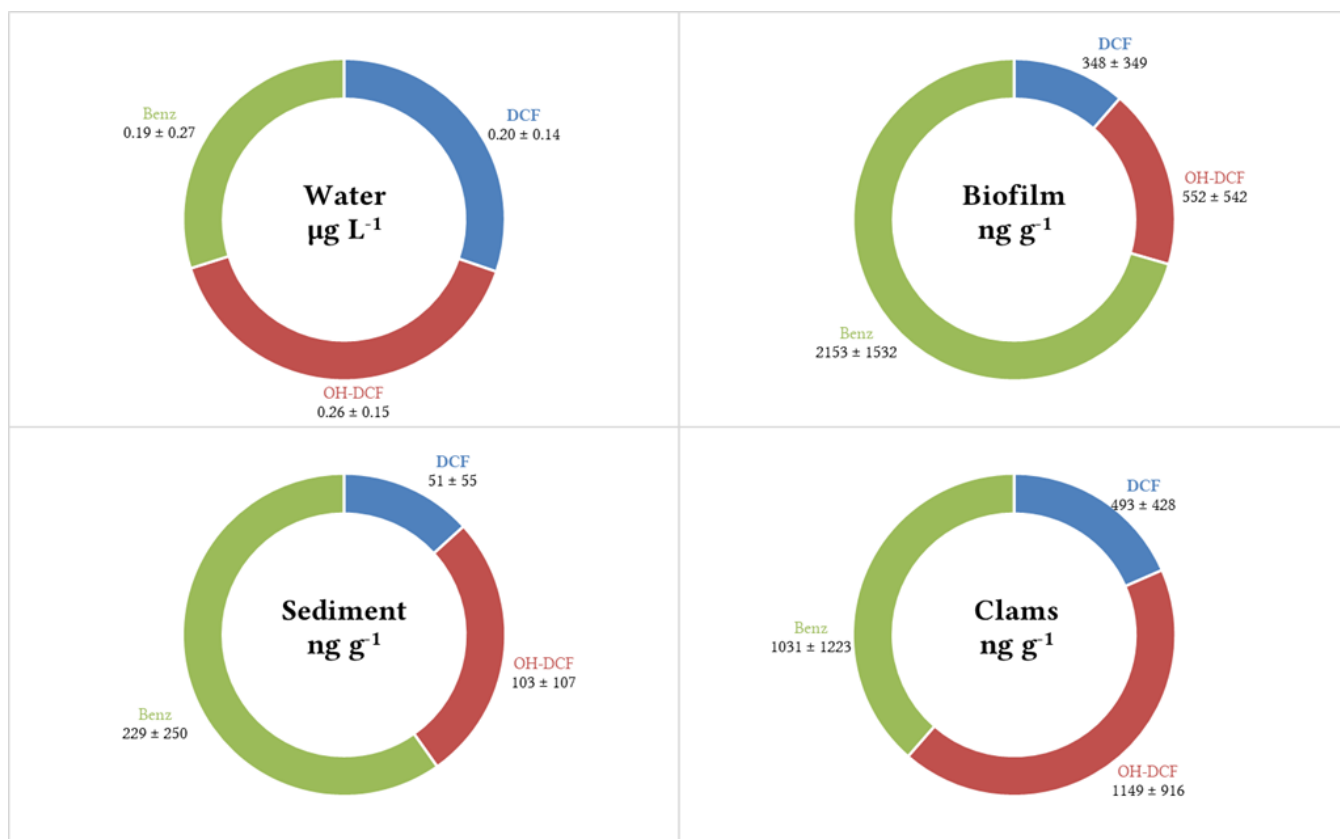
758 **Figure 1: Sampling sites; Up. = upstream, Down. = downstream; Black stars indicate**  
759 **important cities located along the watersheds; Yellow marks indicate the locations of**  
760 **sampling sites.**



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763 **Figure 2: Mean levels ( $\pm$  standard deviation) of DCF and its associated metabolites and**  
 764 **TPs in the compartments of interest, along the three studied rivers.**

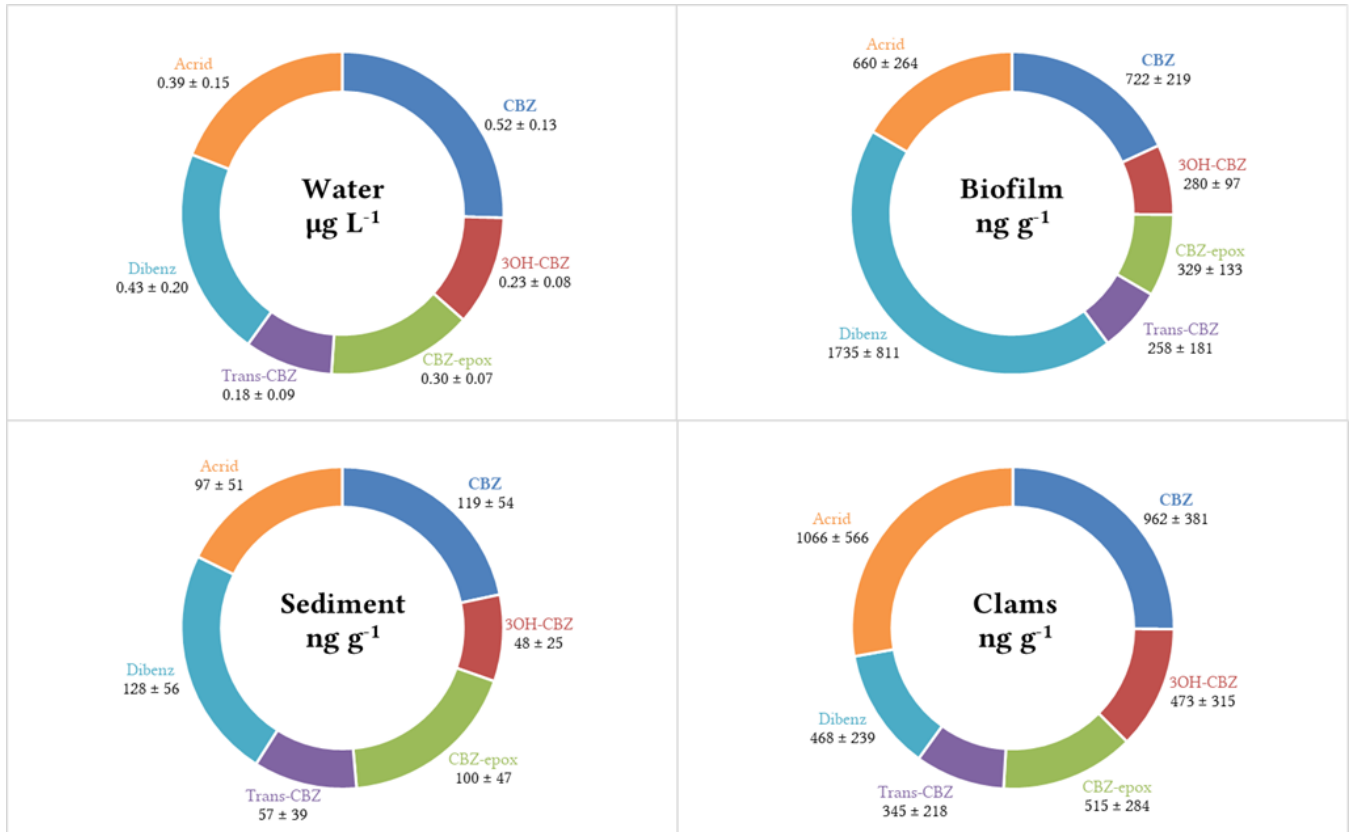


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768 **Figure 3: Mean levels of CBZ ( $\pm$  standard deviation) and its associated metabolites and**  
 769 **TPs in the compartments of interest, along the three studied rivers.**



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773 **Figure 4: Ecotoxicity of targeted compounds and mixtures with respect to**  
 774 ***Pseudokirchneriella subcapitata*, *Daphnia magna* and *Pimephales promelas*. The values**  
 775 **were calculated using the QSAR Toolbox trend analysis approach. The lowest toxicity**  
 776 **endpoints are highlighted in red, and the highest in green, indicating the most and the**  
 777 **least toxic product from each studied family towards each species. Ecotoxicities of**  
 778 **individual compounds were used to estimate mixture ecotoxicities by trend analysis,**  
 779 **considering independent modes of actions and molar fractions observed from the**  
 780 **average concentrations measured in the studied waters.**

	EC <sub>50</sub> (mg L <sup>-1</sup> ) Growth rate t = 96 h
DCF	6
OH-DCF	3.42
Benz	8.62
Mixture	5.63



*Pseudokirchneriella subcapitata*

CBZ	10.4
3OH-CBZ	28.7
CBZ-epox	107
Trans-CBZ	707
Dibenz	5.29
Acrid	278
Mixture	37

	EC <sub>50</sub> (mg L <sup>-1</sup> ) Growth inhibition t = 72 h
ATZ	0.14
DEA	1.96
DIA	1.7
DEDIA	22.8
OH-ATZ	0.26
Aniline	17

	LC <sub>50</sub> (mg L <sup>-1</sup> ) Mortality t = 48 h
DCF	80.1
OH-DCF	2.3
Benz	2.88E+15
Mixture	1710000



*Daphnia magna*

CBZ	111
3OH-CBZ	35.4
CBZ-epox	37.6
Trans-CBZ	7110
Dibenz	1.58
Acrid	99.9
Mixture	40.8

	LC <sub>50</sub> (mg L <sup>-1</sup> ) Mortality t = 48 h
ATZ	6.9
DEA	97.3
DIA	152
DEDIA	464
OH-ATZ	3.41
Aniline	0.08

	LC <sub>50</sub> (mg L <sup>-1</sup> ) Mortality t = 96 h
DCF	11.2
OH-DCF	10.7
Benz	2.71
Mixture	6.67



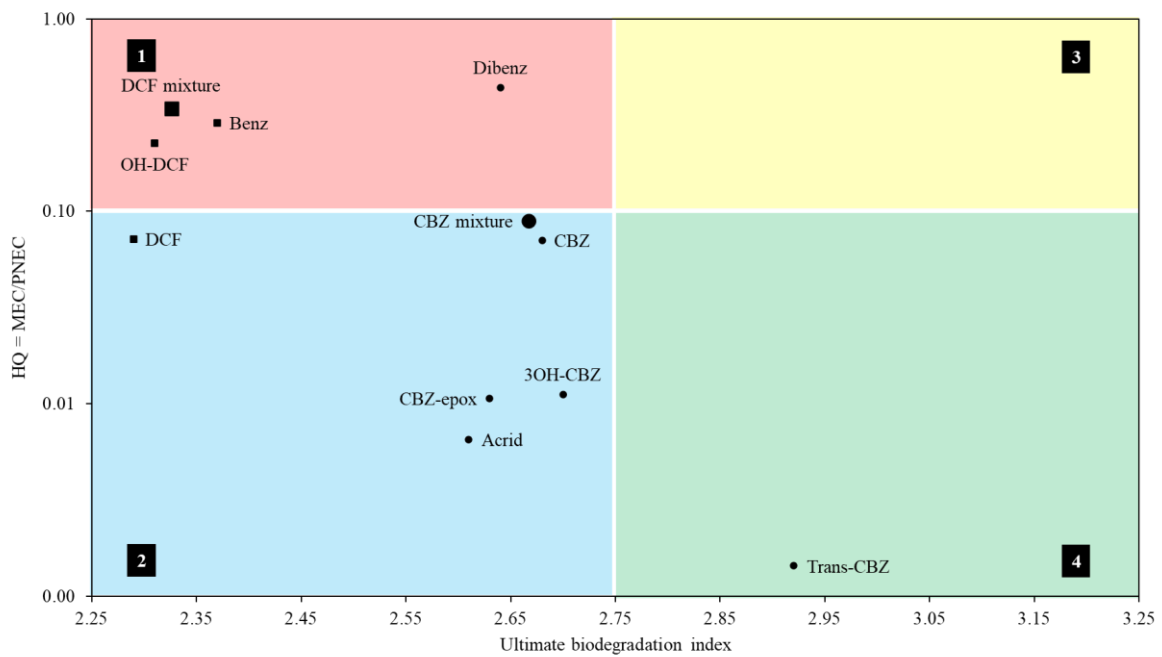
*Pimephales promelas*

CBZ	37.3
3OH-CBZ	120
CBZ-epox	52.1
Trans-CBZ	206
Dibenz	3.27
Acrid	233
Mixture	41.5

	LC <sub>50</sub> (mg L <sup>-1</sup> ) Mortality t = 96 h
ATZ	4.1
DEA	15.6
DIA	28
DEDIA	120
OH-ATZ	19.5
Aniline	32

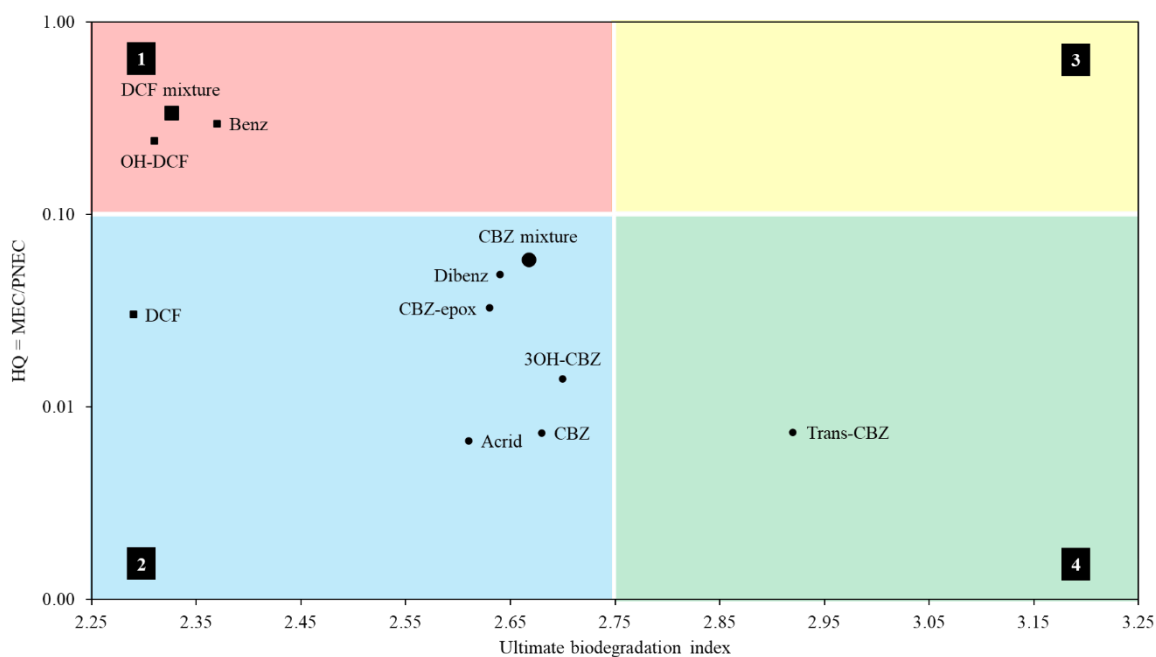
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782 **Figure 5: Highlighting problematic compounds in the waters of the Vienne, the Clain**  
783 **and the Thouet Rivers according to the associated risk (HQ) but also their level of**  
784 **persistence (ultimate biodegradation half-life) (inspired by Mazellier *et al.* (2018))**



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787 **Figure 6: Highlighting problematic compounds in the sediments of the Vienne, the Clain**  
 788 **and the Thouet Rivers according to the associated risk (HQ) but also their level of**  
 789 **persistence (ultimate biodegradation half-life)**



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