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Exposure Assessment of Pharmaceuticals and Their Metabolites in the Aquatic Environment: Application to the French Situation and Preliminary Prioritization

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1 **Exposure Assessment of Pharmaceuticals and Their Metabolites in the**
2 **Aquatic Environment: Application to the French Situation and Preliminary**
3 **Prioritization**

4 Besse, J.P., Kausch Barreto, C., Garric, J.

5
6 **ABSTRACT**

7
8 Low levels of pharmaceuticals have been detected in many countries in surface waters. As
9 a wide range of pharmaceuticals can reach aquatic environments, a selection of molecules to
10 survey is the first step before implementing a monitoring program. We used a simple equation to
11 calculate Predicted Environmental Concentrations (PECs), adapted from the European Medicine
12 Agency model used for the Environmental Risk Assessment (ERA) of human pharmaceutical.
13 Excretion fractions for pharmaceuticals were determined for 76 compounds. Using year 2004
14 French drug consumption data, we determined aquatic PECs for 112 parent molecules and
15 several metabolites. Considering excretion fractions of pharmaceuticals can lead to drastically
16 reduce predicted concentrations reaching the aquatic environment and help to target
17 environmentally relevant pharmaceuticals and metabolites. Calculated PECs using the described
18 methodology are consistent with French field measurements. The simple model for calculating
19 PECs can be used as a valuable estimation of the exposure. Risk quotient ratios were also
20 calculated. Due to the lack of ecotoxicological data, the use of PEC/PNEC ratios is not enough
21 informative to prioritize pharmaceuticals likely to pose a risk for surface waters. Alternative ways
22 to prioritize risk to pharmaceuticals, combining PEC, pharmacological, and ecotoxicological data
23 available from the literature, should be implemented.

24
25 **INTRODUCTION**

26
27 It is now recognized that pharmaceutical compounds reach the environment and can be
28 considered as environmental contaminants. A wide range of drugs including antibiotics,
29 antidepressants, non steroidal anti-inflammatories (NSAIDs), blood lipid-lowering agents, anti-
30 epileptics, and β -blockers have been found in wastewater effluents and surface waters of several
31 countries (Halling-Sorensen *et al.* 1998; Ternes 1998; Kümmerer 2000; Kolpin *et al.* 2002).
32 These observations have contributed to a growing interest in targeting and quantifying these
33 substances in terrestrial and aquatic environments. In France, there is a concern for monitoring
34 pharmaceuticals in freshwaters as this country shows the highest consumption of pharmaceutical
35 drugs in Europe (DREES 2006). This concern is addressed in the framework of the French Plan
36 National Santé Environnement (PNSE 2004) and in Europe, in the context of preventing
37 deterioration and protecting and enhancing the status of aquatic ecosystems, within the European
38 Water Framework Directive. River basin authorities therefore need to establish a list of priority
39 pharmaceuticals prior to implementing a comprehensive survey in surface waters. In this aim, we
40 developed a prioritization approach to identify human pharmaceuticals to be monitored in French
41 surface waters. As a first step in this prioritization strategy, we used a simple equation to
42 calculate Predicted Environmental Concentrations (PECs) adapted from the model proposed by
43 the European Medicine Agency (EMA) guideline (EMA 2006). This equation takes into
44 account three main parameters: the amount of active ingredients consumed by the population
45 over a year, the removal fraction in wastewater treatment plants (WWTPs), and the excretion
46 fraction of the active molecule. Pharmaceuticals enter surface waters mainly from WWTPs

47 (Kümmerer 2000, 2001; Bound and Voulvoulis 2004). The majority of human pharmaceuticals
 48 probably reach surface waters after being excreted from the body, either as parent compounds or
 49 metabolites. Consequently, we reviewed metabolism data in order to estimate values of the
 50 excreted fraction of pharmaceuticals. We also targeted metabolites that present significant
 51 pharmacological activity and can be found in the environment in non-negligible concentrations.
 52 Our study focused on the most widely used human pharmaceuticals in France. We excluded
 53 steroid estrogens from this work, as the risk of endocrine disruption has been previously
 54 discussed (Langston *et al.* 2005; Mills and Chichester 2005; Fent *et al.* 2006). Cytotoxic
 55 compounds were also excluded because these drugs have specific toxic properties (mutagenesis
 56 and carcinogenicity), and need to be assessed in a specific prioritization approach. PECs for
 57 parent compounds and metabolites were calculated using the methodology presented here and
 58 were compared with field measurements. Next, the reliability of the applied methodology was
 59 discussed. Finally, we determined risk quotient ratios, according to the EMEA guideline. Results
 60 and use of the PEC/PNEC (Predicted No Effect Concentration) risk quotient for prioritizing and
 61 ranking pharmaceuticals are discussed.

62

63 **I. MATERIALS AND METHODS**

64

65 **I.1. Model Used for Calculating PECs**

66

67 The EMEA guideline (EMEA 2006) proposes an approach to estimate PEC values for
 68 pharmaceuticals in surface water. PECs are calculated by using the following general equation:
 69

70

$$PEC_{surface\ water} = \frac{ELOCAL_{wat} \times F_{stp}}{WW_{inhab} \times CAP_{stp} \times Factor \times Dilution} \quad (1)$$

71

with: $ELOCAL_{wat} = DOSE_{ai} \times F_{excreta} \times F_{pen} \times CAP_{stp}$ (2)

72

and $F_{pen} = \frac{consumption \times 100}{DDD \times hab \times 365}$ (3)

73 where PEC is expressed in mg·l⁻¹ using the following parameters: *consumption* is the quantity
 74 (mg·year⁻¹) of an active molecule consumed by the population during 1 year in a defined zone
 75 (generally a country); *hab* is the number of inhabitants and 100 the correction factor for the
 76 percentages; 365 is the number of days per year (day·year⁻¹); *DOSE_{ai}*: maximum recommended
 77 daily dose of an active molecule consumed per inhabitant (mg. inhabitants⁻¹ day⁻¹); *DDD*:
 78 Defined Daily Dose for an active molecule (mg. inhabitants⁻¹ day⁻¹); *WW_{inhab}*: volume of
 79 wastewater per person per day (default value = 200 l. inhabitants⁻¹ day⁻¹); *CAP_{stp}*: capacity of
 80 local sewage treatment plant (inhabitants); dilution is the dilution factor from WWTP effluent to
 81 surface waters (default value set to 10). *Factor* stands for the fraction of the molecule adsorbed to
 82 the suspended matter; *F_{pen}* (%): market penetration factor; *F_{pen}* is the proportion of the
 83 population being treated daily with a specific drug substance; *F_{excreta}*: excretion fraction of the
 84 active molecule; *F_{stp}*: fraction of emission of the drug from wastewater treatment plants
 85 (WWTP) directed to surface water, *F_{stp}* can be defined as (1-WWTP removal fraction).
 86 Combination of Eqs. (1), (2), and (3) gives the following:
 87

88
$$PEC_{surfacewater} = \frac{consumption \times F_{excreta} \times F_{stp} \times DOSE_{ai} \times 100}{WW_{inhab} \times hab \times Factor \times Dilution \times DDD \times 365} \quad (4)$$

89
90 In the aim of our prioritization strategy, we simplified this last equation. Eq. (4) is finally
91 transformed into Eq. (5) as follows:
92

93
$$PEC_{surfacewater} = \frac{consumption \times F_{excreta} \times F_{stp}}{WW_{inhab} \times hab \times Dilution \times 365} \quad (5)$$

94
95 all PEC calculations presented in this study will refer to Eq. (5).
96

97 **I.2. Consumption Data Sources**

98
99 The French medicine agency (Agence Française de Sécurité Sanitaire des Produits de
100 Santé, AFSSAPS, Paris) kindly shared yearly sales data compulsory provided by the
101 pharmaceutical firms to AFSSAPS. These data cover both sale quantities of all prescribed drugs
102 delivered in France and over-the-counter drugs for the year 2004 for hospitals and pharmacies. In
103 the scope of this work we assume that delivered quantities represent quantities effectively
104 consumed by the French population and that the consumed amount was evenly distributed
105 throughout the year, as assumed in Carlsson *et al.* (2006). The candidate list of pharmaceuticals
106 was established as follows: a first set of molecules was selected from the top 100 pharmaceuticals
107 used in France (AFSSAPS 2006). To this first set of molecules, we added those that were
108 reported in previous studies to be detected in the aquatic environment or to be of high aquatic
109 ecotoxicity, and finally the molecules known to be persistent in the environment. The list of
110 selected molecules is displayed in Table 1; compounds are sorted by decreasing consumption. As
111 discussed in the introduction, neither steroid estrogens nor cytotoxics were included in this set of
112 molecules.
113

114 **I.3. Determination of the Parameter Fexcreta**

116 *Implications of Pharmaceutical Metabolism for Environmental Considerations*

117
118 In the human body, active molecules pass through several biotransformation mechanisms
119 ending in their elimination from the organism. Schematically, biotransformations can be
120 summarized into two different steps: phase I and phase II biotransformations. Phase I metabolites
121 can show a pharmacological activity similar or not to the parent compound and be biologically
122 active. As an example, norfluoxetine, a phase I metabolite of the serotonergic antidepressant
123 fluoxetine, shows the same pharmacological activity as the parent compound. Phase II
124 metabolites are phase I metabolite or parent compound conjugated with a functional group (such
125 as glucuronide, sulphate, or acetate), which enhance elimination from the organism and inactivate
126 the molecule. It has been shown for estrogens that glucuronide phase II metabolites could be
127 cleaved in the environment and thus regenerate the parent compound (Ternes *et al.* 1999;
128 D'Ascenzo *et al.* 2003). Moreover, sulphate conjugates appear to be more stable in the
129 environment (D'Ascenzo *et al.* 2003). As shown for estrogens, we can reasonably assume that

130 glucuronide conjugates of pharmaceutical compounds are subjected to the same degradation
131 pathway and are cleaved in the environment. Therefore, active metabolites and glucuronide
132 conjugates have to be considered in an Environmental Risk Assessment (ERA) or a prioritization
133 approach.

134

135 *Fexcreta Calculation*

136

137 To provide reasonable Fexcreta values, we made the following assumptions: We assumed
138 that all glucuronide conjugates are cleaved in the environment. Consequently, Fexcreta was
139 determined by summing the excreted proportion of the unchanged active molecule (in urine
140 and/or in feces) and the proportion of the parent molecule existing as a glucuronide conjugate. If
141 no information was given on the nature of the conjugate, we assumed a worst case hypothesis
142 considering that all conjugates were glucuronide conjugates. Rates of molecule not absorbed by
143 the digestive tract were added to the other excretion rates to give the final Fexcreta value.
144 Modifications in metabolism rates that can occur in unhealthy people were not taken into
145 account. When more than one excretion value was given, we always chose the greater one,
146 assuming a worst case scenario (decimal values were rounded up or down to the nearest value).
147 When information such as “negligible excretion of unchanged drug” was given with no other
148 specific information, we assumed a Fexcreta value minimum of 0.01. When no reliable data were
149 found, no Fexcreta value was calculated. Finally, when data were available, we calculated the
150 Fexcreta value for active metabolites using the same methodology with the assumptions
151 described earlier. The methodology used to determine Fexcreta values is synthesized in Figure 1.

152

153 **I.4. Reference Books and Databases Reviewed**

154

155 In order to determine metabolism pathways of selected pharmaceuticals and to calculate
156 Fexcreta values, we reviewed data from several sources commonly used by healthcare services
157 (hospitals, pharmacovigilance services, pharmacists, *etc.*): the Banque Claude Bernard (BCB
158 2006), a complete and free French databank on human pharmaceuticals (<http://www.resip.fr>), the
159 BCB is updated monthly, notably with data from the Marketing Authorisation Application
160 (MAA); the BIAM database (www.biam2.org 2006); the drug database drugs.com
161 (www.drugs.com); the Micromedex Drugdex_R databank (from Thomson Micromedex,
162 available at www.micromedex.com/products/drugdex); the Martindale compendium's *Complete
163 Drug Reference* (Martindale 2002); the Goodman & Gilman's *The Pharmacological Basis of
164 Therapeutic* (Hardman *et al.* 1996); the Merck Index (Merck 2001); and the Dorosz *Guide
165 pratique des médicaments* (Dorosz 2007). The HSDB database ([http://www.toxnet.
166 nlm.nih.gov/](http://www.toxnet.nlm.nih.gov/)) was also investigated.

167

168

169 **II. RESULTS**

170

171 **II.1. Excretion Factor Values**

172

173 Excretion factor values are displayed in Table 1. Reviewing the databases allowed us to
174 determine Fexcreta values for 76 molecules of the 112 selected. From the 76 compounds for
175 which Fexcreta values were determined, 45 showed excretion rates less than 0.5 and 23
176 compounds present excretion rates less than 0.1. Although the data determined here are consistent

177 with previous published excretion rates, some differences can be noted for a few of the
178 compounds studied. We report an excretion value for sulfamethoxazole of 0.4 compared to 0.15
179 (Ternes 1998) and 0.25 versus 0.01–0.08 for ibuprofen (Ternes 1998). In the case of ibuprofen,
180 this difference can be explained by the fact that the proportion of glucuronide conjugates was
181 added in our study. For furosemide, in taking into account the glucuronide ester metabolites, we
182 calculated a Fexcreta value of 1 instead of the value of 0.4 reported by Bindschedler *et al.* (1997).
183 As the glucuronide ester is reported to be the only significant metabolite of furosemide
184 (Micromedex Drugdex_R 2006), we considered that all furosemide was excreted unchanged or as
185 glucuronide metabolite, with 100% of the dose excreted in the environment after cleavage of the
186 ester. Hirsch *et al.* (1999) reported excretion fractions for antibiotics. Our results are in good
187 agreement with these values for doxycycline, amoxicillin, trimethoprim, and erythromycin; for
188 erythromycin, we assumed a worst case value of 1. On the contrary, for roxithromycin and
189 clarithromycin, the results differ significantly. Hirsch reports excretion rates of both unchanged
190 molecules greater than 0.6, whereas we determined a 0.5 value for roxithromycin and a 0.3 value
191 for clarithromycin. For carbamazepine, a Fexcreta of about 0.01 to 0.03 was reviewed from the
192 literature (Ternes 1998; Jjemba 2006), which corresponds to the unchanged fraction excreted in
193 urine. However, carbamazepine is also excreted in faeces and can be metabolized in glucuronide
194 conjugates (Lynn *et al.* 1978; BCB; Micromedex Drugdex 2006). Nevertheless, as no
195 quantitative data were available to allow calculating an accurate Fexcreta value, we chose to
196 consider Fexcreta for this molecule as undetermined rather than giving a wrong estimation. For
197 the active metabolite of simvastatin (β -hydroxy-acid metabolite), we did not find any value in the
198 reviewed databases. However, Carlsson *et al.* (2006) report that 55% of the parent pro-drug
199 simvastatin is excreted under the activated form. Therefore, we assumed a Fexcreta value of 0.55
200 for the β -hydroxy-acid metabolite in our work.

201

202 **II.2. Active Metabolites Entering the Aquatic Environment**

203

204 Reviewing metabolism pathways of pharmaceutical compounds allowed us to target
205 specific metabolites that may be of potential concern for the aquatic environment. These
206 metabolites were selected because they present either a significant pharmacological activity, or a
207 significant excretion fraction (≥ 0.1 , threshold value assumed by the EMEA). Metabolites of
208 interest are listed with their excretion values and their pharmacological properties in Table 2. In
209 addition, searching for active metabolites allowed us to highlight specific compounds that are
210 both active molecules and metabolites. Two important drugs exemplify this particular profile:
211 oxazepam and prednisolone. Oxazepam is an anxiolytic drug belonging to the benzodiazepine
212 class.

213

214 Oxazepam shows a very particular profile because it is both an active molecule and a
215 metabolite of the following active molecules: prazepam, diazepam, nordazepam, and clorazepate
216 dipotassium (Figure 2). Prazepam, diazepam, and clorazepate are metabolized in humans to
217 desmethyldiazepam, an active metabolite also used as a drug (nordazepam), which is
218 subsequently transformed to oxazepam. Oxazepam then undergoes direct glucuronidation before
219 its excretion. Glucuronide conjugates of oxazepam should then undergo subsequent cleavage in
220 the environment and then regenerate the active oxazepam. This case is interesting because
221 diazepam, the benzodiazepine commonly searched for in the aquatic environment, is not expected
222 to reach surface waters in significant concentrations. Diazepam was detected at low frequency in
223 German WWTP effluents with a maximum concentration of 40 ng·l⁻¹ (Ternes 1998), at very low

224 concentration in surface waters, less than 5 ng·l⁻¹ (Fent *et al.* 2006) and once in drinking waters
225 at level up to 10 ng·l⁻¹ (Waggott *et al.* 1981, cited by Halling-Sorensen 1998). Nevertheless,
226 most of the studies did not detect diazepam in effluents (Clara *et al.* 2005; Carballa *et al.* 2005;
227 Rabiet *et al.* 2006) or in surface waters (Ternes 1998; Zuccato *et al.* 2005). On the contrary,
228 oxazepam, which is potentially excreted up to 100% (taking into account the glucuronide
229 conjugates) of the dose absorbed for the five different active molecules, is expected to be present
230 at greater levels in the aquatic environment. A very recent study on occurrence of
231 pharmaceuticals in aquatic systems in France (Togola *et al.* 2007) did not detect diazepam in
232 surface waters, whereas oxazepam was measured at significant concentrations (up to 1500
233 ng·l⁻¹). Therefore, we assume that oxazepam is the benzodiazepine to search for in the
234 environment, and that this drug could be used as an indicator of contamination of the aquatic
235 environment by benzodiazepines.
236

237 Prednisolone belongs to the glucocorticoid therapeutic class. Glucocorticoids are natural
238 (hydrocortisone or cortisol) or synthesized (prednisolone, prednisone,
239 betamethasone, or dexamethasone) steroid compounds. They present immunosuppressant
240 properties and are used in many different pathologies (inflammation, allergy, auto-immune
241 disease, *etc.*). Reviewed metabolism data are incomplete but available data show that only a small
242 portion (less than 5%) of glucocorticoids are excreted in unchanged form (Schorderet 1998),
243 except for prednisolone, which is excreted in a greater fraction: up to 24% in case of a large dose
244 (Schorderet 1998; Martindale 2002). Prednisolone is also the active form of prednisone.
245 Therefore, non-negligible levels of prednisolone can reach the environment. Consequently,
246 prednisolone should be searched for in the environment and could be used as a marker of
247 contamination for other glucocorticoids. This assumption is partially confirmed by the results of
248 Chang *et al.* (2007), who report that prednisolone is the synthetic glucocorticoid frequently found
249 in surface waters. These results indicate that metabolism data are useful for selecting relevant
250 pharmaceuticals or metabolites to survey in surface waters.
251

252 **II.3. Calculated PECs for Parent Compounds**

253

254 PECs were calculated using Eq. (5). As all required data were not available (especially
255 WWTP removal rates data) for all the selected molecules, three PEC values were calculated
256 corresponding to varying conservative levels. PECA is the conservative PEC calculated with
257 actual amounts of pharmaceuticals and not refined by Fexcreta and Fstp values. PECB are PECA
258 refined by Fexcreta values. PECC are PECB refined by Fstp values. Results for PECA and PECB
259 are displayed in Table 1. Considering PECA values, only 15 compounds show a value greater
260 than 1 µg·l⁻¹ and our results highlight paracetamol, ibuprofen, dextropropoxyphene, amoxicillin,
261 and aspirin. PECA values of troxerutin and diosmin, two flavonoid compounds with vitamin P
262 properties (used against venous insufficiency of the lower limbs), are also in the top 10 because
263 they are widely prescribed in France, contrary to other European countries; these last two drugs
264 may be specific to the French consumption profile.
265

266 For compounds such as sertraline, dextropropoxyphene, omeprazole, and pantoprazole,
267 the PECB values are drastically reduced. For example, for the antidepressant sertraline, PECA is
268 equal to 142 ng·l⁻¹ but drops to 20 ng·l⁻¹ when excretion rates are taken into account.
269 Comparison of PECA and PECB values confirms that metabolism is one of the most important

270 processes that can reduce the quantity of parent pharmaceuticals reaching the aquatic
271 environment.

272
273 Therefore, metabolism data and excretion rates have to be taken into account in PEC
274 calculation (Huschek *et al.* 2004). As data on metabolism rates are currently available through
275 databases, they can help to prioritize pharmaceuticals of greater concern.

277 **II.4. Comparison of PEC Values with Field Measurements**

278
279 WWTP removal rates data are limited. Reviewing literature data, we found only data for
280 24 molecules by the 111 selected in this work (Table 3); some of them show a high variability,
281 depending on the study. This is especially the case for metoprolol and diclofenac with removal
282 fractions varying from less than 0.1 to 0.83 and from less than 0.1 to 0.75, respectively.
283 Considering the heterogeneity of data, we determined PECC values by two methods. First, we
284 calculated extreme PECC values by taking into account the minimal and maximal WWTP
285 removal rates reviewed. Second, we calculated a refined PECC using the WWTP removal rates
286 reported by Paffoni *et al.* (2006), as this study provides data for almost all 24 compounds. Our
287 first objective was to compare PEC values for surface water with field measurements, but, as only
288 few measurements of pharmaceuticals in French surface waters are available, we used data on
289 occurrence in WWTP effluents. Consequently, in order to compare the calculated PEC with real
290 measurements, we used Eq. (5) without the default dilution factor of 10. Calculated PECC for
291 WWTP effluents with WWTP effluents measurements performed in France are compared in
292 Table 4.

293
294 Calculated PECs for pharmaceuticals appear to correlate well with effluents
295 measurements. This is the case for diclofenac, ibuprofen, naproxen, ketoprofen, bezafibrate,
296 metoprolol, and propranolol. As a general rule, calculated PECs for antibiotics were in the range
297 but slightly greater than the measured concentrations in effluents. For two compounds, however,
298 PECs are very different from the effluent measurements (more than one order of magnitude); this
299 is the case for amoxicillin and doxycycline. For amoxicillin, the difference could be explained by
300 the fact that this molecule is rapidly degraded in water, which has been previously suggested
301 (Zuccato *et al.* 2005). Another β -lactam, piperacillin, showing a PECB value for WWTP effluent
302 of about $1\mu\text{g}\cdot\text{l}^{-1}$ has been searched for but not detected in WWTP effluents or surface waters in
303 the French aquatic environment (Paffoni *et al.* 2006). These results suggest that β -lactam
304 antibiotics may undergo rapid environmental degradation but this hypothesis must be confirmed.
305 For doxycycline, previous studies have reported that tetracyclines should be bound to suspended
306 matter and sediment due to their complexing properties (Hirsch *et al.* 1999). Therefore
307 doxycycline is unlikely to be found under dissolved form but could still be in the water column if
308 bound to suspended materials, especially colloids.

309
310 PECBs of diazepam and simvastatin (a prodrug) are very low ($2\text{ ng}\cdot\text{l}^{-1}$) and therefore
311 those molecules were not expected to be found in effluents or surface waters, which is confirmed
312 by field measurements (Paffoni *et al.* 2006; Rabiet *et al.* 2006). We also investigated PEC values
313 in surface water for a few metabolites of potential concern (Table 5), considering excretion rates
314 of metabolites and consumed amounts of the respective parent compound. Only a few of the
315 metabolites considered in this work have already been measured in aquatic environments (but not
316 in France), which allowed us to compare PEC and field measurements. The calculated PECB for

317 acetylsulfamethoxazole is in the range of the measured concentrations reported in WWTP
318 effluents and surface waters downstream from a WWTP (Ashton *et al.* 2004).

319
320 The anti-inflammatory drug ibuprofen can generate two main metabolites: carboxy and
321 hydroxy-ibuprofen. Our calculated PECC values reported here are consistent with field
322 measurements for the two metabolites, if we consider a WWTP removal fraction of 0.95 for the
323 two compounds (Bendz *et al.* 2005). The PECC value of fenofibric acid calculated with
324 a WWTP removal fraction of 0.82 (Paffoni *et al.* 2006) is in the range of the surface water levels
325 reported by Paffoni (Table 5). For other metabolites, no field data were available to allow any
326 comparison; the following active metabolites—hydroxymetronidazole, norpropoxyphene, and
327 demethyltramadol (excreted from metronidazole, dextropropoxyphene, and tramadol,
328 respectively)—show PECB values for surface waters of approximately 250 ng·l⁻¹. For oxazepam
329 (benzodiazepine), a final PECB value was calculated by summing the PECs of different sources
330 of oxazepam, which reached roughly 200 ng·l⁻¹. Recent field measurements (Togola *et al.* 2007)
331 reported an average concentration of oxazepam in surface water of about 200 ng·l⁻¹, which is in
332 good agreement with our calculated PEC.

334 II.5. Calculation of Risk Quotients and Risk Assessment for Pharmaceuticals

335
336 As a first attempt to prioritize pharmaceuticals, we calculated risk quotient ratios.
337 According to the EMEA guideline (EMEA 2006), PNECs are calculated using assessment
338 factors, as described in the European Technical Guidance Document (TGD 2003). Unlike TGD,
339 the EMEA guideline enforces the use of chronic toxicity data and requires long-term NOEC for
340 the base set (*i.e.*, three NOEC values from three different trophic levels, applying an assessment
341 factor of 10 to the lowest value). The review of available ecotoxicity data showed that only six
342 compounds bring together the conditions required by the EMEA guideline: clofibrac acid,
343 propranolol, carbamazepine, sulfamethoxazole, fluoxetine, and diclofenac. If we do not use only
344 the EMEA guideline but also refer to the European TGD (2003) for pharmaceuticals with limited
345 chronic data (1 or 2 NOECs from different trophic levels), it is then possible to calculate PNEC
346 values and PEC/PNEC quotients for a further 16 compounds.

347
348 As all data required for calculating PECC values for pharmaceuticals were not always
349 available, we used PECB values for furosemide, sertraline, paroxetine, citalopram, and fluoxetine
350 and PECA values for carbamazepine, aspirin, and fluvoxamine. Results are displayed in Table 6.
351 All risk quotients are less than one, except for amoxicillin, which presents a very high ratio of 62,
352 suggesting a risk for the aquatic environment. Nevertheless, a number of pharmaceuticals show a
353 risk quotient close to 1. This is the case for propranolol, ofloxacin, and erythromycin. For these
354 molecules, a risk cannot be ruled out.

355
356 Three other molecules showed a risk ratio near 1, carbamazepine, sertraline, and
357 furosemide. However, calculated PEC for carbamazepine is conservative and PNEC values for
358 the two other compounds were calculated with an assessment factor of 100; therefore risk ratio is
359 overestimated. Although reported to be one of the most toxic compounds, the serotonergic
360 antidepressant fluoxetine shows a low PEC/PNEC ratio, due to the very low PEC of 9 ng·l⁻¹
361 calculated for this molecule.

362
363

364 **III. DISCUSSION**

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366 **III.1. Reliability of the PEC Calculation, Uncertainties, and Flaws**

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Although field measurements are available for a limited number of pharmaceuticals, preliminary results indicate that PEC calculation is in accordance with environmental levels for pharmaceuticals. Therefore, the simple equation proposed here is valuable for predicting aquatic concentrations for pharmaceuticals in any country. Previously published works on exposure assessment of pharmaceuticals came to the same conclusion. Bound and Voulvoulis (2006) used EMEA guidelines to calculate PEC values and compared them with their own field measurements. They concluded that PEC calculation using the EMEA model could provide useful information for the prioritization of pharmaceuticals. The same conclusion was drawn by Liebig *et al.* (2006), who quoted that even if the EMEA model does not reflect the complexity of the real environment, it permits calculating PECs in accordance with field measurements. This study also reported that the factor with the highest impact and uncertainty was the production volume, estimated in the EMEA model in using *F_{pen}* and *DOSE_{ai}*. Using actual amounts of consumption, as in this study, and *F_{excreta}* values give a reliable exposure assessment for pharmaceuticals.

Nevertheless, some uncertainties remain in the model we used. PEC values were calculated based on human consumption data during 1 year. As some pharmaceutical compounds are used both in human and veterinary medicine, there are still uncertainties about the actual amounts of pharmaceuticals reaching surface waters. This is particularly the case for antibiotic and antiprotozoal compounds. Theoretically, including veterinary consumption is likely to ensure a more comprehensive PEC. However, as the routes of administration and ways of reaching the aquatic environment differ between veterinary and human pharmaceuticals, this study only focused on human use.

Another major uncertainty remains on the quantity actually consumed by patients. Data provided by the AFSSAPS give information on the quantities delivered in hospitals and pharmacies per year but cannot give any information on the patient compliance. Patients' non-compliance can be quite large, and quantities actually consumed by people may be less than the quantities delivered, especially for drugs that do not require medical prescription as NSAIDs. Moreover, if data shared by the AFSSAPS provided a complete profile of the national annual consumption, no data were available regarding local consumptions of pharmaceuticals, which are likely to differ from one region to another. Finally, temporal variations of consumption (especially for pharmaceuticals used in acute treatments, such as antibiotics), and of quantities reaching the aquatic environment cannot be taken into account. Uncertainties also lie in the parameter *F_{stp}*: review of available data shows a high heterogeneity in removal rates. Moreover, WWTP efficiency toward pharmaceuticals can be influenced by the season (Castiglioni *et al.* 2006), therefore leading to varying surface water concentrations throughout the year.

At last, great uncertainties lie in the default values proposed by the model. For example, the default value for quantities of effluent is set to 200 l·inhab⁻¹·day⁻¹, which is a mean value that can be accepted at the national scale in France. However, for some specific French regions, this value may drop to 150 l·inhab⁻¹·day⁻¹. Using this last value in our calculation significantly

410 increases PEC values by 25%. To this extent, PEC values calculated for the WWTP effluents are
411 more reliable than surface water PECs.

412
413 Equation (5) does not take into account the fraction of the molecule sorbed to sediment or
414 suspended matter, which is a flaw in the proposed model. The EMEA model (Eq. [1]) includes
415 this factor (FACTOR), but its calculation requires Koc values that are very scarce at the moment.
416 In addition, Eq. (5) does not take into account abiotic and biotic degradation processes that can
417 occur in surface waters. Abiotic processes are reported to be most important ones (Fent *et al.*
418 2006). Photolysis and hydrolysis were suggested to be rapid ways of removal of amoxicillin in
419 the environment (Andreozzi *et al.* 2004). This statement was supported by the fact that
420 amoxicillin was only detected in surface waters at low levels (Zuccato *et al.* 2005; Paffoni *et al.*
421 2006). The β -blocker propranolol was reported to be rapidly photodegraded and therefore is not
422 expected to be persistent in surface waters (Qin-Tao and Williams 2007). Nevertheless,
423 calculated PEC for propranolol was in the range of field measurements. This could be partially
424 explained by the fact that propranolol and most of the pharmaceuticals are continuously released
425 in the environment. This fact could balance the degradation processes in the environment,
426 therefore some authors have suggested that pharmaceuticals should be considered as “pseudo-
427 persistent” contaminants due to this continuous release (Daughton and Ternes 1999).

428

429 **III.2. Relevance of *Fexcreta* Values**

430

431 As excretion rates range from 0.1 to 1, they are to be taken into account in a prioritization
432 or a risk assessment approach. Metabolism of pharmaceuticals is one of the first steps that limit
433 the concentrations reaching the environment. Consequently, it is valuable to review such data and
434 to search for the most accurate excretion values that would allow us to calculate more realistic
435 PECs.

436

437 In this work, two main assumptions were made in the determination of the *Fexcreta*
438 values. First, we did not take into account modifications of metabolism than can occur in
439 unhealthy people (specifically people with hepatic or renal impairment). As an example: chronic
440 renal failure can result in a decrease in propranolol metabolism via downregulation of hepatic
441 cytochrome P450 activity (www.drugs.com, 2006), therefore potentially leading to increase
442 excretion of unchanged drug. However, as people affected by renal or hepatic impairment may
443 only represent a small part of the population, we considered that the resulting variability of the
444 excretion fractions can be neglected when calculating PEC for the aquatic environment. Accuracy
445 of PECs with field measurements presented here seems to confirm this assumption.

446

447 Second, we considered a worst case scenario for the *Fexcreta* values when more than one
448 excretion fraction was given. This assumption, however, did not lead to high over-predictions of
449 concentrations, as the variability of recovered values was limited and rarely exceeded 10%;
450 contrary to WWTP removal fractions. Only 4 compounds of the 76 showed a greater variability:
451 sotalol (excretion rates values between 0.66 and 0.9), clarithromycin (0.18 to 0.3), ranitidine (0.3
452 to 0.5), and rifampicin for which excretion in urine is dose-dependant (Micromedex Drugdex_R
453 2006).

454

455

456

457 **III.3. Environmental Relevance of Active Metabolites**

458
459 The metabolites reviewed should not be all of environmental concern. However, as no
460 data exist about the ecotoxicity of these compounds, it is rather difficult to draw any conclusions.
461 Moreover, it is not clear that pharmacologically inactive metabolites will not have any biological
462 effects or toxicity on aquatic non-target organisms, especially lower invertebrates. Nevertheless,
463 we will make a few suggestions to select the metabolites to search for in surface waters:
464

- 465 • The amount of the parent molecule consumed should be taken into account when
466 selecting for relevant metabolites.
- 467 • Active metabolites that are pharmacologically equipotent to the parent compound should
468 be considered unless they are excreted at low rates. Because it is not possible to propose a
469 threshold excretion value using available data (metabolism, occurrence, and ecotoxicity),
470 we assume a threshold value of 10%, which is the value proposed by the EMEA (2006) to
471 assess the relevance
- 472 • of a metabolic fraction of a pharmaceutical compound.
- 473 • Active metabolites showing a mechanism of action that is different from the parent
474 compound should also be considered if their excretion fraction is equal to or greater than
475 0.1.
- 476 • Because there are no data on the toxicity of pharmacologically inactive metabolites on
477 aquatic organisms, such metabolites should be considered on a case-by case approach
478 considering the amount of parent compound and their excretion fraction.
- 479 • Metabolites with an excretion fraction greater than the parent compound should be
480 considered relevant for the aquatic environment.
- 481 • Active metabolites of prodrugs should be searched for in the environment.
482

483 Finally, it should be noted that no data are available on the potential toxicity of inactive
484 metabolites, considering that the term “pharmacologically inactive metabolite” does not
485 necessarily mean that such a metabolite has no effects on an aquatic organism. Considering these
486 assumptions, we selected 30 environmentally relevant metabolites (Table 2) for which risk
487 assessment should be implemented.
488

489 Only human metabolites were considered in this work. However, other degradation products
490 can be generated in the environment, especially by photolysis. Photodegradation metabolites can
491 be more toxic than the parent compound (Della- Greca *et al.* 2004; Isidori *et al.* 2005, 2006); and
492 attention should also be paid on such metabolites.
493

494 **III.4. Considerations on Risk Assessment for Pharmaceuticals**

495
496 As quoted by several authors (Jones *et al.* 2002; Carlsson *et al.* 2006; Fent *et al.* 2006),
497 ecotoxicological data, and notably chronic data, are lacking and thus limit the outcome of ERA
498 based on risk quotients. Relevant chronic data are needed. Preliminary results presented here can
499 nevertheless give some insight on the risk assessment of pharmaceuticals. Antibiotics and notably
500 amoxicillin could present a risk for the aquatic environment. However, two points must be noted
501 for this molecule. First, the NOEC value used to derive the PNEC is based on growth inhibition
502 testing on the cyanobacteria *Synechococcus leopoliensis* (Andreozzi *et al.* 2003). If other

503 available data on amoxicillin confirm that cyanobacteria seem very sensitive to amoxicillin
504 (Holten-Lutzoft *et al.* 1999), data on green algae species (Holten-Lutzoft *et al.* 1999) and on
505 invertebrates (Garric *et al.* 2006) only indicate limited toxicity.
506

507 Second, previous studies seem to indicate that amoxicillin is rapidly degraded in the
508 aquatic environment (Andreozzi *et al.* 2004; Zuccato *et al.* 2005). Consequently, the risk ratio for
509 amoxicillin could be smaller than predicted here. Nevertheless, antibiotics remain one of the
510 more hazardous pharmaceutical classes for the aquatic environment due to their antimicrobial
511 activity. Among antibiotics, macrolides, which are not only toxic toward cyanobacteria but also
512 toward green algae (Isidori *et al.* 2005), may represent a class of compounds of high concern for
513 the aquatic environment.
514

515 Although reported to be one of the most toxic compounds, the serotonergic
516 antidepressant fluoxetine shows a low PEC/PNEC ratio (0.09), due to the very low PEC of 9
517 ng-l⁻¹ calculated for this molecule. It should be noted that the active metabolite norfluoxetine,
518 with similar pharmacological activity, may act additively with fluoxetine. It therefore seems
519 reasonable to consider the sum of the two PEC values (fluoxetine + norfluoxetine) in the
520 calculation of the risk quotient, which increases the fluoxetine risk quotient to 0.26, still less than
521 1 but 3 times greater than the previous value. Metabolites should be considered when performing
522 a pharmaceutical ERA, at least for PEC calculation. However, it is not yet possible to assess the
523 hazard of these metabolites, due to the lack of ecotoxicity data. For metabolites that are reported
524 to be equipotent (the same mechanism of action and pharmacological potency) to the parent
525 compound, using the sum of respective PECs may be appropriate. For active metabolites with
526 differences in pharmacological potency but with the same mechanism of action, it will be
527 necessary to take their relative potency into account. For metabolites with a mechanism of action
528 different from the parent compound, however, ecotoxicological data remain necessary to assess
529 their toxicity. For non-pharmacologically active metabolites that may have toxic effects on
530 aquatic organisms, it is also necessary to build ecotoxicological assays.
531

532 Finally, in the context of an ERA, an effort should be made on the study of the effect of
533 pharmaceutical mixtures on aquatic organisms for several reasons. First, pharmaceuticals with
534 the same mechanism of action may act additively on aquatic organisms (Cleuvers 2003; Fraysse
535 and Garric 2005). Therefore, in a risk assessment framework, such pharmaceuticals should be
536 considered together. As an example, for SSRIs, summed PECs of all parent compounds (and also
537 active metabolites) should be taken into account rather than considering separate PECs; moreover
538 the ecotoxicological effects of such mixtures should be better evaluated.
539

540 Second, interactions between pharmaceuticals are well studied and taken into account in
541 humans as the concomitant use of some pharmaceuticals can lead to severe consequences.
542 Mixtures of pharmaceuticals known to interact with each other should be assessed for
543 ecotoxicity. Third, a number of pharmaceuticals are cytochrome P450 enzyme modulators or
544 Para-glycoprotein-P modulators (proteins that play a key role in the resistance to xenobiotics),
545 and thus are likely to disrupt homeostasis of non-target organism and to increase their sensitivity
546 to other pollutants (Endicoot and Ling 1989; Toomey and Epel 1993). Therefore, one of the
547 major issues when considering concentrations entering the environment should be interactions
548 between pharmaceuticals or between pharmaceuticals and other pollutants: single-compound
549 assays are not sufficient to provide accurate environmental risk assessment of pharmaceuticals.

550 Nevertheless, except for a few laboratory (Cleuvers 2003; Eguchi *et al.* 2004; Fraysse and Garric
551 2005; Chritensen *et al.* 2007), and microcosms studies (Brain *et al.* 2004; Richards *et al.* 2004)
552 the ecotoxicological assays done to date only focus on single compounds.
553

554 **III.5. Considerations on the Prioritization of Pharmaceuticals**

555

556 Equation (5) can be used to assess the environmental exposure for pharmaceuticals. If all
557 data were available, a preliminary prioritization based on PEC values could be conducted.
558 However, WWTP removal rates are scarce and limit such an approach. In addition, the effects are
559 not taken into account, and such a simple approach does not allow to select the compounds
560 showing the highest risk. To fill this lack and to provide an initial hazard assessment of
561 pharmaceuticals, some authors have proposed the use of QSAR and a test battery based on
562 mechanism of action (Escher *et al.* 2006; Lienert *et al.* 2007).
563

564 We consider, as do several authors, that the use of existing pharmacological,
565 toxicological, and pharmacokinetic data is likely to be helpful in assessing the risk of
566 pharmaceuticals, as they could provide a better understanding of the fate and effect of
567 pharmaceuticals in the aquatic environment (Lange and Dietrich 2002; Seiler 2002; Fent *et al.*
568 2006; Jjemba 2006). The use of mammalian pharmacological and toxicological data was
569 proposed to help to prioritize the potential impacts of pharmaceuticals to fish (Huggett *et al.*
570 2003). In practice, pharmacokinetic parameters such as bioavailability, half-life in the human
571 body or excretion rates may be used as indicators of the environmental behaviour of
572 pharmaceuticals in the aquatic environment. Jjemba (2006) suggested that compounds excreted
573 unchanged in low amounts may also show low degradability in the environment. As the
574 pharmacokinetic behavior is influenced by the same parameters that can modify environmental
575 behavior such as pH and pKa, it makes sense to draw a parallel between pharmacokinetic and
576 environmental criteria. Williams *et al.* (2006) very recently studied the correlation between the
577 environmental partitioning coefficient Kd and the distribution volume Vd, which measures the
578 distribution of a pharmaceutical within the body. These results suggest that pharmacokinetic
579 parameters should be helpful to estimate environmental behavior for pharmaceuticals. Known
580 side effects of pharmaceuticals may also be valuable to indicate potential harmful effects on non-
581 target organisms as it as already been shown for diclofenac in vultures (Oaks *et al.* 2004), and in
582 fish (Schwaiger *et al.* 2004; Triebkorn *et al.* 2004). Taking into account such effects could make
583 it possible to target the harmful impacts of these compounds, at least on non-target vertebrates.
584

585 Comparative pharmacology could also be useful to understand toxicity pathways of
586 pharmaceuticals. At the moment, studies have only considered the major mechanism of action
587 (MoA) of pharmaceuticals in ecotoxicity assays. However, evidence shows that compounds
588 belonging to same pharmacological and chemical classes (*e.g.*, compounds with same
589 mechanisms of action), can display a high variability in toxic values on same species and
590 endpoints (Huggett *et al.* 2002; Henry *et al.* 2004; Dzialowski *et al.* 2006; Garric *et al.* 2006).
591 Indeed, pharmaceuticals are not only characterized by their principal MoA but also by some
592 additional pharmacological characteristics that should be taken into account. In the case of β -
593 blockers, several authors (*e.g.*, Fraysse and Garric 2005; Fent *et al.* 2006) have suggested that
594 differences in toxicity should be partially explained by pharmacological properties specific to
595 these compounds such as receptor selectivity or membrane-stabilizing activity. For SSRIs, results
596 from Henry *et al.* (2004) show that NOEC values on the reproduction of *C. dubia* range more

597 than two orders of magnitude. Pharmacological data indicate that even if SSRIs have a greater
598 selectivity for blocking serotonin reuptake (their principal MoA), they also have affinities to
599 some other receptors and reuptake inhibitor activities on other systems such as the noradrenergic
600 or dopaminergic systems (Hyttel 1993; Dulin *et al.* 2002). Such “secondary” MoAs could help to
601 understand toxic responses. Sertraline is both the most toxic SSRI on *C. dubia* reproduction
602 (Henry *et al.* 2004) and the most potent inhibitor of serotonin reuptake (Hyttel 1993); however, it
603 is not the most selective SSRI, as it also exerts an activity on dopamine and noradrenaline
604 reuptake (Hyttel 1993). Citalopram, which is the less toxic SSRI (Henry *et al.* 2004), is not the
605 less potent molecule but the most selective one. Such pharmacological data suggest that the toxic
606 response observed for *C. dubia* could not only be linked to serotonin reuptake but also to other
607 MoAs; this hypothesis remains to be confirmed.

608
609 A prioritization methodology for pharmaceuticals could therefore cross PEC values with
610 relevant pharmacological data, such as specific mechanism of action or relevant chronic adverse
611 effects, to give a reliable estimation of the risk. Such a methodology should allow to bypass the
612 lack of ecotoxicological data and could provide useful information for building ecotoxicological
613 assays.

614 **CONCLUSION**

615
616 The main objective of this study was to assess the exposure concentration of
617 pharmaceuticals in surface water with an emphasis on metabolites. The PEC calculation proposed
618 here is reliable, as calculated PECs are in good agreement with field measurements. Metabolism
619 data are important to take into account as they allow a more relevant selection of pharmaceuticals
620 and metabolites to survey in aquatic ecosystems. Moreover, there is a need to consider the
621 specificity of drugs consumption profile for each country, as such a specificity can lead to
622 different priority compounds. Another objective was to perform a preliminary prioritization using
623 risk quotients ratios. Given the lack of relevant ecotoxicological data, it was not possible to
624 prioritize pharmaceuticals using risk ratios. As it will take time to build enough ecotoxicological
625 data, we consider that the use of pharmacokinetic and pharmacological data can help to prioritize
626 pharmaceuticals. Moreover, comparative pharmacology based on mechanism of action, adverse
627 effects, and specific activities could be usefully taken into account to better understand the
628 mechanisms of toxicity of pharmaceuticals and to assess their environmental risk. The next step
629 in our work is to propose a sound selection of pharmaceuticals to be monitored in surface waters
630 by implementing a prioritization methodology based on an exposure assessment crossed with a
631 biological affect assessment using ecotoxicological and pharmacological data.

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633
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640
641

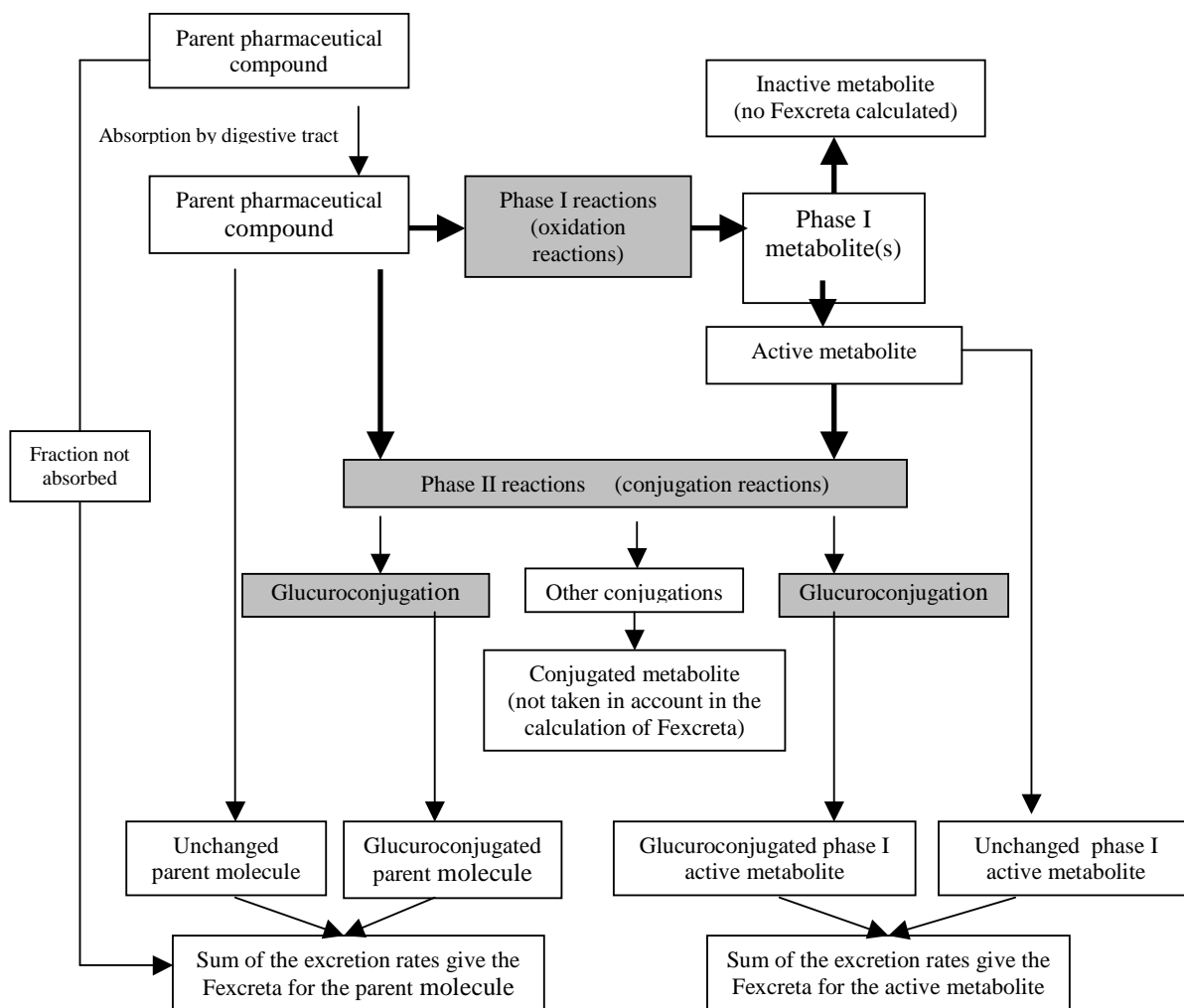


Figure 1. Methodology used to calculate Fexcreta values for pharmaceutical compounds and active metabolites. As glucuroconjugated metabolites can be cleaved in the environment and regenerate the active compound, their excretion rates were taken into account in the calculation of the Fexcreta value.

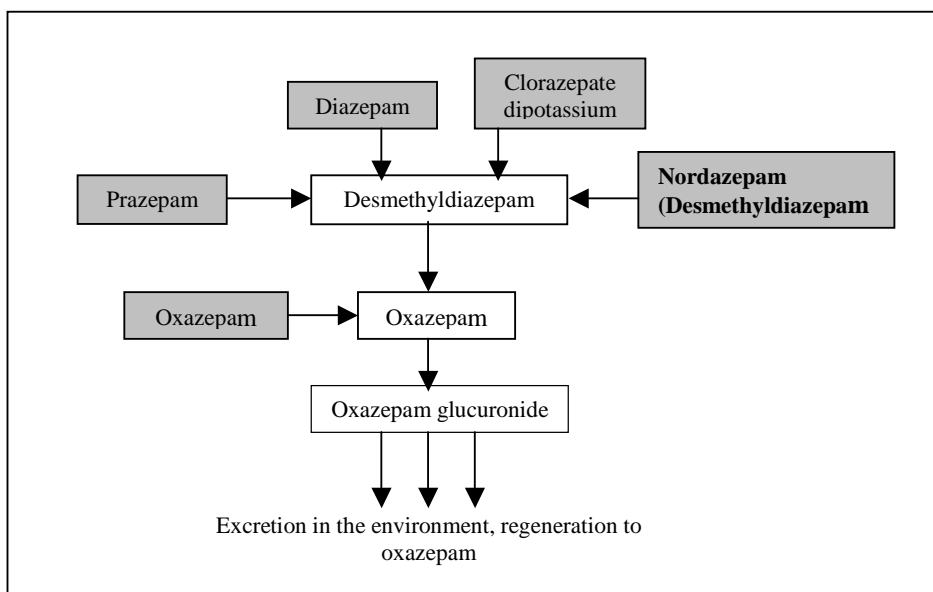


Figure 2. Simplified scheme of the different sources of oxazepam. Grey squares indicate an active molecule used as a commercialized product; white squares indicate metabolites.

Table 1. Consumption, excretion fractions (Fexcreta) and calculated PECs for pharmaceuticals used in France in 2004 (Data from AFSSAPS 2006). Active molecules are sorted by decreasing consumption amounts. PEC are calculated using equation 5 and are expressed in $\text{ng}\cdot\text{l}^{-1}$. PEC_A are calculated using actual amounts only. PEC_B are PEC_A refined by Fexcreta values.

Compound name	Therapeutic use	consumption of active ingredient in the year 2004 (kg)	Fexcreta	PEC_A ($\text{ng}\cdot\text{l}^{-1}$)	PEC_B ($\text{ng}\cdot\text{l}^{-1}$)
paracetamol	analgesic	3303077	0.85	75413	64101
metformin	antihyperglycaemic	716858	1	16367	16367
troxerutin	used in venous insufficiency	444339	-	10145	-
aspirin	analgesic	396212	-	9046	-
diosmin	used in venous insufficiency	373544	-	8528	-
amoxicillin	antibiotic	333223	0.9	7608	6847
ibuprofen	anti-inflammatory	240024	0.25	5480	1370
carbocistein	mucolytic	232308	-	5304	-
sodium valproate	anti-epileptic	112162	0.53	2561	1357
acetylcystein	mucolytic	96759	-	2209	-
fenofibrate	lipid regulating	85670	0.01	1956	20
allopurinol	antigout	54247	0.12	1239	149
dextropropoxyphene	analgesic	51963	0.05	1186	59
buflomedil	anti-ischaemic	50958	0.25	1163	291
naftidrofuryl	anti-ischaemic	45523	-	1039	-
benfluorex	lipid regulating	40730	-	930	-
pristinamycin	antibiotic	39855	-	910	-
naproxen	anti-inflammatory	37332	0.7	852	597
metronidazole	antiprotozoal	36545	0.18	834	150
carbamazepine	anti-epileptic	33514	-	765	-
heptaminol	used in orthostatic hypotension	28423	-	649	-
tramadol	analgesic	25897	0.3	591	177
levodopa	management of parkinsonism	24996	-	571	-
amiodarone	anti-arryhtmic	24318	-	555	-
trimebutine	antispasmodic	23550	-	538	-
clavulanic acid	β -lactamase inhibitor	22699	-	518	-
ketoprofen	anti-inflammatory	21697	0.85	495	421
furosemide	diuretic	21288	1	486	486
bezafibrate	lipid regulating	20852	1	476	476
atenolol	anti-hypertensive (β -blocker)	18337	1	419	419
amphotericin B	antifungal	18179	1	415	415
sulfamethoxazole	antibiotic	16730	0.4	382	153
trimetazidine	anti-ischaemic	16480	-	376	-
clarithromycin	antibiotic	15105	0.18	345	62
ceftriaxone	antibiotic	13603	1	311	311
josamycin	antibiotic	12802	0.2	292	58
propranolol	anti-hypertensive (β -blocker)	12487	0.24	285	68
ciprofloxacin	antibiotic	12186	0.5	278	139
ranitidine	anti-ulcer	11656	0.5	266	133
pravastatin	lipid regulating	10969	0.5	250	125
diclofenac	anti-inflammatory	9896	0.15	226	34
cefepodoxime	antibiotic	9283	0.8	212	170
metoprolol	anti-hypertensive (β -blocker)	8786	0.05	201	10
omeprazole	anti-ulcer	8045	0.01	184	1.8
atorvastatin	lipid regulating	7924	0.01	181	1.8
nicardipine	anti-hypertensive	7800	0.01	178	1.8
simvastatin	lipid regulating	6943	0.01	159	1.6
fosfomicin	antibiotic	6774	1	155	155
hydroxyzine	anxiolytic	6638	-	152	-
doxycycline	antibiotic	6243	0.72	143	103
sertraline	serotonergic anti-depressant	6224	0.14	142	20
oxazepam	anxiolytic	6195	1	141	141
domperidone	antiemetic (dopamine antagonist)	5861	0.07	134	9
paroxetine	serotonergic anti-depressant	5515	0.03	126	4
cyamemazine	antipsychotic	5441	-	124	-
pantoprazole	anti-ulcer	5287	0.01	121	1
piperacillin	antibiotic	4476	1	102	102

Table 1. continued

Compound name	Therapeutic class	consumption of active ingredient in the year 2004 (kg)	Fexcreta	PEC _A (ng.l-1)	PEC _B (ng.l-1)
ofloxacin	antibiotic	4137	1	94	94
azithromycin	antibiotic	4073	0.5	93	46.5
phenobarbital	anti-epileptic	3915	0.25	89	22.3
prednisolone	corticoid	3743	-	85	-
fluoxetine	serotonergic anti-depressant	3740	0.1	85	8.5
citalopram	serotonergic anti-depressant	3487	0.4	80	32
roxythromycin	antibiotic	3404	0.5	78	38.9
trimethoprim	antibiotic	3346	0.5	76	38.2
zolpidem	hypnotic	3344	0.01	76	0.76
bromazepam	anxiolytic	2604	0.03	59	1.78
mianserine	antipsychotic	2423	-	55.3	-
rifampicine	antibiotic	2383	0.18	54	9.8
prazepam	anxiolytic	2166	0.03	49	1.48
tianeptine	antidepressant	2152	0,08	49	3.93
bisoprolol	anti-hypertensive (β-blocker)	2113	0.6	48	28.94
clorazepate	anxiolytic	2109	0.01	48	0.48
amlodipine	anti-hypertensive	2013	0.1	46	4.60
piroxicam	anti-inflammatory	2008	0.1	46	4.58
zopiclone	hypnotic	1948	0.04	44	1.78
ceftazidime	antibiotic	1832	1	42	41,82
levomepromazine	antipsychotic	1699	-	39	-
prednisone	corticoid	1550	-	35	-
cetirizine	anti-allergic	1442	0.7	33	23.05
fluvoxamine	serotonergic anti-depressant	1121	-	26	-
glibenclamide	antihyperglycaemic	1092	0.1	25	2.49
baclofen	muscle relaxant	1080	0.8	25	19.72
ramipril	antihypertensive	1042	0.02	24	0.48
loxapine	antipsychotic	961	-	22	-
nadolol	anti-hypertensive (β-blocker)	938	1	21	21.42
loratadine	anti allergic	927	-	21	-
vancomycin	antibiotic	918	1	21	20.96
metoclopramide	antiemetic (dopamine antagonist)	913	0.3	21	6.25
fluconazole	antifungal	893	0.8	20	16.30
lorazepam	anxiolytic	585	0.85	13	11.35
tazobactam	β-lactamase inhibitor	560	0.8	13	10.22
diazepam	anxiolytic	526	0.01	12	0.12
perindopril	anti-hypertensive	504	0.1	12	1.15
hydrocortisone	corticoid	453	-	10	-
oxprenolol	anti-hypertensive (β-blocker)	377	0.98	8.60	8.43
tropatepine	management of parkinsonism	355	-	8.11	-
haloperidol	antipsychotic	342	-	7.81	-
loperamide	antidiarrhoeal	318	-	7.26	-
carvedilol	anti-hypertensive (β-blocker)	313	-	7.15	-
buprenorphine	opioid	270	-	6.17	-
trihexyphenidyle	management of parkinsonism	257	-	5.86	-
nordazepam	anxiolytic	237	0.01	5.4	0.05
alprazolam	anxiolytic	178	0.01	4.05	0.04
terbutaline	anti-asthmatic	165	-	3.78	-
betamethasone	corticoid	156	-	3.56	-
teicoplanin	antibiotic	136	1	3.11	3.11
midazolam	hypnotic / amnestic	98	-	2.23	-
levothyroxin	thyroid hormone	59	-	1.34	-
ondansetron	anti-emetic	44	0.1	1.00	0.10
clonazepam	anxiolytic	21	-	0.49	-
escitalopram	serotonergic anti-depressant	4.6	-	0.11	-

Table 2. Review of phase I metabolites of potential interest for the French aquatic environment. Active metabolites are sorted in alphabetical order of parent compounds, except for oxazepam. Underlined metabolites are considered to be of environmental concern (see section about environmental relevance of active metabolites for further details).

Active metabolite	Fexcreta	Parent compound	<i>Pharmacological activity</i>
<u>diacetolol</u>	0.5	acebutolol	equipotent to parent compound
oxypurinol	ND	allopurinol	less active than parent compound
N-desethylamiodarone	ND	amiodarone	equipotent as sodium channel blocker less active as antagonist of the calcium channel
nortryptiline	ND	amitriptyline	active, no further details
<u>salicylic acid</u>	ND	aspirin *	active, related to the pharmacological effect
<u>2-hydroxy-atorvastatin</u>	ND	atorvastatin *	active, related to the pharmacological effect
<u>4-hydroxy-atorvastatin</u>	ND	atorvastatin *	active, related to the pharmacological effect
β -acid metabolite	0.05	baclofen	active, no further details
10,11-epoxy metabolite	ND	carbamazepine	active, partially responsible for carbamazepine intoxication
desmethylcarvedilol	ND	carvedilol	2.5 times more potent in rabbits
4-OH-phenylcarvedilol	ND	carvedilol	13 times more potent in rabbits
4 different metabolites	0.22 (all metabolites)	ciprofloxacin	some metabolites may have an antibacterial activity
<u>14-OH-clarithromycin</u>	0.15	clarithromycin	more active on certain bacterial strains (<i>H influenza</i>), synergistic action with clarithromycin
clofibric acid	0.99	clofibrate *	active, linked to the pharmacological effect
<u>norpropoxyphene</u>	0.25	dextropropoxyphene	substantially less central-nervous-system depressant effect than dextro but a greater local anaesthetic effect
<u>fenofibric acid</u>	0.6	fenofibrate *	active, linked to the pharmacological effect
<u>norfluoxetine</u>	0.2	fluoxetine	equipotent to parent compound
cetirizine	ND	hydroxyzine	active, used as patent medicine
<u>2-OH-ibuprofen</u>	0.25	ibuprofen	no data on pharmacological activity
<u>carboxy-ibuprofen</u>	0.37	ibuprofen	no data on pharmacological activity
desipramine	0.06	imipramine	equipotent to parent compound
OH-metronidazole	0.28	metronidazole	between 30 and 50% of the metronidazole activity
desmethylnaproxen	0.28	naproxen	may be pharmacologically inactive
unidentified metabolites	0.08	norfloxacin	some metabolites may have an antibacterial activity
<u>perindoprilat</u>	0.38	perindopril *	active, linked to the pharmacological effect
4-OH-propranolol	ND	propranolol	equipotent to parent compound
<u>ramiprilat</u>	0.12	ramipril *	6 times more active than ramipril
25-O-deacetyl rifampicin	about 0.5	rifampicin	equipotent to parent compound
β -OH-acid metabolite	0.55 **	simvastatin *	active, linked to the pharmacological effect
<u>acetylsulfamethoxazole</u>	0.6	sulfamethoxazole	no antibacterial activity
<u>demethyltramadol</u>	0.6	tramadol	active, analgesic effect, no further details
<u>O-desmethylvenlafaxine</u>	0.3	venlafaxine	active, no further details
Zopiclone-N-oxide	0.15	zopiclone	less active than parent compound
	1	diazepam	
<u>oxazepam</u>	1	clorazepate	active, used as a patent anxiolytic (see section 3.2)
	1	nordazepam	
	ND	prazepam	

ND : no excretion rate value could be determined.

* Indicates a prodrug.

** Data from Carlsson et al., 2006.

Table 3. Wastewater treatment plants (WWTPs) removal fraction for pharmaceuticals and metabolites

	Wastewater treatment plants removal fraction								min value	max value
	Ternes 1998	Stumpf <i>et al.</i> 1999	Paxéus 2004	Bendz <i>et al.</i> 2005	Clara <i>et al.</i> 2005	Paffoni <i>et al.</i> 2006	Castiglioni <i>et al.</i> , 2006 *	Castiglioni <i>et al.</i> , 2006 **		
amoxicillin						0.29	0.49 - 1 (md = 0.75)	1	0.29	1
atenolol			< 0.1			0.52	0 - 0.21 (md = 0.1)	0.36 - 0.76 (md = 0.55)	0	0.76
azithromycin						0.43				0.43
bezafibrate	0.83	0.5			0.4 - > 0.9	0.72	0 - 0.66 (md = 0.15)	0 - 0.98 (md = 0.87)	0	0.98
carbamazepine	0.07		< 0.1 - 0.53	0.3		0.19	0	0	0	0.19
ciprofloxacin						0.62	0.45 - 0.78 (md = 0.6)	0.53 - 0.69 (md = 0.63)	0.45	0.78
clarithromycin						0.69	0 - 0.24 (md = 0)	0	0	0.69
diclofenac	0.69	0.75	< 0.1 - 0.8	0.22		0.27			0.1	0.75
doxycycline						0.06				0.06
fenofibrate						> 0.01				> 0.1
fenofibric acid	0.64	0.45				0.82			0.45	0.82
furosemide							0 - 0.17 (md = 0.8)	0.15 - 0.62 (md = 0.54)	0	0.62
ibuprofen	0.09	0.75	0.52 - 0.99	0.96	> 0.9	0.96	0.25 - 0.72 (md = 0.38)	0 - 1 (md = 0.93)	0	1
OH-ibuprofen				0.95						0.95
carboxy-ibuprofen				0.96						0.96
ketoprofen		0.69		0.65		0.93			0.65	0.93
metoprolol	0.83		< 0.1 - 0.1			0.1			0.1	0.83
naproxen	0.66	0.78	0.48 - 0.93	0.93		0.88			0.48	0.93
ofloxacin						0.4	0 - 0.62 (md = 0.43)	0.33 - 0.66 (md = 0.57)	0	0.66
propranolol	0.96			0.32		0.22			0.22	0.96
ranitidine							0 - 0.76 (md = 0.39)	0.72 - 0.89 (md = 0.84)	0	0.84
roxithromycin					0.5 - 0.6	0.51			0.5	0.6
spiramycine						0.94	0 - 0.11 (md = 0)	0	0	0.94
sulfamethoxazole					0.5 - 0.6	0.64			0.5	0.64
trimethoprim			< 0.1 - 0.4	0.49		0.51			0.1	0.51
vancomycin						1				1

md : median value.

*: removal fraction determined in winter (Castiglioni *et al.* 2006).

** : removal fraction determined in summer (Castiglioni *et al.* 2006).

Table 4. Comparison of calculated PEC for wastewater treatment plants (WWTP) effluents with field measurements conducted in France.

PEC are determined using equation (5) and are expressed in ng.l⁻¹.

	Pharmaceutical concentrations in French WWTP effluents (ng.l ⁻¹)				Calculated PEC for WWTP effluents	
	Paffoni <i>et al.</i> 2006	Miège <i>et al.</i> 2006	Andreozzi <i>et al.</i> 2003	Rabiet <i>et al.</i> 2006	PEC _C (ng.l ⁻¹) ^a	PEC _C (ng.l ⁻¹) ^b
amoxicillin	40				0 - 48615	48615
atenolol	570				1000 - 4190	2010
azithromycin	101					260
bezafibrate	840		ND - 1070		95 - 4760	1330
carbamazepine	1020		980 - 1200	157 - 293	NA	NA
ciprofloxacin	101		60		305 - 765	530
clarithromycin	117				190 - 620	190
diazepam	ND			ND	negligible *	negligible *
diclofenac	810		250 - 410	211 - 486	85 - 305	250
doxycycline	73					965
fenofibrate	310		20 - 120			< 180
ibuprofen	600		20 - 1820	18 - 219	0 - 13700	550
ketoprofen	270		ND - 1620	22 - 1081	295 - 1475	295
metoprolol	100	509 - 1774	80		15 - 90	90
naproxen	350		510 - 1730	42 - 289	420 - 3100	715
ofloxacin	177		330 - 510		320 - 940	565
propranolol	190	416 - 1111			25 - 535	535
roxithromycin	50				155 - 195	190
simvastatin	ND				negligible *	negligible *
sulfamethoxazole	205		70 - 90		550 - 765	550
trimethoprim	72		20 - 40		185 - 345	185
vancomycin	ND					negligible **

ND: not detected in WWTP effluents

NA: not applicable: carbamazepine Fexcreta values were not determined in this study.

a: distribution of PEC calculated using minimal and maximal WWTP removal rates reviewed (see table 3 for details)

b: PEC calculated using WWTP removal rates calculated by Paffoni *et al.*, 2006.

*: PEC_C are considered negligible considering the very low Fexcreta values of 0.01 assumed for diazepam and simvastatin.

** : PEC_C is considered negligible as Paffoni *et al.* (2006) reports a WWTP removal fraction of 1.

WWTP measured concentrations from Paffoni *et al.* (2006) are mean concentrations.

Table 5. Calculated surface water PEC values for relevant metabolites.

Metabolite	PEC _B (ng.l ⁻¹)	PEC _C (ng.l ⁻¹)	measured concentration	sample	reference
salicylic acid	NA	NA	25	surface water	Ternes, 1998
acetylsulfamethoxazole	230	NA	161 ^a	WWTP effluent	Ashton <i>et al.</i> 2004
hydroxy-ibuprofen	1370	70	70 ^a	downstream WWTP	
			50	WWTP effluent	
			20	downstream WWTP	Bendz <i>et al.</i> 2005
carboxy-ibuprofen	2025	100	430	WWTP effluent	
			230	downstream WWTP	
14-OH-clarithromycin	50	NA	ND	-	-
OH-metronidazole	235	NA	ND	-	-
25-O-deacetyl-rifampicin	25	NA	ND	-	-
norfluoxetine	17	NA	ND	-	-
oxazepam	205 ^c	NA	1500	surface water	Togola <i>et al.</i> , 2007
zopiclone N-oxide	7	NA	ND	-	-
norpropoxyphene	295	NA	ND	-	-
demethyltramadol	355	NA	ND	-	-
β-OH-acid metabolite *	90	NA	ND	-	-
			1260 ^a	WWTP effluent	Paffoni <i>et al.</i> 2006
fenofibric acid	1175	2068	380 ^b	WWTP effluent	
			45 ^b	surface water	Ternes, 1998
perindoprilat	4	NA	ND	-	-
ramiprilat	3	NA	ND	-	-

PEC values were calculated using equation 5. Sample and references report to measured concentrations.

All PECs are calculated for surface waters except the PEC for fenofibric acid which is calculated for WWTP effluents.

*: active metabolite of simvastatin.

a: mean values.

b: median values.

c: oxazepam PEC was calculated by summing different sources for this compound.

ND: not detected or not already searched in the aquatic environment.

NA: not applicable due to lack of data.

Table 6. Determination of risk quotients for pharmaceutical compounds (compounds are sorted by decreasing risk ratios values).

compound	most sensible specie	taxa	endpoint	reference of the ecotoxicological assay	AF used	PNEC final value (ng.l ⁻¹)	PEC _A value (ng.l ⁻¹)	PEC _B value (ng.l ⁻¹)	PEC _C value (ng.l ⁻¹)	PEC/PNEC ratio
amoxicillin	<i>S. leopoliensis</i>	cyanobacteria	growth	Andreozzi <i>et al.</i> 2004	10 *	78			4860	62.3
aspirin	<i>Daphnia. magna</i>	cladoceran	reproduction	Marques <i>et al.</i> 2004	100	10000	9046			0.9
ofloxacin	<i>S. leopoliensis</i>	cyanobacteria	growth	Ferrari <i>et al.</i> 2004	50	100			56.5	0.56
propranolol	<i>Hyallolela azteca</i>	gammaridae	reproduction	Huggett <i>et al.</i> 2002	10 *	100			54.5	0.54
carbamazepine	<i>Ceriodaphnia dubia</i>	cladoceran	reproduction	Ferrari <i>et al.</i> 2004	10 *	2500	765			0.31
furosemide	<i>Ceriodaphnia. dubia</i>	cladoceran	reproduction	Isidori <i>et al.</i> 2006	100	1560		486		0.31
clarithromycin	<i>D. magna</i>	cladoceran	reproduction	Yamashita <i>et al.</i> 2006	50	62			19	0.3
diclofenac	<i>Ceriodaphnia dubia</i>	cladoceran	reproduction	Ferrari <i>et al.</i> 2004	10 *	100			25	0.25
sertraline	<i>Ceriodaphnia. dubia</i>	cladoceran	reproduction	Henry <i>et al.</i> 2003	100	90		20		0.22
sulfamethoxazole	<i>S. leopoliensis</i>	cyanobacteria	growth	Ferrari <i>et al.</i> 2004	10 *	590			55	0.1
fluoxetine	<i>unspecified</i>	chlorophyceae	growth	FDA-CDER 1996	10 *	100		9		0.09
fenofibrate	<i>Daphnia. magna</i>	cladoceran	reproduction	Garric <i>et al.</i> 2006	50	140			< 18	< 0.13
paroxetine	<i>Ceriodaphnia. dubia</i>	cladoceran	reproduction	Garric <i>et al.</i> 2006	50	600		4		6.67 10-3
fluvoxamine	<i>Ceriodaphnia. dubia</i>	cladoceran	reproduction	Henry <i>et al.</i> 2004	50	7400	26			3.5 10-3
citalopram	<i>Ceriodaphnia. dubia</i>	cladoceran	reproduction	Henry <i>et al.</i> 2003	100	8000		32		4 10-3
ibuprofen	<i>Daphnia.magna</i>	cladoceran	reproduction	Han <i>et al.</i> 2006	100	2. 10 ⁵			55	2.7 10-4
trimethoprim	<i>S. capricornutum</i>	chlorophyceae	growth	Eguchi <i>et al.</i> 2004	100	2,55. 10 ⁵			19	7.45 10-5
acebutolol	<i>Ceriodaphnia dubia</i>	cladoceran	reproduction	Garric <i>et al.</i> 2006	50	1,25. 10 ⁶	NA	NA	NA	NA
erythromycin	<i>S. capricornutum</i>	chlorophyceae	growth	Eguchi <i>et al.</i> 2004	50	206	NA	NA	NA	NA
clofibric acid	<i>Ceriodaphnia dubia</i>	cladoceran	reproduction	Ferrari <i>et al.</i> 2004	10 *	64	negligible	negligible	negligible	negligible
clofibrate	<i>Daphnia.magna</i>	cladoceran	reproduction	Köpf 1995 ***	50	200	negligible	negligible	negligible	negligible

AF: Assessment Factor.

Taxa species and references refer to the assay that showed the lowest NOEC.

* Indicates that risk quotients for these compounds were calculated applying the EMEA methodology (three NOEC values from three different taxonomic groups). Other risk ratios were conducted using TGD (2003) recommendations (with only one or two NOEC values). When three NOEC values for three different taxonomic groups were available, a factor of 10 was applied to the lowest NOEC value; when two NOECs were available, a factor of 50 was applied; a factor of 100 was applied when only one NOEC value was available.

** Quoted by Webb 2001, in Kummerer 2001.

ND: not determined.

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