Occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: Removal and ecotoxicological impact of wastewater discharges and sludge disposal

J. Martín, D. Camacho-Muñoz, J.L. Santos, I. Aparicio, E. Alonso

Department of Analytical Chemistry, Escuela Politécnica Superior, University of Seville, C/Virgen de África 7, E-41011 Seville, Spain

1. Introduction

Pharmaceutically active compounds have become environment pollutants of emerging concern because their intrinsic biological activity may cause adverse effects to aquatic and terrestrial ecosystems, particularly at chronic exposure. Pharmaceutical compounds reach wastewater treatment plants (WWTPs) mainly through excreta and disposal of unused or expired drugs. Pharmaceutical compounds are not completely removed during wastewater treatments and, as a result, they are discharged into the receiving streams [1,2] and can end up onto soils when sewage sludge generated is employed as fertilizer [3,4]. The removal of pharmaceutical compounds in WWTPs is commonly evaluated as a mass balance between concentrations in influent and effluent wastewater [5–7]. Two processes are mainly responsible for the removal of pharmaceutical compounds: biodegradation and sorption onto sludge. An accurate determination of the biodegradation rates is extremely difficult due to the large number of degradation products, most of them unknown [4]. Sorption onto sludge depends on the physical and chemical properties of the compounds and on the characteristics of sewage sludge. The influence of sorption onto sludge requires experimental studies through the solid–water partition coefficients ($K_D$) [8–13]. In most cases, $K_D$ values are estimated using batch experiments [8,9]. In other cases, $K_D$ values are predicted using different equations developed for lipophilic compounds [10,11]. Only a few authors, calculate $K_D$ values from the measurements of the concentrations of the pharmaceutical compounds in wastewater and sewage sludge [12,13].

The first aim of this study was to evaluate the occurrence and distribution of pharmaceutical compounds in wastewater and sludge lines in wastewater treatment plants. To achieve this aim, sixteen pharmaceutical compounds belonging to seven therapeutic groups were monitored during 1-year period in influent and effluent wastewater and in primary, secondary and digested sludge from four WWTPs. The second aim was to evaluate the effectiveness of wastewater and sludge treatment lines to remove these compounds. $K_D$ values were calculated, from measured concentrations in wastewater and sludge, to evaluate the contribution of sorption onto sludge in the removal. The third aim was to evaluate the ecotoxicological risk due to effluent wastewater discharges to the receiving streams and to the disposal of sewage sludge as fertilizer onto soils. Pharmaceutical compounds monitored were five non-steroidal anti-inflammatory drugs (diclofenac, ibuprofen, ketoprofen, naproxen and salicylic acid), two antibiotics...
(sulfamethoxazole and trimethoprim), a β-blocker (propranolol), two lipid regulators (clofibric acid and gemfibrozil), an antiepileptic drug (carbamazepine), four estrogens (17α-ethinylestradiol, 17β-estradiol, estril and estrone), and a nervous stimulant (caffeine). Salicylic acid and clofibric acid are metabolites of pharmaceuticals (acetylsalicylic and clofibrate, respectively) extensively metabolized. The presence of caffeine in wastewater is due, in a lower extent, to its presence in pills and, in a higher extent, to its presence in food-related sources, as coffee and soft drinks.

2. Experimental

2.1. Chemicals and reagents

HPLC-grade water, acetonitrile and methanol were purchased from Romil Ltd. (Barcelona, Spain). Hexane, acetone (HPLC grade) and sulfuric acid of analytical grade were obtained from Panreac (Barcelona, Spain). Potassium dihydrogen phosphate of analytical grade was purchased from Merck (Darmstadt, Germany). Caffeine was obtained from Sigma–Aldrich (Steinheim, Germany). Clofibric acid, gemfibrozil, ibuprofen, propranolol hydrochloride, 17α-ethinylestradiol, 17β-estradiol, estril, estrone, sulfamethoxazole and trimethoprim (99% purity) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Three-milliliters solid phase extraction (SPE) cartridges, packed with 60 mg of Oasis HLB, were purchased from Waters (Milford, MA, USA).

Stock solutions of each pharmaceutical compound, at a concentration level of 1000 mg/L, were prepared in methanol and stored at 4 °C. Working solutions were prepared by diluting the stock standard solutions in methanol.

2.2. Wastewater and sludge sampling

Influent and effluent wastewater and primary, secondary and digested sludge were sampled from four WWTPs namely north (350,000 equivalent inhabitants, 62,000 m³/day), south (950,000 equivalent inhabitants, 164,500 m³/day), east (200,000 equivalent inhabitants, 40,900 m³/day) and west (200,000 equivalent inhabitants, 23,150 m³/day) WWTP sited in Seville city (south of Spain). Treatments in the studied WWTPs involve pretreatment, primary (settling) and secondary (activated sludge) treatments. Fig. 1 shows a diagram scheme of wastewater and sludge treatment lines with the sampling points marked in bold. Pretreated wastewater is subjected to settling in primary clarifiers where primary sludge is obtained. Secondary treatment is based on activated sludge processes in a biologic reactor and settling in secondary clarifiers where secondary sludge is produced. Mixed sludge (a mixture of primary and secondary sludge) is then anaerobically digested and dehydrated for sludge stabilization.

Sixteen influent and effluent samples (four samples from each WWTP) were collected from January 2008 to January 2009 in studied WWTPs. Daily-composite samples were obtained by mixing the aliquots collected every hour by an automatic device operating during 24 h. Sample volumes collected each hour were proportional to influent and effluent flows. A total sample volume of 2.5 L was transferred to amber glass bottles and stored at 4 °C until analysis.

Sixteen sludge samples (four samples from each WWTP) were collected from each sampling point (Fig. 1). Two liters of primary and secondary sludge and one kilogram of digested sludge and compost were collected in glass bottles. Sludge samples were firstly decanted and then filtered to remove their water content. Finally, sludge samples were lyophilized in a Cryodos-50 lyophilizer (Telstar, Terrasa, Spain), sieved (particle size <100 μm) and stored, when necessary, in glass bottles at −30 °C until analysis. The water content of sludge samples after lyophilization was calculated by drying lyophilized sludge in an oven at 105 °C. Their moisture content was taken into account to refer concentrations to sludge dry matter.

2.3. Analytical methods

Pharmaceutical compounds were analyzed according to previously reported and validated methods [14,15]. Extraction of the pharmaceutically active compounds from wastewater was carried out by solid-phase extraction according to Camacho-Muñoz et al. [14]. Sludge analysis was based on the extraction of the pharmaceutical compounds by ultrasonic-assisted extraction and clean-up by solid-phase extraction according to Martín et al. [15]. In both cases, the chromatographic analysis was performed using an HPLC 1200 Series instrument (Agilent, USA) equipped with an ultraviolet diode array (DAD) and a rapid scan fluorescence (Fl) detector connected on line. Separations were carried out using a Zorbax Eclipse XDB-C18 (150 mm × 4.6 mm, 5 μm) cartridge column (Agilent, USA) protected by a XDB-C18 (4 mm × 4 mm, 5 μm) guard column (Agilent, USA). Chromatographic analysis was carried out by gradient elution with acetonitrile and a 25 mM potassium dihydrogen phosphate solution as previously reported [14].

2.4. Solid–water partition coefficients, \( K_d \)

Solid–water partition coefficients (\( K_d \),) corresponding to the distributions of the pharmaceutical compounds between primary sludge and influent wastewater and between secondary sludge and effluent wastewater, were calculated by the Eq. (1):

\[
K_d = \frac{C_{\text{solid}}}{C_{\text{water}}}
\]

where \( K_d \) is expressed in L/kg, \( C_{\text{solid}} \) is the concentration of the pharmaceutical compound in the solid phase (μg/kg dry matter (dm)) and \( C_{\text{water}} \) is the concentration of the pharmaceutical compound in the aqueous phase (μg/L).

2.5. Ecotoxicological risk assessment

Ecotoxicological risk assessment was evaluated by means of risk quotient (RQ) values in effluent wastewater, in the receiving stream, in digested sludge and in digested-sludge amended soil. Risk quotient values are usually expressed as the ratio between the predicted environmental concentration (PEC), or the measured environmental concentrations (MEC values) when available, and the predicted no-effect concentration (PNEC). RQ values lower than 1 indicate that the compound does not imply a significant risk to the environment.

PECwater Values were estimated from the concentration levels measured (MEC values) in effluent wastewater applying a dilution factor of 100. The dilution factor was estimated from the flow rates of effluent wastewater and the receiving stream. PECsoil Values, estimated one year after one sludge-dose application, were calculated applying the Eq. (2) from the European Commission Technical Guidance Document on Risk Assessment EUR 20418 EN/2 [16];

\[
\text{PEC}_{\text{soil}} = \frac{C_{\text{sludge}} \times \text{APPL}_{\text{sludge}} \times \text{DEPTH}_{\text{soil}}}{\text{RHO}_{\text{soil}}}
\]

where \( C_{\text{sludge}} \) is the concentration measured in digested sludge expressed as μg/kg dm; \( \text{APPL}_{\text{sludge}} \) is the application rate of dry-sludge onto soils (0.5 kg/m² year for agricultural soils); \( \text{DEPTH}_{\text{soil}} \) is the mixing depth (0.20 m for agricultural soils) and \( \text{RHO}_{\text{soil}} \) is the bulk density of wet soil (1700 kg/m³ for agricultural soils).
PNEC\textsubscript{water} values were calculated from the lowest values of chronic and acute toxicity data (lethal concentration (LC), effect concentration (EC) and non-observed effect concentration (NOEC)) reported in literature to several aquatic organisms: bacteria, algae, invertebrate and fish species [2] by applying an assessment factor of 1000 to consider the toxicity to other aquatic species more sensitive than those selected in toxicity studies [16,17]. PNEC\textsubscript{solid} values were estimated from PNEC\textsubscript{water} values because no toxicological data of pharmaceutically active compounds in the terrestrial compartment was found in literature. PNEC\textsubscript{solid} values were estimated applying the equilibrium partition approach [17]:

\[
PNEC_{\text{solid}} = PNEC_{\text{water}} \times K_d
\]

where \(K_d\) is the solid–water partition coefficient which takes into account the two main mechanisms of sorption into sludge: adsorption and adsorption [4]. In fate and contaminant transport calculations, \(K_d\) is defined by the United States Environmental Protection Agency as the ratio of the contaminant concentration associated with the solid to the contaminant concentration in the surrounding aqueous solution when the system is at equilibrium. \(K_d\) values from literature [9,12,13,18–23] were used (Table 1).

### 3. Results and discussion

#### 3.1. Occurrence of the pharmaceutically active compounds in wastewater and sludge

The distribution of the pharmaceutical compounds in wastewater treatment line is shown in Table 2. Gemfibrozil, ketoprofen, naproxen, propranolol and salicylic acid were detected in all of the wastewater samples whereas clofibric acid, estrone, sulfamethoxazole and trimethoprim were not detected in wastewater. The anti-inflammatory drugs were the compounds at the highest concentration levels, being ibuprofen and salicylic acid the ones at the highest concentration levels with mean values, in the four WWTPs sampled, of 32.0 and 27.2 \(\mu\)g/L, respectively, in influent wastewater and 4.04 and 1.00 \(\mu\)g/L, respectively, in effluent wastewater. Their high concentrations can be related to their wide use enhanced because no medical prescription is necessary for their sale. In Spain, the total amount of ibuprofen sold per year has been estimated to be about 276 tons, while antibiotics are sold at a lesser extent, for example, trimethoprim is being sold at 3.7 tons per year [24]. The nervous stimulant caffeine, which not only comes from pharmaceuticals but also from coffee consumption, reached concentration levels up to 8.97 \(\mu\)g/L in influent wastewater, and up to 0.94 \(\mu\)g/L in effluent wastewater. The estrogenic compounds were found at lower concentration than mean values from below the limits of detection to 0.30 \(\mu\)g/L, in influent wastewater; and from below the limits of detection to 0.41 \(\mu\)g/L in effluent wastewater. Alongside wastewater treatment line, the concentrations of the pharmaceuticals decreased but all the compounds detected in influent wastewater were still present in effluent wastewater.

The distribution of the pharmaceutical compounds in sewage sludge is shown in Table 3. The pattern of occurrence of the pharmaceutical compounds in sewage sludge was similar to that observed in wastewater. The highest mean concentrations, in the four WWTPs sampled, correspond to ibuprofen (2206 \(\mu\)g/kg dm in primary sludge, 1584 \(\mu\)g/kg dm in secondary sludge and 1170 \(\mu\)g/kg dm in digested sludge). The other compounds at high concentrations in primary sludge were gemfibrozil, salicylic acid and caffeine (mean concentration in primary sludge: 873 \(\mu\)g/kg dm, 560 \(\mu\)g/kg dm and 446 \(\mu\)g/kg dm, respectively). The pharmaceuticals with high \(\log K_{ow}\) values and low \(pK_a\) values as diclofenac, ketoprofen and gemfibrozil were mainly detected in wastewater instead of in sludge. At wastewater pH, they are mainly in their ionized form and, therefore, present in the aqueous phase.

The pattern of occurrence of the pharmaceutical compounds in primary sludge was different to that observed in secondary sludge. For example, the pharmaceutical compounds at the highest concentrations in primary sludge were ibuprofen, gemfibrozil, salicylic acid and caffeine while, in secondary sludge, the pharmaceutical compounds at the highest concentrations were carbamazepine, 17\(\alpha\)-ethinylestradiol, estradiol and propranolol. This fact could be explained by the different physicochemical characteristics of the studied compounds (Table 4) and the different composition of primary and secondary sludge, resulting in different sorption behaviors. After the anaerobic digestion, the concentration levels of most of the compounds decreased (Table 3). The concentration
Table 1
Partition coefficients and ecotoxicological data of the pharmaceutically active compounds.

| Pharmaceutically active compound | Partition coefficient Log(Kd, sludge) Log(Kd, soil) | Ecotoxicological data Organism Test Toxicological value (mg/L) | PNECwater (μg/L) PNECsludge (μg/kg) PNECtestsite (μg/kg) |
|---------------------------------|-----------------------------------------------|-------------------------------------------------------------|-----------------------------------------------------------------|------------------------------------------------------|---------------------------------|
| Anti-inflammatory drugs | | | | | |
| Diclofenac | 2.70a | 2.21b | V. fischeri (bacteria) EC50 (15 min) | 9.70 | 9.70 | 4862 | 1595 |
| Ibuprofen | 2.10a | 1.45f | H. attenuata (invertebrate) EC50 (96 h) | 1.65 | 1.65 | 1141 | 256 |
| Ketoprofen | 2.40a | 0.95d | V. fischeri (bacteria) EC50 (15 min) | 15.6 | 15.6 | 3919 | 140 |
| Naproxen | 1.55d | 0.95d | H. attenuata (invertebrate) EC50 (96 h) | 2.62 | 2.62 | 752 | 189 |
| Salicylic acid | 1.38a | 1.91d | V. fischeri (bacteria) EC50 (15 min) | 43.1 | 43.1 | 992 | 3520 |
| Antibiotics | | | | | |
| Sulfamethoxazole | 1.04d | 0.90d | P. subcapitata (algae) EC50 (96 h) | 0.15 | 0.15 | 1.64 | 1.19 |
| Trimethoprim | 1.83a | 1.41d | D. magna (invertebrate) EC50 (96 h) | 121 | 121 | 10273 | 3102 |
| Antiepileptic drug | | | | | |
| Carbamazepine | 1.15d | 1.40d | D. magna (invertebrate) EC50 (48 h) | 13.8 | 13.8 | 195 | 347 |
| β-Blocker | | | | | |
| Propranolol | 2.52d | 1.76d | D. subspicatus (algae) EC50 (48 h) | 0.70 | 0.70 | 232 | 40.3 |
| Nervous stimulant | | | | | |
| Caffeine | 2.30a | 1.40d | Leuciscus idus (fish) EC50 (96 h) | 87.0 | 87.0 | 17357 | 2185 |
| Estrogens | 17α-Ethinylestradiol | 2.46f | 1.75f | S. purpuratus (invertebrate) EC50 | 0.12 | 0.12 | 8.75 | 1.69 |
| 17β-Estradiol | 2.56f | 1.83f | S. purpuratus (invertebrate) EC50 | 0.01 | 0.01 | 5.14 | 0.99 |
| Estradiol | 2.67f | 2.67f | S. purpuratus (invertebrate) EC50 | 1.52 | 1.52 | 711 | 711 |
| Estrone | 2.45a | 1.40f | T. buddugla (invertebrate) EC50 (10 days) | 0.10 | 0.10 | 28.2 | 2.51 |
| Lipid regulators | | | | | |
| Clofibric acid | 0.70d | 0.95d | D. magna (invertebrate) EC50 (48 h) | 72.0 | 72.0 | 361 | 642 |
| Gemfibrozil | 1.29b | 0.11f | H. attenuata (invertebrate) EC50 (96 h) | 1.18 | 1.18 | 203 | 13.3 |

*Okuda et al. [12].
*Drilla et al. [18].
*Stuer-Lauridsen et al. [19].
*Barron et al. [20].
*Carballa et al. [9].
*Sarmah et al. [21].
*López de Alda et al. [22].
*Radjenović et al. [13].
*Krassensens et al. [23].

Table 2
Mean concentrations (n = 4) of the analyzed pharmaceutical compounds in influent and effluent wastewater from each WWTP.

<table>
<thead>
<tr>
<th>Pharmaceutically active compound</th>
<th>Mean concentration (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent wastewater</td>
<td>Effluent wastewater</td>
</tr>
<tr>
<td>North WWTP</td>
<td>South WWTP</td>
</tr>
<tr>
<td>Anti-inflammatory drugs</td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>0.72</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>50.6</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>1.69</td>
</tr>
<tr>
<td>Naproxen</td>
<td>4.09</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>27.8</td>
</tr>
<tr>
<td>Antibiotics</td>
<td></td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>&lt;LOQ0.017</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>&lt;LOQ0.012</td>
</tr>
<tr>
<td>Antiepileptic drug</td>
<td></td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>0.51</td>
</tr>
<tr>
<td>β-Blocker</td>
<td></td>
</tr>
<tr>
<td>Propranolol</td>
<td>0.39</td>
</tr>
<tr>
<td>Nervous stimulant</td>
<td></td>
</tr>
<tr>
<td>Caffeine</td>
<td>0.89</td>
</tr>
<tr>
<td>Estrogens</td>
<td></td>
</tr>
<tr>
<td>17α-Ethinylestradiol</td>
<td>&lt;LOQ0.017</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>&lt;LOQ0.014</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.12</td>
</tr>
<tr>
<td>Estrone</td>
<td>&lt;LOQ0.012</td>
</tr>
<tr>
<td>Lipid regulators</td>
<td></td>
</tr>
<tr>
<td>Clofibric acid</td>
<td>&lt;LOQ0.001</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>2.64</td>
</tr>
</tbody>
</table>

LOD: limit of detection; LOQ: limit of quantification. LOD and LOQ superscripts correspond to LOD and LOQ concentration values [14].
Table 3  
Mean concentrations ($n = 4$) of the analyzed pharmaceutical compounds in primary, secondary and digested sludge from each WWTP.

<table>
<thead>
<tr>
<th>pharmaceutically active compound</th>
<th>Mean concentration ($\mu$g/kg dry matter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary sludge</td>
</tr>
<tr>
<td></td>
<td>N-WWTP</td>
</tr>
<tr>
<td><strong>Anti-inflammatory drugs</strong></td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>$&lt;$LOD1.60</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>4.1</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>2.1</td>
</tr>
<tr>
<td>Naproxen</td>
<td>1.2</td>
</tr>
<tr>
<td><strong>Antibiotics</strong></td>
<td></td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Antiepileptic drug</strong></td>
<td></td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>1.5</td>
</tr>
<tr>
<td>Trimeprin</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>$\beta$-Blocker</strong></td>
<td></td>
</tr>
<tr>
<td>Propanol</td>
<td>1.2</td>
</tr>
<tr>
<td>Caffeine</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Estrogens</strong></td>
<td></td>
</tr>
<tr>
<td>17$\alpha$-Ethynylestradiol</td>
<td>1.5</td>
</tr>
<tr>
<td>17$\beta$-Estradiol</td>
<td>1.5</td>
</tr>
<tr>
<td>Estradiol</td>
<td>1.5</td>
</tr>
<tr>
<td>Estriol</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Lipid regulators</strong></td>
<td></td>
</tr>
<tr>
<td>Clofibric acid</td>
<td>1.5</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>1.5</td>
</tr>
</tbody>
</table>

N-WWTP: north WWTP; S-WWTP: south WWTP; E-WWTP: east WWTP; W-WWTP: west WWTP. 
LOD and LOQ superscripts correspond to LOD and LOQ concentration values.

of 17$\beta$-estradiol increased after digestion, mean values, in the four WWTPs sampled, from 15 $\mu$g/kg dry matter in primary sludge to 294 $\mu$g/kg dry matter in digested sludge. This fact could be explained by the cleavage of conjugated steroid estrogens [25–27] and by the concentration that undergo persistent pollutants due to the loss of weight of sludge after digestion process. There is also a possibility of pharmaceutical compounds to enter the food chain being transferred from soil to plants via root systems when wastewater is employed to irrigate agricultural soils or sludge is used as fertilizer. Such uptake by plants has been reported for carbamazepine [28,29], sulfamethoxazole [28], trimetoprim [28], ibuprofen [30] and 17$\alpha$-ethynylestradiol [31].

Table 4  
Physicochemical properties ($\log K_{ow}$ and $\log K_{p}$ values) and calculated $\log K_{d}$ values of the pharmaceutically active compounds.

<table>
<thead>
<tr>
<th>pharmaceutically active compound</th>
<th>Physicochemical properties</th>
<th>Calculated $\log K_{d}$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Primary sludge</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N-WWTP</td>
</tr>
<tr>
<td><strong>Anti-inflammatory drugs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Naproxen</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Antibiotics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Antiepileptic drug</strong></td>
<td></td>
<td></td>
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<tr>
<td>Sulfamethoxazole</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Trimeprin</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>$\beta$-Blocker</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propanol</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Caffeine</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Estrogens</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17$\alpha$-Ethynylestradiol</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>17$\beta$-Estradiol</td>
<td>1.5</td>
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</tr>
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<td>Estradiol</td>
<td>1.5</td>
<td>1.5</td>
</tr>
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<td>Estriol</td>
<td>1.5</td>
<td>1.5</td>
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<tr>
<td><strong>Lipid regulators</strong></td>
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<td></td>
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<tr>
<td>Clofibric acid</td>
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<td>1.5</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>1.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

$\log K_{ow}$, $\log K_{p}$ and $\log K_{d}$ are physicochemical properties of the compounds used to calculate the $\log K_{d}$ values.

$\log K_{d}$ values are calculated from the physicochemical properties and concentration data.

$\log K_{d}$ values are calculated from the physicochemical properties and concentration data.
3.2. Solid–water partition coefficients, $K_d$

$K_d$ values, in primary sludge and secondary sludge, calculated from experimental concentrations measured in wastewater and sludge are shown in Table 4. Log $K_d$ values in secondary sludge were higher than those in primary sludge. This fact, depending on the physicochemical properties of the compound ($\log K_{ow}$ and $pK_a$ values), can be due to a better sorption onto secondary sludge than onto primary sludge or to a fast biodegradation. The acidic compounds, characterized by low $pK_a$ values, are expected to have low $\log K_d$ values because at wastewater pH they are mainly in their ionized form and, consequently, mainly dissolved in the aqueous phase. Nevertheless, $\log K_d$ values of the anti-inflammatory drugs, all of them with carboxylic groups, are just slightly lower, in primary sludge, and similar, in secondary sludge, than those of the other pharmaceutical compounds with no carboxylic group. The unexpectedly high $\log K_d$ values of the anti-inflammatory drugs cannot be associated to a high potential of sorption onto sludge but to a fast biodegradation that reduce the measured concentrations in the aqueous phase. The same phenomenon was reported, for the anti-inflammatory drug acetaminophen, by Radjenović et al. [13]. The high $\log K_d$ values of carbamazepine, 17β-ethinylestradiol and estriol indicate that the compounds tend to be retained onto sludge which is consistent with their high $pK_a$ values. This fact is in agreement with that reported in the literature [8,9,20,32].

3.3. Removal of pharmaceutically active compounds from wastewater

The removal rates achieved in each WWTP and the average removal rates in the four WWTPs evaluated are shown in Fig. 2. Significant variation was observed from one compound to another but no significant different removal behavior was observed among the four WWTPs evaluated. The main mechanisms of removal of pharmaceutical compounds from wastewater are biodegradation or sorption onto sludge. The anti-inflammatory drugs salicylic acid and ibuprofen were the best removed pharmaceutical compounds (mean removal rates: 99 and 87%, respectively). Both compounds have low $pK_a$ values (Table 4) so they are expected to be mainly in the aqueous phase. Their removal from wastewater could be explained by biodegradation instead of by sorption onto sludge. This fact is consistent with that reported by several authors [6,33,34] who propose biodegradation as the most probable mechanism for their removal. Diclofenac was the anti-inflammatory drug poorest removed, mean removal rate: 14%. The poor removal of diclofenac can be probably due to the combination of degradation in wastewater together with the liberation of additional diclofenac molecules by de-conjugation of glucuronidated or sulfated diclofenac and/or its desorption from particles [7]. Similar poor removal rates (<16%) were observed for the lipid regulator gemfibrozil and the β-blocker propranolol. Carbamazepine is a pharmaceutical compound with a high chemical stability to the point that has been proposed as a anthropogenic marker [5,6]. Therefore, the removal of carbamazepine from wastewater could be explained by sorption onto sludge due to its hydrophilic nature ($\log K_{ow}$: 2.4). Elimination rates of caffeine vary significantly, not only from one WWTP to another, but also in the same WWTP at different time periods what can be associated to its temporal-conditioned consume. The low polarity of the estrogenic compounds, characterized by $\log K_{ow}$ values between 2.8 and 4.2 (Table 4), makes sorption onto sludge to be the most likely process responsible for their removal from wastewater (17β-estradiol: 55%, 17α-ethinylestradiol: 47% and estriol: 26%) [35].

3.4. Risk assessment

Fig. 3 shows RQ values of each pharmaceutical compound in four different scenarios: effluent wastewater, receiving streams, digested sludge and digested-sludge amended soil. To estimate RQ values in effluent wastewater and digested sludge, the highest concentration levels measured in effluent wastewater and digested sludge were used as MEC values. To estimate RQ values in receiving streams and in digested-sludge amended soils, the highest PEC values were applied. The compounds responsible for the highest environmental risks in effluent wastewater and digested sludge were 17β-estradiol (RQ values 12 and 359, respectively), 17α-ethinylestradiol (RQ values 12 and 22, respectively) and ibuprofen (RQ values 3.2 and 4.4, respectively). RQ values of
the other pharmaceutical compounds varied between 0.01 and 0.7 in effluent wastewater and 0.02–0.26 in digested sludge so no risk is suspected to occur in these two scenarios.

The dilution effect that takes place after the discharge of effluent wastewater into the receiving stream results in a decrease of the concentrations of the pharmaceutical compounds. The concentrations of all the pharmaceutical compounds in the receiving stream were low enough that no risk to aquatic organisms is suspected to occur. All the RQ values were lower than 1 (Fig. 3). Therefore, acute toxic effect in the aquatic environment, with the current use of pharmaceutical compounds, is unlikely. However, toxic effects due to chronic environmental exposure, mainly by aquatic species with a long-life cycle, could still be present. In a previous paper [2], the occurrence and risk assessment of the presence of pharmaceutical compounds in Guadalquivir River were reported. The results provided in the present paper shows that the low RQ values estimated in the previous paper are due to the dilution effect of wastewater discharges into the receiving stream. Regarding to sludge samples, after sludge application onto soil, a drastic decrease of RQ values was observed. The only toxicological effect expected is the one caused by 17β-estradiol since the RQ value of 17β-estradiol exceeds the limit value of 1 for the most sensitive species (RQ value: 2.7). This fact means that an ecotoxicological risk is still present to terrestrial ecosystems in spite of the important decrease of the concentration of 17β-estradiol from digested sludge to digested-sludge amended soil.

4. Conclusions

The highest concentrations in wastewater and sludge were observed for the anti-inflammatory drugs ibuprofen and salicylic acid. The pharmaceutical compounds detected in wastewater were also detected in sludge, except diclofenac which was only detected in wastewater. In wastewater treatment line, the most persistent compounds were diclofenac, propranolol, estriol and gemfibrozil. The best removed compounds were ibuprofen and salicylic acid. In sludge line, different behavior of the pharmaceutical compounds was observed as well. The concentrations of some compounds as naproxen and salicylic acid decrease from primary sludge to digested sludge; the concentrations of other compounds as 17β-estradiol, increase; and the concentration of the other compounds as 17α-ethinylestradiol and estriol, do not vary significantly. The concentrations measured in sludge indicate that sorption can be one of the key factors controlling the removal of pharmaceutical compounds. The removal of the anti-inflammatory drugs and the lipid regulator gemfibrozil cannot be explained by sorption onto sludge but by biodegradation in the aqueous phase. Environmental risk assessment revealed a high ecotoxicological risk due to ibuprofen, 17α-ethinylestradiol and 17β-estradiol both for aquatic and terrestrial ecosystems. After disposal of wastewater in the receiving stream and sludge disposal onto soils, a significant decrease of the ecotoxicological risk occurs. Nevertheless, RQ value of 17β-estradiol in digested-sludge amended soil is high enough to cause ecotoxicological risk to the most sensitive species in the terrestrial ecosystem. The results obtained show the necessity to improve wastewater treatments to reduce the input of pharmaceutical compounds in surface water and soils. Special attention should be paid to the presence of ibuprofen and the estrogenic compounds in digested sludge mainly when sludge is used as fertilizer onto agricultural soils because they could even enter in the food chain after the uptake of the pharmaceuticals by plants.

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