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Identification of the pharmaceuticals for human use contaminating the Italian aquatic environment

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Abstract

A predictive approach seems useful to identify pharmaceuticals in the environment and give an idea of overall levels of contamination, so as to restrict monitoring to those molecules most likely to be contaminants. We propose an approach based on two parts. The first is to rank the molecules according to the predicted environmental loads; the second is to refine the list by analysing the pharmaceuticals in sewage treatment plants (STPs) and comparing the concentrations with levels previously measured in surface water. This approach identified a restricted group of priority pollutants (ofloxacin, furosemide, atenolol, hydrochlorothiazide, carbamazepine, ibuprofen, spiramycin, bezafibrate, erythromycin, lincomycin and clarithromycin) in the aquatic environment in Italy, for further studies and monitoring.

Keywords: Pharmaceuticals; Environment; Surface water; River; STP

1. Introduction

The term "pharmaceuticals" covers a complex class of widely used compounds. Thousands of different active molecules are currently used in Italy [1] and in the world to treat or to prevent diseases, with hundreds of new molecules synthesized every year to replace obsolete compounds. Once administered, pharmaceuticals can be excreted as the parent compound or active metabolites, and can reach the environment to variable extents [2]. The amounts reaching surface water depend on several factors, some theoretically predictable, like metabolism and degradation, some unpredictable, such as improper disposal. Monitoring environmental contamination by pharmaceuticals is advisable for several reasons, including reliable assessment of risks for the environment and, through the food chain, for man. However, blanket monitoring is difficult because of the excessive number of pharmaceuticals and metabolites, with different chemical structures and physico-chemical properties [3]. It is therefore best to focus on the molecules of concern for the environment. The tendency now is to establish priorities so as to restrict monitoring to a limited number of hazardous molecules, but proposals on how to do this selection are scarce. Pharmaceuticals are usually ranked according to tonnage, though some molecules with low sales volumes but high biological activity and toxicity are included (hormones, anti-cancer drugs) [4–6]. However, the number of molecules is often still excessive for a reliable monitoring program. This is because some data necessary to shorten the list are lacking, such as the degradation rates in sewage treatment plants (STPs) and surface water [7,8].

Here we propose an approach based on two parts, one on theory and one practical, to identify the molecules of concern for the environment in Italy. The first step is to pre-select the pharmaceuticals according to tonnage (prescription) or to biological activity, and the second is to refine the list by measuring them in STPs and in surface water.

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2. Materials and methods

2.1. Pre-selection

This comprises a preliminary screening of the pharmaceuticals for human use most likely to cause environmental problems in Italy. We ranked the predicted environmental loads of the active substances, calculated by multiplying sales figures, based on official Ministry of Health prescription data [1], by the rate of metabolism in man. By correcting for the percentage of excretion as parent compound, annual sales figures were converted into predicted environmental loads [3]. Only the drugs with the highest predicted loads were considered for analysis in the environment. We also included a group of "historical" drugs with long persistence (diazepam and carbamazepine), and a group of molecules with high activity and potential toxicity but low environmental load, like estrogens and anti-cancer drugs (ethinylestradiol, cyclophosphamide, methotrexate).

2.2. Sampling in STPs

The selected pharmaceuticals were measured in effluents of the following STPs in Italy: Cagliari, Latina, Cuneo, Varese, Cosenza, Palermo, Naples, Monza. For each plant a 24-h composite sample was obtained by pooling effluents collected every 20 min by an automatic sampling device. Water samples were stored at 4 °C. Before extraction and analysis, samples were filtered on a glass micro-fiber filter GF/D 2.7 μm (Whatman, Kent, UK).

2.3. Sampling in surface water (Po and Lambro rivers)

Concentrations measured in STPs were compared with previously published levels in the rivers Po and Lambro [6]. In some cases (when data were not available) we added new measurements. For this purpose, composite water samples (pools of five samples collected each 30 min) were collected from the river Po (sampling sites Mezzano, Piacenza and Cremona) and the river Lambro (sampling site Lambro park, in Milan), in September 2004. Water was stored at 4 °C. Before extraction and analysis, samples were filtered on a glass micro-fiber filter GF/D 2.7 μm (Whatman, Kent, UK).

2.4. Analysis

STP and river water samples were analysed as previously reported [6]. Briefly, samples were extracted by Oasis MCX at pH 1.5–2.0 and Lichrolut EN at pH 7.0. Eluates were dried under an air stream and redissolved in 0.01% acetic acid in MilliQ water (pH 3.5), then centrifuged, transferred to glass vials, and 10-μL samples were injected with an auto sampler. The HPLC system consisted of two Perkin-Elmer series 200 pumps and a Perkin-Elmer series 200 auto sampler. A Luna C8 column, 50 mm × 2 mm i.d., 3 μm particle size (Phenomenex, Torrance, CA, USA), was used for chromatographic separation. For analysis in the positive ion mode eluent A was 0.1% formic acid in MilliQ water (pH 2) and eluent B was acetonitrile. The elution started with 100% of eluent A, followed by a 10 min linear gradient to 100% of eluent B, 2 min isocratic elution and a 2 min linear gradient to 100% of eluent A, which was maintained for 6 min to equilibrate the column. Analysis in the negative ion mode was done with TEA 0.05% in water as eluent A and acetonitrile as eluent B, and the same elution gradient as above.

Estrogens were analyzed in the negative ion mode with the same eluent but a different gradient. Analysis started with 100% of eluent A, followed by a 6-min gradient to 70% of eluent A and 30% of eluent B and a 7-min gradient to 100% of eluent B, maintained for 2 min and then back to the initial conditions within 1 min. During analysis the flow rate was 200 μL/min and the column was kept at room temperature.

2.5. Mass spectrometry (HPLC tandem MS–MS)

An Applied Biosystem-SCIEX API 3000 triple quadrupole mass spectrometer equipped with a turbo ion spray source (Applied Biosystems—Sciex, Thornhill, Ontario, Canada) was used. The analyses were done in the negative ion mode for bezafibrate, ibuprofen, furosemide, hydrochlorothiazide, sulphamethoxazole and estrogens and in the positive ion mode for the other compounds. Mass spectrometry analyses were done in the multiple reaction monitoring (MRM) mode, measuring the fragmentation products of the protonated or deprotonated pseudo-molecular ions of each drug and internal standard. Each compound was quantified by MRM, using the two highest characteristic precursor ion/product ion transitions. Comparison of the retention times with the corresponding reference standards also helped identify the compounds. The internal standards were salbutamol-D_3, to quantify the pharmaceuticals analyzed in the positive ion mode, and ibuprofen-D_3 and 17β-estradiol-D_2 for compounds analyzed in the negative ion mode.

3. Results

The first step in selecting pharmaceuticals "of concern" was to identify molecules that might pose an environmental risk from a quantitative point of view, and this was done using national prescription data. Before excretion, pharmaceuticals may be partially or completely metabolised in the body. By correcting for the percentage of excretion as parent compound, annual prescription figures were converted into predicted environmental loads. Only the drugs with the highest loads were considered for analysis in the environment (Table 1). This group, therefore, included top-ranking pharmaceuticals by prescription, though we did not include drugs with high prescription volumes but extensively metabolized and degraded before excretion. A second group of pharmaceuticals was also added, comprising some

Table 1
Prescriptions, metabolic rate, and predicted environmental loads of some top-ranking pharmaceuticals in Italy in 2001

Pharmaceuticals	Prescriptions (tonnes, active substance)	Excretion as parent compound (%)	Predicted environmental load (tonnes)
Amoxycillin	209.58	60	125.75
Atenolol	22.07	90	19.86
Hydrochlorothiazide	14.66	95	13.93
Ranitidine	26.67	40	10.67
Clarithromycin	33.87	25	8.47
Ceftriaxone	8.47	70	5.93
Furosemide	6.40	90	5.76
Bezafibrate	7.60	50	3.80
Ciprofloxacin	14.82	20	2.96
Enalapril	4.91	30	1.47
Spiramycin	5.11	20	1.02
Omeprazole	3.34	20	0.67
Erythromycin	3.92	10	0.39
Ibuprofen	1.90	10	0.19

Predicted environmental loads were obtained by correcting the prescription figures for the excretion rate as parent compound in humans.

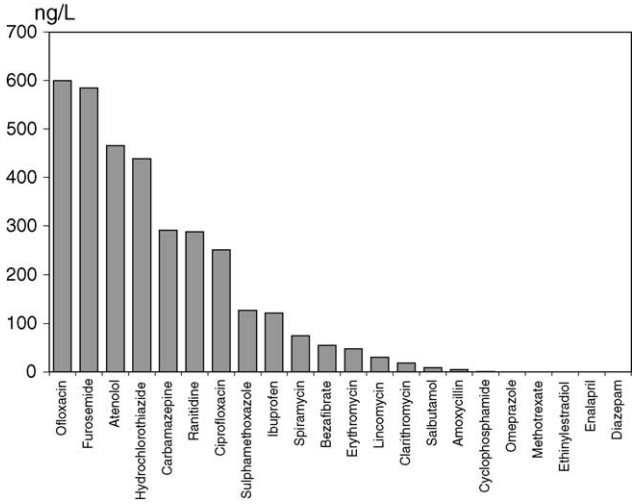

Fig. 1. Pharmaceuticals in effluents of urban sewage treatment plants (STP) in Italy. Median of nine STPs (values are expressed in ng/L).

molecules with low sales volumes but high biological activity and toxicity (hormones, anti-cancer drugs), and some with a long history of use and persistence in the environment (diazepam and carbamazepine) [2]. Table 2 shows the final list of drugs selected, with their therapeutic categories. It includes several antibacterial drugs, belonging to the penicillins, quinolones, macrolides, lincosamides and sulfamides classes, some diuretics, cardiovascular, gastrointestinal and central nervous system drugs, an anti-inflammatory, a bronchodilator, a lipid regulator drug, estrogens and anti-cancer drugs.

Fig. 1 reports the median of the concentrations of the pharmaceuticals measured in effluents of nine STPs spread over Italy, with three plants in the north (Cuneo, Varese, Monza), one in central Italy (Latina), two in the south (Cosenza, Naples) and two in the islands (Cagliari, Palermo).

Amoxycillin, in spite of very high-predicted environmental loads (125 tonnes per year) had low concentrations in STPs effluents, probably because of rapid environmental degradation. The same was observed for omeprazole, salbutamol, enalapril, diazepam, cyclophosphamide, methotrexate and ethinylestradiol. Concentrations of ofloxacin, furosemide, atenolol, hydrochlorothiazide, carbamazepine, ranitidine, ciprofloxacin, sulphamethoxazole and ibuprofen were in the hundreds of ng/L, spiramycin, bezafibrate, erythromycin, lincomycin and clarithromycin were in the tens.

Table 2
Pharmaceuticals for human use selected for analysis

Penicillins	Amoxycillin
Quinolones	Ciprofloxacin ofloxacin
Macrolides-lincosamides	Clarithromycin erythromycin lincomycin spiramycin
Sulfamides	Sulphamethoxazole
Diuretics	Furosemide hydrochlorothiazide
Cardiovascular	Atenolol enalapril
Gastrointestinal	Omeprazole ranitidine
CNS drugs	Carbamazepine diazepam
Anti-inflammatory	Ibuprofen
Bronchodilators	Salbutamol
Lipid regulators	Bezafibrate
Estrogens	Ethinylestradiol
Anti-cancer drugs	Cyclophosphamide methotrexate

Effluents of STPs, with their content in pharmaceuticals, enter surface waters, thus contributing to environmental contamination. However, the amounts reaching surface water are affected by several factors, including environmental degradation. The degradation rates of several pharmaceuticals in the environment are not known, and can only be estimated from degradation data in laboratory conditions, sometimes available from the literature. Table 3 reports degradation rates of pharmaceuticals in water, as described in the literature. Some molecules have short half-lives and are rapidly degraded (amoxycillin, ceftriaxone, ibuprofen, bezafibrate, omeprazole, sulphamethoxazole), while others are reported to be stable in fresh water for considerable periods (atenolol, carbamazepine, ciprofloxacin, enalapril, erythromycin, furosemide, ofloxacin, ranitidine). No data were available for other potentially relevant molecules, such as lincomycin, clarithromycin, hydrochlorothiazide, salbutamol, spiramycin.

Table 4 compares levels of pharmaceuticals in effluents of STPs, with concentrations in the river Po and river Lambro, as reported recently [6]. It is, therefore, useful for estimating the environmental persistence of these drugs, which is an indication of their hazardous potential. This comparison confirms ofloxacin, furosemide, atenolol, hydrochlorothiazide, carbamazepine, ibuprofen, spiramycin, bezafibrate, erythromycin, lincomycin and clarithromycin as priority pollutants in Italy. Sulphamethoxazole, ciprofloxacin, ranitidine and salbutamol can be considered “second line” pollutants because in spite of high levels in STPs they are not detectable – or only at low levels – in the rivers.

Table 3
Stability of some pharmaceuticals in water [7,8]

Pharmaceuticals	Stability in water	Comments
Amoxycillin	t_{90} < 2 d	Low stability
Atenolol	Stable for 40 d (5–25 °C) t_{50} 45.2 h pH 7.4 (UV ray)	Moderate stability
Bezafibrate	83% degraded in 6 d in STP	Low stability
Carbamazepine	t_{50} 100 d	Prolonged stability
Ceftriaxone	t_{90} 250 h (pH 6, 20 °C)	Low stability
Ciprofloxacin	Stability > 40 d in closed bottle test t_{50} 90.2 min (xenon lamp 200 W/m^2) t_{50} 1.6–2.5 d in STP	Moderate stability
Clarithromycin	t_{50} 1.3 h pH 2, 37 °C	Excessively acidic conditions
	t_{50} 17 min pH 1.39	Excessively acidic conditions (no data available)
Enalapril	Stable 56 d (25 °C) Stable 91 d (4 °C)	Prolonged stability
Erythromycin	$t_{50} \geq 1$ y 11.5 d (20 °C) t_{50} 3 s pH 1.39	Prolonged stability
Furosemide	Stable 90 d pH 5.2 Stable 96% 240 d pH 5.2	Prolonged stability
Ibuprofen	t_{50} < 1 d 90% degraded in 6 d STP	Low stability
Ofloxacin	t_{50} 10.6 d	Moderate stability
Omeprazole	70% 1–2 d pH 5.9–7.0 26% 14 d pH 7.8 94% > 100 d pH 11 73% 6d pH 7.0	Low stability
Ranitidine	Stable 160 h pH 6.18, 65°	Prolonged stability
Sulphametoxazole	t_{50} 2.4 d	Low stability

4. Discussion

Until recently, information on medicinal substances released into the environment was scant but numerous studies have now measured pharmaceuticals in effluents of STPs, and surface and ground water in Europe and USA [8–14]. Environmental concentrations of the pharmaceuticals are generally in the μg/L range in STP effluents and, because of dilution or degradation, in the ng/L range in rivers and ground water. However, because of differences in the prevalence of diseases, treatment habits and options, or simply for market reasons, the pollution profile in different countries can vary.

To identify pharmaceuticals of environmental concern in Italy a comprehensive analytical method measuring all possible substances would be ideal, but this is difficult with hundreds of candidate molecules. We, therefore, took a different approach, starting by ranking the molecules with the highest likelihood of causing environmental problems and refining the group by analysis. By correcting for the percentage of excretion as parent compound after administration in man, annual prescription figures were converted into predicted environmental loads, and only the drugs with the highest loads were considered [6]. The list also included some molecules with low tonnage, but suspected of causing environmental problems because of their high biological activity and toxicity (hormones, anti-cancer drugs), or their long history of use and persistence in the environment (diazepam and carbamazepine) [2].

Table 4
Pharmaceuticals in effluents of urban sewage treatment plants (STP) (median of nine STPs), and in surface water from the River Po (median and maximum value of seven sampling sites), and River Lambro (sampling site Milan)

ng/L	STPs	Lambro river	Po river	
	Median		Maximum	Median
Ofloxacin	600.0	306.1	37.0	33.1
Furosemide	585.0	254.7	67.2	3.5
Atenolol	466.0	241.0	41.7	17.2
Hydrochlorothiazide	439.1	255.8	24.4	4.6
Carbamazepine	291.1	175.3	34.2	23.1
Ranitidine	288.2	38.5	4	1.3
Ciprofloxacin	251.0	14.4	26.2	Nd
Sulphamethoxazole	127.2	Nd	Nd	Nd
Ibuprofen	121.2	20.0	17.4	13.0
Spiramycin	75.0	74.2	43.8	9.8
Bezafibrate	54.8	57.2	2.7	1.9
Erythromycin	47.4	4.5	15.9	3.2
Lincomycin	30.5	24.4	248.9	32.6
Clarithromycin	18.1	8.3	20.3	1.6
Salbutamol	8.7	2.5	1.7	1.1
Amoxycillin	4.7	Nd	Nd	Nd
Cyclophosphamide	0.6	Nd	Nd	Nd
Diazepam	0.0	Nd	Nd	Nd
Enalapril	0.0	0.5	0.1	0.1
Ethinylestradiol	0.0	Nd	Nd	Nd
Methotrexate	0.0	Nd	Nd	Nd
Omeprazole	0.0	Nd	Nd	Nd

Values are expressed in ng/L.

Urban STPs collect waste water and sewage from densely inhabited areas. Some pharmaceuticals are poorly removed in these plants and are consequently detectable in surface waters. STPs might, therefore, be considered important point sources of contamination. The list was first checked, therefore, by measuring pharmaceuticals in effluents of nine STPs spread over Italy. This provided preliminary data on pharmaceuticals in wastewaters entering surface waters. However, to constitute an environmental problem, a drug must also persist enough to be found in surface water.

We, therefore, compared levels in STPs and in the Po and Lambro rivers, as recently reported [6]. The Po is the largest Italian river and collects sewage from a vast industrialized area in northern Italy [14]. The river Lambro collects waste water from Milan, a city with more than a million inhabitants which, at the time of the study, had no wastewater treatment plant. They can, therefore, be considered representative of Italy and serve to estimate the contamination of surface waters.

This comparison was useful to identify ofloxacin, furosemide, atenolol, hydrochlorothiazide, carbamazepine,

ibuprofen, spiramycin, bezafibrate, erythromycin, lincomycin and clarithromycin as priority pollutants. For several of these molecules this was expected, because of their high-predicted environmental loads and reported stability in water, but it was unexpected for others, like ibuprofen and bezafibrate, because of their short half-lives and rapid degradation in STPs [7]. Sulphamethoxazole, ciprofloxacin, ranitidine and salbutamol can be considered "second line" pollutants and could probably be removed from the priority list because their concentrations in river water are low.

5. Conclusions

We used a two-part approach, one theory and one practical, to identify pharmaceuticals of concern for the environment in Italy. The first part involved ranking the molecules according to predicted environmental loads, and the second part was a refinement of the list by analysis of the pharmaceuticals in water from nine STPs and two major rivers in Italy. We identified a short list of priority pollutants for the Italian environment, to which studies and further monitoring could be restricted.

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