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Impact of wastewater treatment plants on receiving surface waters and a tentative risk evaluation: the case of estrogens and betablockers

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Abstract

Five estrogenic hormones (unconjugated + conjugated fractions) and 10 betablockers were analyzed in three wastewater treatment plant (WWTP) effluents and receiving river waters in the area of Lyon, France. In the different samples, only two estrogens were quantified: estrone and estriol. Some betablockers, such as atenolol, acebutolol and sotalol, were almost always quantified, but others, e.g. betaxolol, nadolol and oxprenolol were rarely quantified. Concentrations measured in river waters were in the ng/L range for estrogens and between 0.3 and 210 ng/L for betablockers, depending on the substance and the distance from the WWTP outfall. The impact of the WWTP on the receiving rivers was studied, and showed a clear increase in concentrations near the WWTP outfall. For estrogens, the persistence in surface waters was not evaluated given the low concentrations levels (around 1 ng/L). For betablockers, concentrations measured downstream of the WWTP outfall were up to 16 times higher than those measured upstream. Also, the persistence of metoprolol, nadolol and propranolol was noted even 2 km downstream of the WWTP outfall. The comparison of betablocker fingerprints in the samples collected in effluent and in the river also showed the impact of WWTP outfall on surface waters. Finally, a tentative environmental risk evaluation was performed on 15 sites by calculating the ratio of receiving water concentrations to predicted non-effect concentrations (PNEC). For estrogens, a total PNEC of 5 ng/L was considered, and these substances were not linked to any potential environmental risk

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(only one site showed an environmental risk ratio above 1). Unfortunately, few PNECs are available, and risk evaluation was only possible for 4 of the 10 betablockers studied: acebutolol, atenolol, metoprolol and propranolol. Only propranolol presented a ratio near or above 1, showing a possible environmental risk for 4 receiving waters out of 15.

Keywords: estrogens, betablockers, wastewater treatment plant, surface water, risk evaluation

Abbreviations: E1 (estrone), αE2 (17α-estradiol), βE2 (17β-estradiol), E3 (estriol), EE2 (ethynylestradiol), E1-D4 (estrone-D4), E2-D2 (17β-estradiol-D2), E3-D2 (estriol-D2), EE2-D4 (17α-ethynylestradiol-D4), Eff. (effluent), SW (surface water), WWTP (wastewater treatment plant), ACE (acebutolol), ATE (atenolol), BET (betaxolol), BIS (bisoprolol), MET (metoprolol), NAD (nadolol), OXP (oxprenolol), PROP (propranolol), SOT (sotalol), TIM (timolol), ATE-D7 (atenolol-D7), MET-D7 (metoprolol-D7), PROP-D7 (propranolol-D7), MEC (measured environmental concentration), PNEC (predicted non-effect concentration), LC-MS/MS (liquid chromatography coupled with tandem mass spectrometry), MRM (multiple reaction monitoring), PEC (predicted environmental concentration), QMNA5 (5-year lowest water flow discharge), NOEC (chronic non-observed effect concentration), EC50 (half maximal effective concentration)

1. Introduction

Pharmaceuticals have been quantified worldwide at the ng/L level in surface waters (Grujic *et al*., 2009; Kasprzyk-Hordern *et al*., 2009; Zhou *et al*., 2009). Wastewater treatment plants (WWTPs) are recognized as the main entryway of these substances into the aquatic environment (Bendz *et al*., 2005; Castiglioni *et al*., 2006; Nakada *et al*., 2006). WWTPs are not designed to treat and remove pharmaceuticals, which are only degraded to some degree during sewage

treatment (Miege *et al*., 2009b). Betablockers are a class of drugs used for various indications such as cardiac arrhythmias, hypertension and cardioprotection after a heart attack. These pharmaceuticals are widely used in France, where, for example, more than 18 t of atenolol were consumed in 2004 (Besse *et al*., 2008). Once consumed, betablockers are excreted partly unchanged (Vieno *et al*., 2006; Hernando *et al*., 2007; Maurer *et al*., 2007). The consequences of their presence in the aquatic environment are not well-documented but the problem arised since beta-adrenergic receptors were found in fish (Haider and Baqri, 2000). Also, growth dysfunctions were observed on invertebrates in the presence of 0.5 mg/L of propranolol (Huggett *et al*., 2002). Unlike betablockers, estrogens can have a natural origin and are secreted daily by the human body (Ternes and Joss, 2006). They can also be consumed to treat, for example, menopausal problems or for contraception. In Europe, EE2, a synthetic estrogen, is commonly used in contraceptive pills: in 2004 in France, 40 kg of EE2 was consumed (Besse *et al*., 2008). These substances act as endocrine-disrupting compounds and can induce effects on fish reproduction from the ng/L level (Hansen *et al*., 1998; Larsson *et al*., 1999; Jobling *et al*., 2003; Gutjahr-Gobell *et al*., 2006).

Betablockers in WWTP effluents and surface waters have already been studied (Ternes, 1998; Andreozzi *et al*., 2003; Gros *et al*., 2006; Miege *et al*., 2006; Vieno *et al*., 2006), but most of the analytical methods used are multi-residue methods (i.e. with a lower sensitivity) and only aimed at analyzing 4 or 5 betablockers. In France, few data for concentrations in rivers are available (Andreozzi *et al*., 2003; Miege *et al*., 2006; Coetsier *et al*., 2009) and to our knowledge, no data are available for atenolol, nadolol and sotalol, which are among the most hydrophilic betablockers, and widely used in France. Many studies have focused on estrogens and their analysis in effluents and surface waters (Baronti *et al*., 2000; Kuch and Ballschmiter, 2001; Lagana *et al*., 2004; Vethaak *et al*., 2005; Morteani *et al*., 2006; Vigano *et al*., 2006; Loos *et al*., 2007; Kuster *et al*., 2008), but few have been conducted in France (Cargouet *et al*., 2004; Labadie and Budzinski, 2005; Vulliet *et al*., 2008; Miege *et al*., 2009c). Although some estrogens are often

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analyzed (such as E1, βE2 and EE2), others, such as αE2 and E3 are less frequently studied. Labadie *et al*. (2005) studied the impact of a WWTP on its receiving river by analyzing estrogens. Samples were collected from 30 m to 10 km downstream of a WWTP outfall, and the work focused on temporal and spatial steroid distributions along the river. Also, Castiglioni *et al*. (2006) studied the distribution and fate of pharmaceuticals (including atenolol, estrone, 17β-estradiol and ethynylestradiol) in surface water receiving effluents from a WWTP; percentage attenuation in river water was also evaluated. However, these studies focused only on one site. In addition, Vieno *et al*. (2006) studied phamaceuticals in two rivers impacted by a WWTP, but only 4 betablockers were analyzed (acebutolol, atenolol, metoprolol and sotalol). The authors compared concentrations at different sampling points in the river and estimated loss of the compounds by comparing the load in the downstream river with the loads from all the WWTPs located on the river. Finally, Miege *et al*. (2009) proposed a risk evaluation study on 5 rivers impacted by effluent release. However, the study was based only on predictive environmental concentrations in rivers calculated from measured concentrations in effluent. Hence further investigations are needed to improve our knowledge of the extent of river contamination caused by WWTP outfall, which may vary according to the geographical location or the type of WWTP.

The objective of this study was to evaluate the impact of selected French WWTPs on the receiving waters. We analysed 10 betablockers: acebutolol (ACE), atenolol (ATE), betaxolol (BET), bisoprolol (BIS), metoprolol (MET), nadolol (NAD), oxprenolol (OXP), propranolol (PROP), sotalol (SOT) and timolol (TIM), and 5 estrogens: estrone (E1), 17α-estradiol (αE2), 17β-estradiol (βE2), estriol (E3) and ethynylestradiol (EE2) selected for their high consumption, the data available in the scientific literature or their toxicity. To evaluate the impact of WWTPs on receiving rivers, three sites located in the Lyon area were studied and both effluent and river samples were analyzed. For each site, concentrations measured in rivers upstream and downstream of the WWTP outfalls were compared. A tentative risk evaluation was performed for these three

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sites, using either measured concentrations in rivers or predicted concentrations in rivers from measured concentration in effluents. This tentative risk evaluation was completed using results obtained on 12 WWTP effluents previously analysed (Gabet-Giraud *et al*, 2010), and after calculation of predicted concentrations in downstream rivers.

2. Materials and methods

2.1. Sampling

To study the impact of wastewater treatment plants (WWTPs) on surface waters, river water and effluent samples were collected at three sites (Beaujeu, Bourgoin-Jallieu and Fontaines-sur-Saône, Figure 1). Automatic 24 h composite samples were collected for effluent according to Gabet-Giraud *et al* (2010). Grab surface water samples were collected in 2.5 L amber glass bottles previously rinsed twice with the sample water. All the samples were stored at 4 °C during transport to the laboratory. Filtering and extractions were performed within 24 h after sampling.

Three rivers located in the area of Lyon (France) were selected for this study (Figure 1). The River Saône (length 480 km) is the main tributary of the River Rhône. The sampling site, near Fontaines-sur-Saône, is impacted by domestic and industrial contaminations. The River Ardières (length 9.9 km) is a tributary of the River Saône. The site, near the town of Beaujeu, is impacted by different contamination sources: wastewater treatment plants, industry and agriculture (mainly vineyards). The River Bourbre (length 72.2 km) is another tributary of the River Rhône. This site, near the town of Bourgoin-Jallieu, is impacted by domestic and industrial contamination.

Figure 1.

The characteristics of the WWTPs studied are presented in Table 1. Two are equipped with a primary treatment system (primary settling). Biological treatments consist of activated sludge (conventional or medium rate) or a biological filter.

Table 1.

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To study the impact of WWTPs, samples were collected in effluents and in rivers up- and downstream of the effluent outfall, as described in Table 1. For the River Bourbre a more complex configuration required a different sampling methodology (Figure 2). The River Bourbre was sampled at one sampling point located 20 m upstream, and two sampling points located 5 m and 2000 m downstream of the confluence with the Bion, which receives the WWTP effluent. The Bion was also sampled 2000 m upstream of the WWTP outfall.

To obtain representative results, sampling was performed once per week for at least three consecutive weeks. The Saône River was sampled on 6 different days in November 2007 and June 2008. The River Ardières was sampled 3 times in June 2008. The River Bourbre was sampled 4 times in September 2008. The Bion River samples are not considered in this paper (River Bion flow is negligible compared with the WWTP effluent flow). A total of 42 surface water samples (18 for Saône, 12 for Ardières and 12 for Bourbre) and 8 effluent samples (2 for Saône, 2 for Ardières and 4 for Bourbre) were collected between November 2007 and September 2008.

Figure 2.

2.2. Sample preparation and analysis

Water samples were first filtered through pyrolyzed (450 °C, 1 h) glass fiber filters (GF/F, 0.7 µm pore size).

The analytical method for the 5 estrogens is described elsewhere (Miege *et al*., 2009a). Briefly, aliquots of filtrate spiked with deuterated estrogens (E1-D4, E2-D2, E3-D2 and EE2-D4) were extracted by solid phase extraction (SPE) on Oasis HLB® cartridges and purified on Florisil cartridges. Extracts were evaporated to dryness and reconstituted in a solution of E2 acetate, used as an internal standard. For the analysis of total estrogens (i.e. after hydrolysis of conjugated forms), enzymatic cleavage was performed using β-glucuronidase isolated from *Helix pomatia* before extraction.

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The 10 betablockers were analysed as described in Gabet-Giraud *et al* (2010). Briefly, aliquots of acidified filtrate were extracted by SPE on Oasis MCX® cartridges, evaporated to dryness and reconstituted in a solution of metoprolol impurity A, used as an internal standard. Aliquots of each sample were also spiked with the 10 betablockers to define recoveries for each type of sample (i.e. river or effluent).

Analysis of estrogens and betablockers was performed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) with acquisition in multiple reaction monitoring (MRM) mode. As recommended in the EU Commission Decision 2002/657/EC, the MS-MS conditions included the use of two ionization transitions for each compound (except for the deuterated surrogates), one for quantification and one for identity confirmation.

Final estrogen concentrations were calculated using recoveries obtained for the internal deuterated surrogates (αE2 and βE2 were both corrected by E2D2). For betablockers, final concentrations were calculated using recoveries obtained for the non-deuterated betablockers in spiked samples: concentrations were corrected only if the recoveries were below 80% or above 120%.

Method limit of quantification (LOQ) was estimated for each substance/sample pair as described elsewhere (Miege *et al*., 2009a). For E1, αE2, βE2 and E3, LOQ values ranged between 0.3 and 2.7 ng/L, it can reached 9.0 ng/L for EE2. For betablockers, LOQ ranged between 0.2 and 1.1 ng/L.

2.3. Environmental risk evaluation

To evaluate the potential impact of each substance on the aquatic environment, we determined a quotient risk calculated as the ratio of a measured environmental concentration (MEC) to a predicted non-effect concentration (PNEC). When MEC is not available, a predicted

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environmental concentration (PEC) can be used (European Commission, 2003). An ecological risk is suspected when the ratio (MEC or PEC)/PNEC equals or exceeds 1 for a given substance.

A tentative risk evaluation was compared using either the MEC or the PEC obtained in the three rivers studied (part 3.5). This tentative risk evaluation was completed (part 3.6) for receiving rivers of 12 WWTPs located in France (Paris area, Lyon area and in the south of France) using the PEC in rivers calculated from mean concentrations (n = 2 or 3) measured in effluent samples (AMPERES project, Gabet-Giraud *et al*., 2010).

For PEC calculation, fluxes of micropollutants were calculated (from concentrations measured in effluents and WWTP flow) and divided by the 5-year lowest water flow discharges of the receiving river (European Commission, 2003).

The 15 WWTPs (including the three studied in this paper) have various capacities (between 2900 and 700,000 PE) and discharge their effluents into rivers of different sizes (from 0.02 to 600 m^3/s).

3. Results and discussion

3.1. Occurrence of estrogens

The frequency of quantification and measured concentrations are presented in Table 2. Three estrogens (αE2, βE2 and EE2) were never quantified in effluents or in surface waters. The most frequently quantified estrogen was E1, present in all the effluent samples and in 98% of surface water samples. Concentrations of E1 ranged from 1.7 to 20 ng/L (mean 1.6 ng/L) in effluent samples and from 0.3 to 3.9 ng/L (mean 9.3 ng/L) in surface waters. Also, E1 is one of the estrogen present at the highest concentrations in influent samples (Gabet-Giraud *et al*., 2010); it is produced by biodegradation of βE2 and EE2 (Ternes *et al*., 1999a; Czajka and Londry, 2006; Ren *et al*., 2007). E3 was quantified only in the effluent samples from Fontaines-sur-Saône WWTP, and in 1 of the 9 samples collected in the River Saône downstream of the WWTP. The measured

concentrations in the effluent samples were high (between 202 and 218 ng/L), but decreased to 26 ng/L in surface waters downstream from the WWTP.

In the literature, as here, αE2, βE2 and EE2 have not been quantified in effluent or in surface water (Boyd *et al*., 2003; Rodriguez-Mozaz *et al*., 2004; Labadie and Budzinski, 2005; Farre *et al*., 2006; Kuster *et al*., 2008; Vulliet *et al*., 2008). A recent review (Miege *et al*., 2009b) showed that E1 was quantified in 93% of the effluent samples studied (n = 79), at concentrations ranging between 0.6 and 95 ng/L (mean value 20.9 ng/L), in agreement with our results. This review also showed that E3 was quantified in more than 90% of the effluent samples (n = 33); that is in the range of the maximum values reported in our study. Results from the literature confirmed a low quantification frequency of E3 in surface waters (Rodriguez-Mozaz *et al*., 2004; Labadie and Budzinski, 2005; Farre *et al*., 2006; Kuster *et al*., 2008). Reported concentrations of E3 in surface waters ranged from 1 to 50 ng/L (Morteani *et al*., 2006; Vigano *et al*., 2006; Kuster *et al*., 2008; Peng *et al*., 2008). A study conducted on the Mississippi River in the United States (a site outside the direct influence of discharge points from WWTPs) and on Lake Pontchartrain (Louisiana USA), showed that E1 was never detected (n = 4, LOD = 0.3 ng/L) (Boyd *et al*., 2003). In a recent survey conducted on several European rivers, including the Rivers Ardières and Bourbre, a quantification frequency of 16% was reported for E1 (n = 122, average concentration of 4 ng/L) (Loos *et al*., 2009); E1 was quantified at 3 ng/L in the River Bourbre, but was not quantified in the River Ardières. However, in this study the LOD was relatively high for E1 (2 ng/L). By contrast, in a study conducted in Italy in the Rivers Po and Lambro, E1 was quantified in all samples (n = 3) between 4 and 47 ng/L (Vigano *et al*., 2006); E3 was also quantified in all samples (n = 3) at concentrations ranging between 4 and 50 ng/L. In samples collected in the River Tamagawa and Lake Kasumigaura (Japan) receiving WWTP outfall, E1 was quantified systematically at concentrations between 0.2 and 3.8 ng/L (n = 8) and E3 was never detected (LOD of 1.5 ng/L) (Isobe *et al*., 2003). In France, a study conducted on an urban dam (receiving effluents from

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various WWTPs and industrial effluents) and a lake (supplied by different rivers from rural zones) in the Rhône-Alpes area, showed that E1 was only quantified in one lake sample at 0.3 ng/L (Vulliet *et al.*, 2008). E1 was never detected in the River Seine, between 200 and 355 km downstream of the city of Paris (n = 6, LOD between 0.3 and 8.0 ng/L depending on the analyte and the matrix) (Labadie and Budzinski, 2005), while it was quantified from 1.1 to 3.0 ng/L in all samples (n = 6) collected in the Rivers Seine and Oise upstream and downstream (about 60 km) of Paris (Cargouet *et al.*, 2004). However, some studies reported higher concentrations: up to 65 ng/L in a Chinese river located in one of the most developed and densely populated areas of China (Peng *et al.*, 2008).

The differences between total (i.e. conjugated + unconjugated fractions) and unconjugated fractions of estrogens were not significant. The proportion of free (i.e. unconjugated) estrogens represented on average about 80% of the total estrogens for effluent and surface waters. The differences between estrogen concentrations measured in samples with and without hydrolysis could be linked to the analytical uncertainty, which was estimated in the range 20–50% depending on the measured concentration. Thus we can consider that most of the estrogens in surface water and effluent samples are present in the free form. Comparable results were obtained in surface water samples (Belfroid *et al.*, 1999). Estrogens are excreted by bodies in conjugated forms (glucuronide or sulfate) which are more soluble, but in activated sludge processes, cleavage (E1-3S in E1, and E2-17G in βE2) was observed (Ternes *et al.*, 1999b; Baronti *et al.*, 2000).

Table 2

3.2. Occurrence of betablockers

Betablockers were analyzed in 8 effluents and 34 surface water samples (Table 2); they were not measured in the River Saône sampled in June 2008.

In effluent samples, ACE, ATE, BIS, MET, PROP and SOT were always quantified. The other betablockers, TIM, NAD, OXP and BET were quantified in 88, 50, 38 and 25% of effluent samples, respectively. The highest concentrations were observed for ATE, SOT, ACE and PROP, with median concentrations above 138 ng/L. Median concentrations were lower than 50 ng/L for NAD, MET, BIS and OXP, with only traces of TIM.

In surface waters, ACE, ATE and SOT were the most frequently quantified betablockers (>90%), while NAD, BET and OXP were only quantified in fewer than 32% of the samples. BIS, MET, PROP and TIM were quantified at intermediate frequencies (44–82%) in surface water samples, respectively. ATE, ACE and SOT were present at the highest median concentrations: 17, 14 and 5.5 ng/L, respectively. Median concentrations ranged between 0.3 and 4.6 ng/L for other betablockers.

In the literature, ACE, ATE, BIS, MET, PROP and SOT are generally quantified in effluent samples (>86%) with mean concentrations ranging between 10 and 990 ng/L (Andreozzi *et al*., 2003; Vieno *et al*., 2006; Miege *et al*., 2009b). Reported concentrations for a substance can vary widely from one country to another. For example, while MET was quantified at 80 ng/L (n = 2) in France (Andreozzi *et al*., 2003), concentrations between 910 and 1070 ng/L (n = 3) were measured in Finland (Vieno *et al*., 2006). For NAD and OXP, reported detection frequencies were higher than those observed in our study. In a study conducted in the United States of America, NAD was quantified in 71% of the effluent samples (n = 34) at a median concentration of 51 ng/L (Huggett *et al*., 2003). Also, in a WWTP effluent monitoring campaign conducted in 4 European countries including France, OXP was quantified in 71% of the samples (n = 7, median concentration 20 ng/L) (Andreozzi *et al*., 2003). On the contrary, while TIM was quantified in 88% of the effluent samples in our study (mean concentration of 3.6 ng/L), it was only quantified in fewer than 7% of German effluents (n = 29); however, the LOQ in the German study was relatively high (25 ng/L) (Ternes, 1998). For BET, which was only quantified in 25% of the effluent samples, a previous

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study in 4 European countries including France reported concentrations below LOD (Andreozzi *et al*., 2003). However, the LOD were not detailed.

In surface water, various results have been reported. In a study conducted in the Rivers Po and Lambro, in the most densely inhabited and industrialized areas of Italy, ATE was quantified in all river samples (n = 8) between 3.4 and 241 ng/L (Calamari *et al*., 2003). By contrast, ATE was quantified only in 60% of the samples collected in the River Vantaa, located in the most densely populated area of Finland, at concentrations between 12 and 25 ng/L (Vieno *et al*., 2006); the same study reported a quantification frequency of 80% (n = 5) for ACE, SOT and MET, with concentrations between 2 and 8 ng/L, 15 and 52 ng/L and 20 and 116 ng/L, respectively. Conversely, a study performed in the River Seine in the Paris area quantified MET in only 30% of the collected samples (n = 10) at a mean concentration of 10 ng/L (Paffoni *et al*., 2006); ATE, SOT, PROP and BIS were also analyzed (n = 10, quantification frequency of 100%, 100%, 50% and 0, respectively) and measured with mean concentrations of 26, 45, 12 ng/L and <10 ng/L, respectively. For PROP, the same quantification frequency (50%, n = 6) was observed in a study conducted in the Rivers Taff (UK) and Warta (Poland) with measured concentrations between 5 and 6 ng/L (Kasprzyk-Hordem *et al*., 2007); ATE and MET were also found between 3 and 60 ng/L, and 7 and 155 ng/L, respectively (quantification frequency of 67% and 50%, respectively). In surface water collected in Spain in the Ebro river basin, MET and PROP were not detected (n = 10, LOD of 3 and 2, respectively) and SOT and ATE were quantified at up to 70 and 250 ng/L, respectively (Gros *et al*., 2006). Generally, reported concentrations of BIS, BET, NAD, MET, PROP and TIM are below 10 ng/L (Ternes, 1998; Gros *et al*., 2006; Paffoni *et al*., 2006). However, concentrations measured in surface water can reach high values: BIS and MET were quantified at above 2000 ng/L in river water samples (Ternes, 1998).

3.3. Qualitative impact of WWTPs on surface waters

Generally, among the three sites studied, the River Bourbre was the one where the impact of the WWTP outfall was the most obvious (Figure 3, Supplementary material). The WWTP outfall was the least diluted for this river: the average dilution factor (calculated as the ratio River flow / WWTP flow) was 14, against 70 and 7000 for the Rivers Ardières and Saône, respectively.

Figure 3

For estrogens, because of the relatively low concentrations measured in effluent samples, the impact of WWTPs in surface water was not significant; except in the River Bourbre, where the high concentration of E1 in the effluent (maximum of 16 ng/L) lead to a slight increase in E1 concentration in the river (from 1.6 to 2.5 ng/L, on average) immediately downstream from the WWTP. However, 2 km after the outfall of the WWTP (sampling point “Downstream 2”), concentrations of E1 were equivalent to those measured upstream. E3 was only quantified in effluent samples of Fontaines-sur-Saône (average concentration 210 ng/L) and in one sample collected downstream of the outfall (at 26 ng/L). As only low concentrations of E1 and too few data for E3 were measured in river water, the profile study and also the quantitative study of the impact of WWTPs on downstream rivers (see below) were not conducted on these hormones.

For betablockers, the impact of WWTPs was also more visible in the River Bourbre. For example, the average concentration increased after the WWTP outfall from 14 to 99 ng/L for ACE, from 28 to 123 ng/L for ATE and from 8.4 to 120 ng/L for SOT. In the River Saône, the average concentration increased from 14 to 76 ng/L for ACE, from 10 to 93 ng/L for ATE and from 22 to 67 ng/L for SOT. For these two rivers, the increase in concentrations after the WWTP was also measurable for BIS, MET and PROP. In the River Ardières, average concentrations increased from 9.1 to 39 ng/L for ACE and from 2.2 to 29 ng/L for ATE after the WWTP outfall (for ATE, concentration of upstream 1 was considered because of the abnormally high concentration in upstream 2). However, in the River Ardières, the concentration of SOT from WWTP effluent did not lead to a significant increase of concentration in the river (from 3.5 to 5.2 ng/L).

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To evaluate the impact of WWTP outfall on river water quality, we compared the profile of the 10 betablockers in effluent samples with those of river water upstream and downstream of the WWTP outfall. For each sampling point, the relative abundance of each substance was calculated by dividing its concentration by the sum of all betablocker concentrations. This study was only conducted on samples from the River Bourbre as this was the site where the WWTP impact was the most visible (Figure 4). The river water collected immediately after the WWTP outfall (Downstream 1) and the effluent had similar profiles, whereas the profile observed in river water collected upstream of the WWTP was different from the profile of the effluent sample. Moreover, the profiles of water samples collected upstream and downstream 2 were again similar, showing a return to upstream conditions 2 km downstream the WWTP outfall.

Figure 4

3.4. Quantitative impact of WWTP on surface waters

To evaluate quantitatively the observed gradient from upstream to downstream of the WWTP outfall, we calculated for betablockers a ratio of increase as follows:

$$\text{Increase} = \frac{C_{\text{Downstream}} - C_{\text{Upstream}}}{C_{\text{Upstream}}},$$

where $C_{\text{Downstream}}$ is the measured concentration in the river immediately downstream of the WWTP outfall, and C_{Upstream} is the measured concentration in the river immediately upstream of the WWTP outfall.

When a significant increase was noted, an attenuation percentage was calculated as the difference in concentration between downstream 1 and downstream 2 as follows:

$$Attenuation\ (\%) = \frac{(C_{Downstream\ 1} - C_{Upstream}) - (C_{Downstream\ 2} - C_{Upstream})}{(C_{Downstream\ 1} - C_{Upstream})} \times 100,$$

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where $C_{\text{Downstream 1}}$ is the measured concentration in the river immediately downstream of the WWTP outfall, and $C_{\text{Downstream 2}}$ is the measured concentration in the river at the second point downstream of the WWTP outfall.

As shown in Table 3, concentrations of betablockers in surface waters downstream of the WWTP outfall can be up to 13 times higher than concentrations measured upstream. Concerning the 4 substances quantified in effluents at the highest concentrations (ACE, ATE, SOT and PROP), concentrations increased by a factor of 3 to 13 in the River Bourbre, 0 to 12 in the River Ardières and 2 to 8 in the River Saône. Also, the impact of WWTP outfall was generally greater on the River Bourbre than on the Rivers Saône or Ardières (except for ATE) because of the lower effluent dilution for the River Bourbre. For example, MET, which was quantified at similar concentrations in effluent from Beaujeu and Bourgoin Jallieu WWTP (30–40 ng/L), showed a concentration 6 times higher in surface water after WWTP outfall in the River Bourbre, while no concentration variation was noted for the River Ardières. On the contrary, while ATE concentration was multiplied by 3 after WWTP outfall in the River Bourbre, it was, surprisingly, multiplied by 12 in the River Ardières; this result is linked to the high concentration of ATE measured upstream of the WWTP outfall in the River Bourbre (mean concentration 28 ng/L). For NAD and TIM, which were quantified at low concentrations in all effluent samples (<56 ng/L), the impact of the WWTP outfall was not detected on river concentrations.

To study the fate and behavior of betablockers in the downstream rivers, the attenuation of concentration between samples collected immediately after the effluent outfall (5 m) and samples collected further downstream (2000 m for the River Bourbre and 20 m for the River Ardières) was evaluated. MET and PROP seemed to be the most persistent betablockers, since their attenuation was below 10% at Bourgoin. Higher attenuations of concentration were noted for ACE at both sites: around 54%. Considering the low hydrophobicity of this substance (log *K*ow 1.42) (Detroyer *et al*., 2001), it is not likely to be adsorbed on particulate matter. The attenuation of ACE may

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therefore be explained by degradation occurring in river water even only 20 m downstream of the effluent outfall; likewise for SOT, which was half degraded after 20 m. For ATE, while an attenuation of 61% was noted in the River Bourbre (2000 m), it was only 11% in the River Ardières (20 m) because of a lower degradation. We observed that BIS was 61% degraded after 2000 m. No assertion is possible for NAD and TIM, because measurements were too close to the LOQ. The analysis of particulate matter content and of samples collected at several points downstream of the effluent outfall would give a better understanding of the behavior and fate of betablockers at these sites.

A study reported in the literature on several pharmaceuticals (including ATE) also evaluated the attenuation between measured concentrations along the River Olona (Italy), 100 m and 1000 m downstream a WWTP outfall (Castiglioni *et al*., 2006). It showed an attenuation of 29% for ATE. Surprisingly, ATE was also detected in particulate matter, but exact content could not be quantified. Another study focused on the fate and behavior of several pharmaceuticals, including ACE, ATE, MET and SOT, in the River Vantaa in Finland, downstream of several WWTP outfalls (Vieno *et al*., 2006): Results showed the persistence of ATE and SOT (loss $\leq$ 10%) but a significant elimination of ACE and MET (loss > 60%) along the river. The distance between the last WWTP and the sampling point in the river was much higher than in our study (above 10 km).

Table 3

3.5. Calculation of predicted environmental concentration (PEC) and comparison with measured concentration (MEC)

To assess the representativity of PEC values, PECs in rivers (from effluent measurement) were compared with MECs in rivers (from river measurement, minimum and maximum values), for the three sites studied here (Table 4). For E1, PECs were generally found in the same range as

MECs for the River Bourbre. In contrast, for the Rivers Saône and Ardières, PECs for E1 were systematically underestimated compared with MECs (i.e. PECs 20 to 100 times lower than MECs in the River Saône, 3 to 16 times lower in the River Ardières). These differences can be explained by the presence of other WWTPs upstream of the target WWTP that could contribute to the total amount of micropollutants in the river. Concerning E3, which was only quantified in effluents from one of the three sites studied, PECs were close to LOQ, and therefore it is difficult to draw any firm conclusion. For betablockers, in the Rivers Bourbre and Ardières, 77% of PECs were higher than the corresponding MECs by a factor of 1.1 to 4. Nonetheless, for these rivers, a reasonably fit could generally be noted between PEC and MEC. In contrast, PECs calculated for betablockers in the River Saône were generally underestimated, by a factor up to 30 for BIS. To conclude, the use of PEC is not completely reliable and, unexpectedly, it does not always represent the worst possible case; MEC values, when available, are to be preferred to PEC values.

Table 4

3.6. Tentative risk evaluation for the rivers downstream of 15 French WWTPs

To evaluate the environmental risk linked to the presence of estrogens and betablockers in French rivers, MECs for the three rivers studied here and PECs for the receiving rivers of the 12 WWTPs previously studied were compared for each substance with PNEC values found in the literature (Table 5).

The PNEC values were computed from toxicity tests, but are available only for some of the substances studied. For estrogens, a rough estimation of PNEC at 5 ng/L was made (Stuer-Lauridsen *et al*., 2000). For betablockers, PNEC were available for only 4 of the 10 betablockers analyzed. One author estimated EC50 (half maximal effective concentration) for ATE, MET and PROP on different species: an invertebrate (*Daphnia magna*), an alga (*Desmodesmus subspicatus*) and an aquatic plant (*Lemna minor*). The PNECs were obtained by dividing the lowest EC50 by an

assessment factor of 1000 (Cleuvers, 2005). The following results were obtained: PROP was the most toxic substance with a PNEC at 0.73 µg/L; the PNECs of ATE and MET were evaluated at 7.9 and 310 µg/L, respectively. Another study reported a PNEC derived from chronic tests on fish at 10 ng/L for PROP (Ferrari *et al*., 2004). For ACE, a PNEC was evaluated at 1250 µg/L on an invertebrate (*Ceriodaphnia dubia*) by dividing the lowest NOEC (chronic non-observed effect concentration) by 50 (Garric *et al*., 2006). No PNEC value was found for NAD, but the toxicity tests performed on aquatic invertebrates showed that acute exposure to NAD at 100 mg/L resulted in no change in species survival (Huggett *et al*., 2002). Thus the following PNECs were used for this environmental risk evaluation study: 5 ng/L for total estrogens ("total" meaning the sum of estrogens), 1250 µg/L for ACE, 7.9 µg/L for ATE, 310 µg/L for MET and 10 ng/L for PROP.

For estrogens, total risk evaluation ratios evaluated with MECs of the three rivers studied ranged between 0.3 and 5.5 (Table 5). The atypical ratio of 5.5 is linked to the high concentration of E3 measured in one of the samples collected in the River Saône (25.9 ng/L). Among the receiving rivers of the 12 additional WWTPs studied, total risk evaluation ratios never exceeded 0.8. This shows a generally low predicted environmental risk associated with these substances in the systems studied.

Concerning betablockers, for ACE, ATE and MET, the ratio between MEC (or PEC) and PNEC were in most cases below 0.2, showing an non-significant predicted environmental risk. For PROP, the mean ratios were 0.3, 0.8 and 1.7 for the Rivers Ardières, Saône and Bourbre, respectively (ratio between 0.1 and 0.7 for the River Ardières, between 0.3 and 2.5 for River Saône and between 0.8 and 3.5 for River Bourbre). For PROP, among the receiving rivers of the 15 WWTP effluents presented in Table 5, a ratio below 0.1 was calculated for 2 sites, while 8 sites showed a ratio between 0.1 and 1, and 5 sites had a ratio higher than 1 and up to 34.2. These 5 sites correspond to the rivers where the WWTP effluents were the least diluted (Rivers Bourbre, Maurepas, Maldroit, Bouillide and Ardières). From Table 5, PROP may represent a potential

environmental risk up to a dilution ratio of the effluent in the river of 13. A previous study performed on two large French rivers (the Saône and the Rhône) presented lower ratios PEC/PNEC for PROP: between 0.03 and 0.45 depending on the site studied (Miege *et al*., 2006). In the present study, the fluxes of PROP were in the same range as those observed by Miege *et al.* (2006) but the 5-year lowest water flow discharges were 100 times lower.

Table 5

4. Conclusion

Three different sites in the Lyon area of France, were studied, showing that among the 5 estrogens analyzed, only E1 and E3 were quantified in some effluents and river waters. The most frequently quantified estrogen was E1. The concentration of estrogen reached 220 ng/L in effluent (for E3) and 26 ng/L in river water (for E3). However, mean estrogen concentration in surface water was generally at the ng/L level. Among the 10 betablockers analyzed, ACE, ATE and SOT were quantified in almost all the collected samples. Other substances, such as BET, NAD and OXP, were rarely quantified. Betablocker concentrations could reach up to 2450 ng/L in effluent and 240 ng/L in surface water (for ATE), but mean concentrations of individual betablocker in river water were below 50 ng/L.

The impact of WWTPs on the receiving rivers was manifest for all the sites studied, except for estrogens at Fontaines-sur-Saône, where the effluent was highly diluted in the river (dilution by nearly 7000). Concentrations of estrogens and betablockers generally increased downstream of the WWTP outfall; this was particularly the case on the River Bourbre, which was, among the different sites studied, the one where the effluent was the least diluted in the river. Downstream of the effluent outflow, the betablocker concentrations could be up to 13 times higher than upstream of the WWTP. The study of the attenuation of concentration between samples collected immediately after the effluent outfall (5 m) and samples collected further downstream (20 m or 2

km) showed that some betablockers, such as MET and PROP, were not degraded even 2 km after the WWTP outfall, whereas others, like ACE were mostly degraded (more than 50% of attenuation). The similarity of the relative distribution (i.e. fingerprint) of the betablockers was shown between samples of effluent and river samples collected immediately downstream of the effluent outflow.

An environmental risk evaluation was performed on 15 sites (including the three studied here). For estrogens, the total risk evaluation ratios were systematically below 1, except for one site, thus the low predictive environmental risk was low. With a ratio below 0.1, the predictive environmental risk linked to the presence of ATE, ACE and MET in river water was negligible. On the contrary, ratios above 1 were obtained for PROP, showing a possible environmental risk for 5 sites. Betablockers can represent a potential environmental risk up to a dilution ratio of the effluent in the river of 13. Unfortunately, as no PNEC was found for SOT, it was impossible to evaluate the risk linked to its presence in the aquatic environment, although we showed high concentrations in rivers. Even though potential toxicity due to individual betablocker or estrogen was not proved here, the environmental risk evaluation presented here did not take into account synergistic, antagonist or bioaccumulation effects.

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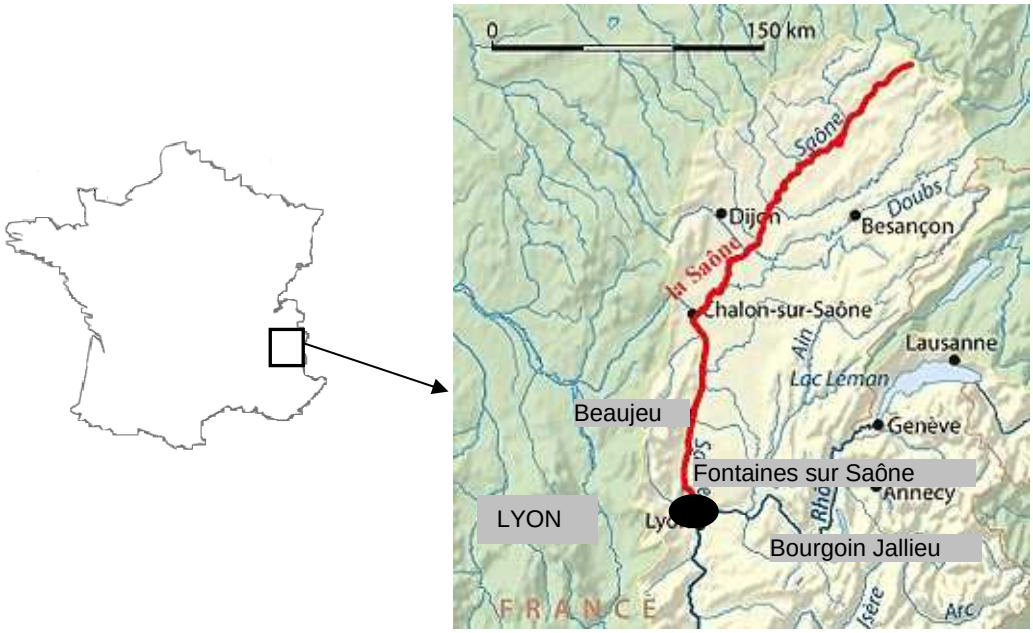

Figure 1. Location of the three sampling sites near Lyon, France

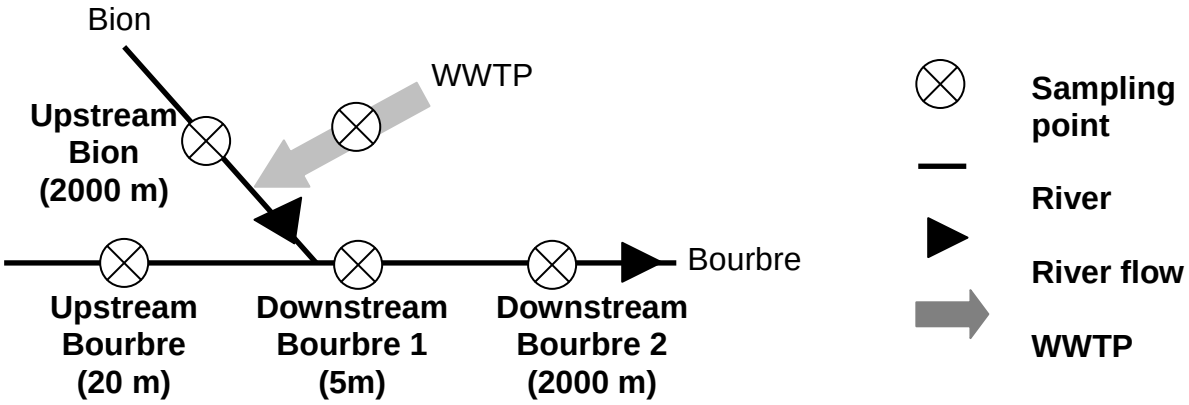

Figure 2. Configuration of the sampling points on the Bourgoin-Jallieu site

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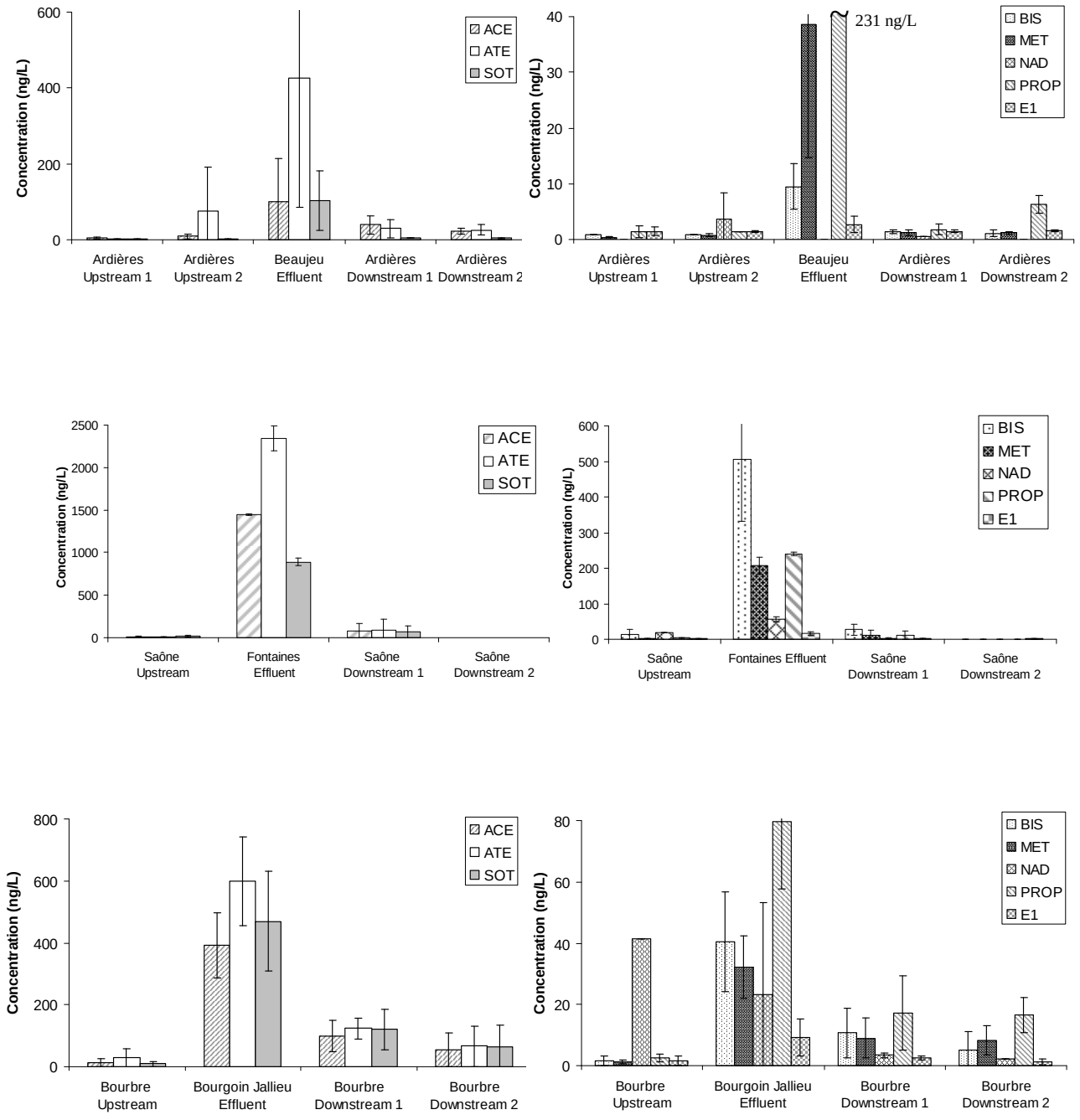

Figure 3. Mean concentrations (± sd) of estrogens and betablockers at the different sampling stations of the three sites studied (a, Ardières; b, Bourbre; c, Fontaines-sur-Saône)

Some data not shown (cf. Table 2): αE2, βE2 and EE2 were never quantified; E3 only quantified in Fontaines-sur-Saône (in all effluents and in one surface water sample); BET and OXP only quantified in the samples from Fontaines-sur-Saône (mean concentrations below 2 ng/L for surface water and of 18 and 30 ng/L in effluents for BET and OXP, respectively); TIM only quantified at low levels (mean concentrations below 3 ng/L in surface water and between 2.9 and 8.7 ng/L in effluent samples)

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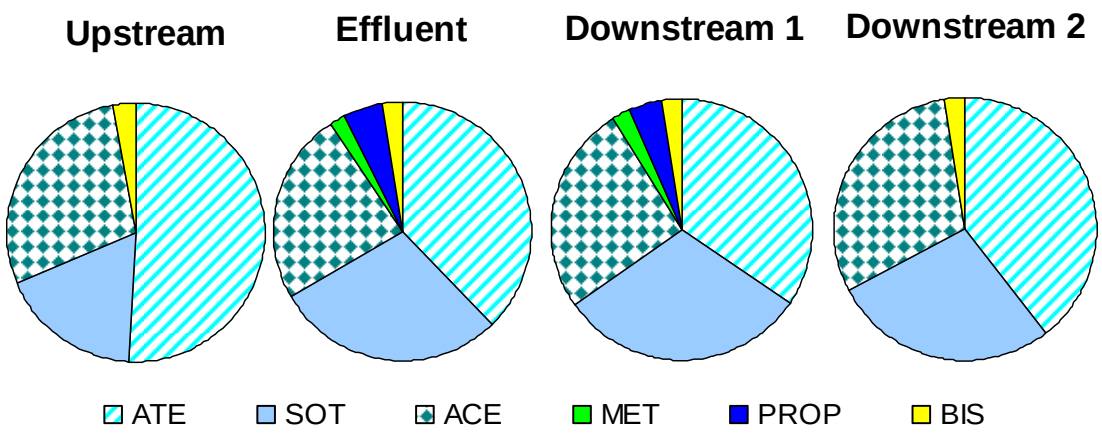

Figure 4. Relative abundance (%) of quantified betablockers for each sampling station in the River Bourbre and in the effluent from the Bourgoin-Jallieu WWTP

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Table 1. Information on WWTP and sampling campaigns

WWTP Treatment process	Population equivalent (PE)	Flow (m3/d)	Receiving River	Sampling points	Sampling period
Low loaded activated sludge (C + N)*	2 900	750	Ardières	Upstream 1 (4000m) Upstream 2 (20m) Effluent Downstream 1 (5m) Downstream 2 (20m)	June 2008
Primary settling + medium-rate activated sludge	78 000	17 500	Bourbre	Cf Figure 2.	Sept. 2008
Primary settling + Biological filter (C + N)	30 000	6 500	Saône	Upstream (200m) Effluent Downstream 1 (50m) Downstream 2 (200m)	Nov. 2007 and June 2008

* conventional activated sludge

Table 2. Concentrations of estrogens and betablockers measured in effluent and surface water samples of three sites in France (Rivers Ardières, Bourbre and Saône and WWTPs) from November 2007 to September 2008

	Quantification frequency (%)		Mean (ng/L)		Median (ng/L)		Min (ng/L)		Max (ng/L)	
	Eff.	SW	Eff.	SW	Eff.	SW	Eff.	SW	Eff.	SW
E1	100	98	9.3	1.6	8.7	1.5	1.7	0.3	20	3.9
E3	25	2	210	26	210	26	202	26	218	26
ACE	100	100	595	35	381	14	68	3.0	1455	183
ATE	100	97	1043	50	705	17	388	0.4	2450	240
BET	25	15	18	0.6	18	0.4	17	0.2	19	1.7
BIS	100	82	149	7.3	37	1.7	6.6	0.3	630	38
MET	100	71	77	4.4	38	1.8	22	0.4	223	29
NAD	50	32	40	9.2	48	4.0	2.1	0.4	61	42
OXP	38	9	20	1.3	27	0.3	0.8	0.1	32	3.4
PROP	100	65	158	8.1	138	4.6	62	0.7	294	35
SOT	100	91	495	34	435	5.5	97	1.6	918	213
TIM	88	44	4.6	1.5	3.6	1.6	1.5	0.8	9.5	2.0

Eff.: effluent (n = 8 for estrogens and betablockers)

SW: surface water (n = 42 for estrogens and 34 for betablockers)

(αE2, βE2, EE2 never detected)

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Table 3. Increase ratio for betablocker concentrations in river samples from upstream to downstream of the WWTP outfall and attenuation (%) measured between the two sampling stations downstream of the WWTP outfall

	WWTP	River	Dilution factor of effluent in river	ACE	ATE	BIS	MET	NAD	PROP	SOT	TIM
Increase ratio	**Bourgoin Jallieu**	**Bourbre**	14	6	3	6	6	-1	6	13	1
	Beaujeu	**Ardières**	70	3	12*	0	1	-1	0	1	0
	Fontaines	**Saône**	7000	4	8	1	5	-1	2	2	1
Attenuation (%)	**Bourgoin Jallieu**	**Bourbre (2 km)**	14	53	61	61	9	nc	5	51	nc
	Beaujeu	**Ardières (20 m)**	70	54	11	nc	nc	nc	nc	47	nc

*calculated with upstream 4000 m before WWTP outfall, because of one suspicious concentration for upstream 20 m before WWTP outfall

nc: attenuation not calculated because measurements were too close to limits of quantification.

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Table 4. Predicted environmental concentrations (PEC) and measured concentrations (MEC, min-max) at the different sampling stations of the three rivers studied downstream of the WWTP outfall

WWTP and River	Sampling day	WWTP flow (m^3/s)	QMNA5 (m^3/s)		Concentration (ng/L)											
					E1 tot	E3 tot	ATE	SOT	NAD	TIM	ACE	MET	OXP	PROP	BET	BIS
Fontaines - Saône River	13/11/07	124	40	PEC	0.03	0.3	3,4	1,3	0,1	0,01	2,2	0,3	0,05	0,4	0,03	1,0
				MEC	0.8 - 3.8	< 0.2	9.8 – 26.5	< 8.9 - 153	< 1.0 - 1.0	< 1.3	11.7 – 23.2	1.8 – 4.4	0.1 – 0.3	< 0.8 - 4.1	0.2 – 0.4	29.6 – 34.7
	16/11/07	267	40	PEC	0.02[a]	0.3[a]	3,5	1,3	0,1	0,01	2,1	0,3	0,04	0,3	0,02	0,5
				MEC[b]	0.7 - 0.8	< 2.6	10.1 – 13.8	24.5 - 28.0	< 1.1	< 1.0	17.9 – 21.0	1.7 – 2.1	< 0.9	4.8 – 5.7	< 0.9	9.0 – 11.2
Beaujeu - Ardières River	10/06/08	0.827	0.444	PEC	0.1	/	32.1	8.0	/	0.23	5.6	1.8	0.1	13.8	/	0.5
				MEC	1.2 - 1.6	/	0.4 - 19.6	< 0.4 - 5.8	< 0.2 - 0.6	1.7 - 2.0	3.0 - 13.2	< 0.3 - 1.8	< 0.3	< 0.4 - 6.9	< 0.3 - 0.5	< 0.2 - 1.6
	24/06/08	0.345	0.444	PEC	0.3	/	65.1	15.6	/	0.3	16.9	4.2	/	22.1	/	0.9
				MEC	0.8 - 1.8	/	< 0.8 - 209	< 0.1 - 5.0	< 0.2 - 7.1	1.6 - 1.8	3.1 - 43.5	0.4 - 1.0	/	< 0.5 - 7.4	/	< 0.3 - 0.4
Bourgoin Jallieu - Bourbre River	02/09/08	2.07[b]	0.105	PEC	1.8	/	165	243	15.6	1.4	151	16.5	/	38.4	/	22.1
				MEC	1.5 - 1.9	/	73.5 - 165	19.2 - 213	2.3 - 41.5	< 0.1 - 1.0	33.3 - 168	1.8 - 18.5	/	3.4 - 34.5	/	3.7 - 20.9
	09/09/08	2.41	0.105	PEC	1.4	/	202	179	/	/	129	10.6	/	24.3	/	16.2
				MEC	0.3 - 2.3	/	11.7 - 125	3.4 - 113	< 0.3 - 2.8	/	7.6 - 110	< 0.2 - 8.3	/	< 0.3 - 16.1	/	0.5 - 13.7
	16/09/08	4.70	0.105	PEC	5.0	/	251	128	/	0.6	118	10.2	/	25.9	/	10.4
				MEC	0.4 - 3.3	/	10.9 - 118	4.5 - 94.3	/	< 0.5	5.8 - 59.5	< 0.3 - 5.0	/	< 0.4 - 10.7	/	0.3 - 3.5
	23/09/08	2.27	0.105	PEC	5.9	/	292	151	0.8	1.2	191	10.9	/	30.8	/	11.8
				MEC	2.1 - 3.9	/	16.3 - 103	5.5 - 80.4	< 0.5	< 0.6	9.5 - 111	0.8 - 4.9	/	1.5 - 12.5	< 0.2 - 0.4	1.9 - 6.4

QMNA5: 5-year lowest water flow discharges

/: not quantified in the samples (in effluent for PEC and in surface water for MEC); LOQ estimated between 0.2 and 1.1 for betablockers and between 0.3 and 3.1 for E3

[a] free concentration

[b] MEC of the 20/11/07

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Table 5. Risk assessment for estrogens (total risk) and four betablockers (ACE, ATE, MET and PROP) in rivers downstream of WWTP outfall et 15 sites in France

WWTP	River	Population equivalent (PE)	WWTP outflow (m^3/d)	5 years lowest water flow discharges (m^3/s)	Calculated dilution factor	MEC (mean value) or PEC (ng/L)										PEC/PNEC ratio			
						E1	αE2	βE2	E3	EE2	ACE	ATE	MET	PROP	Sum of estrogens	ACE	ATE	MET	PROP
Beaujeu	Ardières	2 900	750	0.11	13	1.5	/	/	/	/	19.0	36.2	0.9	3.2	0.3	< 0.1	< 0.1	< 0.1	0.3
Bourgoin Jallieu	Bourbre	78 000	17 500	0.44	2	1.8	/	/	/	/	76.3	94.4	8.8	17.0	0.4	< 0.1	< 0.1	< 0.1	1.7
Fontaines sur Saône	Saône	30 000	6 500	40	532	1.6	/	/	25.9	/	44.7	51.7	6.9	8.4	5.5	< 0.1	< 0.1	< 0.1	0.8
1	Maurepas	36000	4430	0.11	2	3.1	/	/	/	/	18.1	400	28.9	179	0.6	< 0.1	0.1	< 0.1	17.8
2	Seine	250000	26737	63.0	204	$0.3*10^{-2}$	/	/	/	/	0.2	2.0	1.2	1.2	0.0	< 0.1	< 0.1	< 0.1	0.1
3	Maldroit	50000	6486	0.09	1	3.9	/	/	/	/	155	277	34.9	217	0.8	< 0.1	< 0.1	< 0.1	21.7
4	Gave of Pau	110000	14513	14.0	83	$0.9*10^{-2}$	/	/	/	/	1.2	7.3	1.6	1.9	< 0.1	< 0.1	< 0.1	< 0.1	0.2
5	Vallon St Antoine	24000	1459	0.78	46	$0.4*10^{-2}$	/	/	/	/	3.6	9.7	0.5	2.6	< 0.1	< 0.1	< 0.1	< 0.1	0.3
6	Bouillide	26000	3750	0.02	1	0.8	/	/	/	/	518	1378	165	342	0.2	< 0.1	0.2	< 0.1	34.2
7	CanteRane	1000	81	0.03	34	1.5	0.2	0.3	0.7	/	/	/	/	2.2	0.5	< 0.1	< 0.1	< 0.1	0.2
8	Ardières	2900	674	0.10	13	0.6	/	/	/	/	15.6	55.7	2.3	26.3	0.1	< 0.1	< 0.1	< 0.1	2.6
9	Rhône	13000	1061	600	48860	$0.3*10^{-3}$	/	$0.5*10^{-4}$	/	/	$0.4*10^{-2}$	$0.2*10^{-1}$	$0.1*10^{-1}$	$0.3*10^{-2}$	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
10	Rhône	700000	312767	600	166	$0.4*10^{-1}$	/	/	/	/	/	8.2	1.2	1.2	< 0.1	< 0.1	< 0.1	< 0.1	0.1
11	Rhône	88000	7150	600	7250	$0.1*10^{-2}$	$0.3*10^{-3}$	$0.4*10^{-3}$	$0.7*10^{-2}$	$0.3*10^{-3}$	0.4	0.3	0.1	0.1	0.0	< 0.1	0.0	0.0	0.0
12	Arvan	17000	1198	0.74	54	0.1	/	0.3	0.6	/	15.8	8.9	5.0	3.6	0.2	< 0.1	0.0	0.0	0.4

/: not quantified

PNEC: 5 ng/L for estrogens (Stuer-Lauridsen *et al*., 2000), 1250000 ng/L for ACE (Garric *et al*., 2006), 7900 ng/L for ATE (Cleuvers, 2005), 310000 for MET (Cleuvers, 2005) and 10 ng/L for PROP (Ferrari *et al*., 2004).

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Supplementary material. Mean concentrations (min-max, in ng/L) of estrogens and betablockers measured in the different sampling points of the three sites studied

		ACE	ATE	BIS	BET	MET	NAD	OXP	PROP	SOT	TIM	E1
Bourbre	**Upstream (20 m)**	14 (6.0-33)	28 (11-74)	1.6 (0.3-3.7)	/	1.3 [c] (0.8-1.8)	42 [a] (42-42)	/	2.5 [c] (1.5-3.4)	8.4 (3.4-19)	/	1.6 (0.5-3.9)
Bourgoin Jallieu WWTP	**Effluent**	393 (289-521)	599 (470-796)	41 (26-63)	/	32 (25-47)	23 (2.1-45)	/	80 (62-110)	470 (315-695)	2.9 (1.5-3.9)	9.2 (3.5-16.1)
Bourbre	**Downstream 1 (5 m)**	99 (58-168)	123 (85-165)	11 (3.5-21)	/	9.0 (4.4-19)	3.4 [c] (2.8-4.0)	/	17 (7.6-35)	120 (60-213)	1.0 [a] (1.0-1.0)	2.5 (1.9-3.3)
Bourbre	**Downstream 2 (2000 m)**	54 (5.8-111)	65 (10-137)	5.2 (0.3-13)	/	8.3 [c] (4.9-12)	2.3 [a] (2.3-2.3)	/	16 [c] (12-21)	63 (4.5-158)	0.8 [a] (0.8-0.8)	1.2 (0.3-2.1)
Ardières	**Upstream 1 (4000 m)**	5.0 (3.1-7.3)	2.2 [d] (2.1-2.4)	0.9 [d] (0.9-0.9)	/	0.4 [d] (0.4-0.5)	/	/	1.4 [d] (0.7-2.1)	2.3 [d] (1.6-3.0)	1.8 (1.5-2.0)	1.4 (0.8-2.3)
Ardières	**Upstream 2 (20 m)**	9.1 (3.0-16)	76 (0.4-209)	1.0 [b] (1.0-1.0)	/	0.7 [d] (0.5-0.9)	3.7 [d] (0.4-7.1)	/	1.4 [b] (1.4-1.4)	3.5 [d] (3.3-3.6)	1.6 (1.4-1.8)	1.4 (1.2-1.5)
Beaujeu WWTP	**Effluent**	147 (68-226)	628 (388-868)	9.5 (6.6-12)	/	39 (22-55)	/	/	231 (167-294)	153 (97-208)	3.2 (2.8-3.6)	2.7 (1.7-3.7)
Ardières	**Downstream 1 (5 m)**	39 (12-62)	29 (13-56)	1.4 [d] (1.2-1.6)	/	1.2 (0.9-1.8)	0.6 [b] (0.6-0.6)	/	1.8 (1.1-2.9)	5.2 (4.9-5.8)	1.6 (1.3-1.8)	1.4 (1.1-1.6)
Ardières	**Downstream 2 (20 m)**	23 (13-30)	26 (19-41)	1.1 (0.4-1.1)	/	1.2 (1.0-1.4)	/	/	6.3 (4.5-7.4)	4.4 (3.5-5.1)	1.6 (1.5-1.7)	1.6 (1.5-1.8)
Saône	**Upstream (200 m)**	14 (12-18)	10 (9.8 – 10)	14 (1.3-30)	0.2 [b] (0.2-0.2)	1.9 (1.7-2.3)	18 [b] (18-18)	0.1 [b] (0.1-0.1)	3.8 [d] (2.8-4.8)	22 (16-28)	/	1.4 (1.9-0.7)
Fontaines sur Saône WWTP	**Effluent**	1446 (1436-1455)	2345 (2240-2450)	507 (383-630)	18 (17-19)	206 (190-223)	56 (52-61)	30 (27-32)	240 (237-243)	886 (855-918)	8.7 (8.0-9.5)	16.2 (12.4-20)
Saône	**Downstream 1 (50 m)**	76 (21-183)	93 (14-240)	27 (9.0-38)	1.1 [d] (0.4-1.7)	12 (2.1-29)	2.7 [d] (1.0-4.4)	1.9 [d] (0.3-3.4)	11 (4.1-25)	67 (22-153)	1.0 [b] (1.0-1.0)	1.7 (1.3-2.0)
Saône	**Downstream 2 (200 m)**	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.6 (1.0-1.9)

[a] quantification frequency of 25%, [b] quantification frequency of 33%, [c] quantification frequency of 50%, [d] quantification frequency of 66%, (if not specified, quantification frequency of 100%)

/: not quantified (LOQ between 0.2 and 1.1 ng/L depending on the sample and the substance), NA: not analyzed

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