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Prioritization and analysis of pharmaceuticals for human use contaminating the aquatic ecosystem in Italy

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ABSTRACT

A predictive approach is needed to study pharmaceuticals and their loads in the environment so as to restrict monitoring to the molecules most likely to occur widely. A three steps method has been developed for this purpose. The first step is to establish the Predicted Environmental Concentrations (PECs) following the approach proposed by the European Medicines Agency (EMA); then the list is refined taking account of metabolic rates and excretion of the parent compounds in humans. In the third stage the substances are sorted according to their fate in sewage treatment plants (STPs). Finally, the results are compared with real concentrations of the pharmaceuticals in surface waters analyzed by HPLC–MS/MS techniques. This paper reports results of this predictive approach in 2013 for Italy.

1. Introduction

Pharmaceuticals in waste and surface waters have been extensively explored over the last two decades and as analytical techniques have become more sensitive more compounds have been detected, showing the presence of complex mixtures of pharmaceuticals that changes with time as new drugs are synthesized to replace obsolete compounds [1–3]. Monitoring pharmaceuticals in the environment is important to identify sources of contamination, study their behavior and fate and assess the potential risks deriving from their ubiquity. At the concentrations in the environment – generally in the ng/L to the μg/L range – direct effects on the environment or human health cannot be excluded for therapeutic drugs as a whole, though this is unlikely, at least when the compounds are considered individually [4]. The toxic effects of mixtures however are harder to explore [5–7].

Pharmaceuticals – unchanged and/or as metabolites – are discharged into the environment typically through the sewage system receiving consumers' urine or through improper disposal of unused medications, but other local sources of contamination include unauthorized use in cattle breeding and illegal disposal methods [8–10]. Removal by sewage treatment plants (STP), breakdown in surface water, and dilution by river flows and rainfall are all factors that influence the final concentrations in surface water [11,12].

There are so many pharmaceuticals in use that it is hard to monitor them all since analysis would be too complex, expensive, and time-consuming. A strategy is therefore needed to prioritize compounds, so as to restrict monitoring to a limited number of the potentially most hazardous ones.

In the literature, compounds to be monitored are often selected on the basis of their detection in previous studies [13] or on their environmental persistence [14]. Otherwise the selection can be driven by market data [15–17] or can focus on specific classes of pharmaceuticals, such as antibiotics [18], or a combination of these criteria [10–12,19].

An alternative method to prioritize pharmaceuticals involves calculating their Predicted Environmental Concentration (PEC) in surface water, as proposed by the European Medicine Agency (EMA) for the pre-marketing Environmental Risk Assessment (ERA) of new pharmaceuticals for human use [10,20]. The crude PECs only need be refined for compounds that exceed a threshold of 0.01 μg/L [20–22]. This approach can also be applied to pharmaceuticals already marketed. To calculate a PEC for these substances the data required are their sales volumes, which can be estimated from the number of "defined daily doses" (DDD) consumed per inhabitant [20]. PECs can then be refined by considering other parameters, including the excretion rate, i.e. the percentage of the drug excreted as parent compound.

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The PECs calculated from annual sales represent an annual average on a nation-wide scale and do not take into account seasonal and regional variability of consumption. Thus the theoretical values are often not consistent with the measurements in surface waters which usually refer to instant or daily sampling in a defined area [23,24].

The method proposed by EMA has been followed to test the accuracy of the predictions [22,25] or to estimate environmental risks [23]. These studies show that predictions are not always accurate and can differ by more than one order of magnitude from the measurements. Alternative methods have therefore been suggested to improve predictions. Castiglioni et al. [26] proposed refining the crude PECs not only by correcting for the excretion rate, as suggested by the EMA guidelines [27], but also considering the degradation of the compounds in surface water. The accuracy of this predictive model was tested comparing crude and refined PECs with the concentrations measured during a monitoring campaign on the River Po in Northern Italy [26]. Then Zuccato et al. [11] used this approach to identify pharmaceuticals of concern for the environment in Italy.

As more the data have become available it is now possible to refine the crude PECs by considering a further value: the removal rate in STPs. Our group calculated this in 2006 for several priority pharmaceuticals in some STPs in Italy [12] and as increasing information is now found in the literature [28–30] the list of pharmaceuticals can be expanded. For instance, Verlicchi et al. [24] used the removal rates in STPs reported in the literature to refine the predicted concentrations of several pharmaceuticals and to compare them with the concentrations measured in the influent and effluent of a STP and in the receiving body of water.

The aim of our work was to update the predictive approach we used in the past to the present situation in Italy including substances not previously considered. An updated list of priority pharmaceuticals was obtained starting from the sales volumes and from a comprehensive review of the recent literature, which enabled us to update and refine metabolic excretion rates and removal rates in STPs for each pharmaceutical.

Priority pharmaceuticals were measured in the effluent of the Milan Nosedo STP and in surface water collected at the basin closure of the River Lambro, using an up-to-date analytical method based on HPLC–MS/MS. The collected values were also used to calculate and refine the PECs in surface water and in STP effluents and the resulting PECs were then compared with the measurements to assess the reliability of the prediction.

2. Materials and methods

2.1. Selection of pharmaceuticals

Theoretical environmental loads are generally calculated from the sales loads of the substances, which in turn are measured starting from medical prescriptions or from the sales volumes for OTC pharmaceuticals. Prescription data are reported as the numbers of defined daily doses (DDD) per 1000 inhabitants [DDD/1000inh/day] [31]. These data were multiplied by the DDD value [32] of the active ingredient, and normalized to the Italian population (60 million people). Sales volumes of OTC pharmaceuticals are reported as number of packs sold per year [33], so they were multiplied by the amount of active principle in each pack. Finally, the data were converted to tons of active principle per year. After correction for the metabolic excretion rate, the active substances with the highest theoretical environmental loads were selected for the priority list.

By this procedure we updated the previous list of pharmaceuticals, published in 2005 [11], including new recently marketed compounds and excluding compounds no longer sold in Italy. In some cases, substances such as carbamazepine and bezafibrate, which have been found in significant concentrations in the environment in Italy during previous investigations [11,12,26], were included in the priority list to continue the monitoring, although we could not obtain their sales data and calculate theoretical environmental loads. The selected pharmaceuticals are listed in Table 1.

Table 1
Priority list of pharmaceuticals selected from sales load.

Pharmaceutical	CAS number*	Molecular weight*	Therapeutic class
Acetaminophen	103-90-2	151.16	Analgesic-Antipyretic
Amoxicillin	26787-78-0	365.40	Antibiotic-penicillin
Atenolol	29122-68-7	266.33	Cardiovascular drug
Atorvastatin	134523-00-5	558.63	Lipid-regulator
Bezafibrate	41859-67-0	361.81	Lipid-regulator
Carbamazepine	298-46-4	236.26	CNS drug
Ciprofloxacin	85721-33-1	331.34	Antibiotic-quinolones
Clarithromycin	81103-11-9	747.95	Antibiotic-macrolides
Diclofenac	15307-86-5	296.14	NSAID
Enalapril	75847-73-3	376.44	Cardiovascular drug
Furosemide	54-31-9	330.74	Diuretic
Hydrochlorothiazide	58-93-5	297.73	Diuretic
Ibuprofen	15687-27-1	206.28	NSAID
Irbesartan	138402-11-6	428.52	Cardiovascular drug
Ketoprofen	22071-15-4	254.28	NSAID
Lansoprazole	103577-45-3	369.36	Proton-pump inhibitor
Levofloxacin	100986-85-4	361.36	Antibiotic-quinolones
Losartan	114798-26-4	422.91	Cardiovascular drug
Metformin	657-24-9	129.16	Antidiabetic
Naproxen	22204-53-1	230.25	NSAID
Paroxetine	61869-08-7	329.36	Antidepressive
Ramipril	87333-19-5	416.51	Cardiovascular drug
Rosuvastatin	287714-41-4	481.53	Lipid-regulator
Simvastatin	79902-63-9	418.56	Lipid-regulator
Valsartan	137862-53-4	435.51	Cardiovascular drug

* Data from Drugbank.ca.

2.2. Calculation of PECs

According to EMA guidelines [20] PECs in STP effluents and in surface water were calculated using the following formula:

$$\mathrm{PEC}\,(\mathrm{g/L}) = \frac{A(100 - R)}{365 \times P \times V \times D \times 100}$$

where A is the amount of pharmaceutical used in the test area expressed in kg per year, R the removal rate, P the population in the area, V the volume of wastewater per capita per day (0.2 m^3), D a dilution factor in surface water (set at 10 for EU) [10]. Crude PECs in surface water were calculated by setting the removal rate (R) at 0 and in STP effluents were calculated setting the dilution factor (D) at 0 too.

2.3. Refinement of PECs: excretion and removal rate

2.3.1. Excretion rate

To improve predictions we refined the crude PECs using the excretion rate of the pharmaceuticals in human, which indicates the real amount of the active substance entering the environment. Data on human excretion in urine and feces were obtained from a literature review. In this refinement procedure we considered the excretion of both the parental and the conjugated forms because these can be hydrolyzed and reconverted to the parent compound in wastewater. In the calculation we used the median of the values obtained from the literature. Data and references are summarized in Table 2.