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Strategic Survey of Therapeutic Drugs in the Rivers Po and Lambro in Northern Italy

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A survey was done in the river Po (Italy) to check for therapeutic drugs in the environment. A number of pharmaceuticals were selected for analysis on the basis of high consumption and excretion as parent compound in humans. Eight sampling stations along the rivers Po and Lambro made it possible to plot the patterns of contamination in a highly populated region with a large number of animal farms. Atenolol, lincomycin, erythromycin, clarithromycin, bezafibrate, and furosemide were present at all the sampling sites, and other drugs were found only in some. Concentrations ranged from 0.1 to 250 ng/L, and several drugs exceeded the trigger value (10 ng/L) suggested by recent documents from the European Agency for the Evaluation of Medicinal Products (EMEA), assessing environmental risks for these chemicals. The patterns of contamination showed differences among sub-basins which correlated with the presence of large human settlements and/or animal farms. The ratio of measured to predicted concentrations (MEC/PEC) allowed a gross division of the drugs into two groups. The first consisted of pharmaceuticals with a MEC/PEC in the range 0.01−0.3, where the ratio is probably determined by the environmental behavior and the extent of degradation of the molecule. The other group consisted of pharmaceuticals found at concentrations higher than those predicted (MEC/PEC > 1). In this group, which consists of drugs sold without prescription or for veterinary use, market justifications (sales load uncertainty) have more role than chemical properties and environmental fate in explaining the differences between measured and predicted environmental concentrations.

Introduction

In recent years, pharmaceutical residues in the environment have become a subject of public concern. Thousands of tons of pharmacologically active substances are used yearly to treat human and animal illnesses, in farming and aquaculture. These substances can be excreted unmetabolized or as active metabolites; they can escape degradation in sewage treatment plants and enter the environment, and can be detected in sewage, surface, ground, and drinking waters (*1*−*4*). Improper disposal of expired medications also contributes to this contamination. Little is known about the extent of environmental occurrence, transport and ultimate fate of most pharmaceuticals after use. Until recently there were few analytical methods capable of detecting these compounds at the low concentrations expected in the environment. Another major difficulty is that thousands of pharmaceutical compounds are registered in most of the European countries and their market changes every year. Finally, the prediction of partition in environmental media, according to the classical approach, does not work properly for these compounds (e.g., soil/sediment sorption or suspended solid sorption in sewage treatment plants in relation to log K_{oc} or log K_{ow}). A recent review suggests that a number of independent mechanisms such as cation exchange, cation bridging at clay surface, surface complexation, and hydrogen binding are involved, other than hydrophobicity partitioning, in sorption of veterinary pharmaceuticals (*5*).

Pharmaceuticals are designed to stimulate a response in humans and animals at low doses, with a very specific target, so the implications for human health and the environment need to be assessed. Few attempts have been made to propose remedial actions or management measures (*6*−*8*). In a few cases the effects have been investigated, as for estrogens or antibiotics. Steroids, and in particular estrogens used in oral contraceptives, have high potency and can cause biological effects even at very low concentrations. These compounds may be responsible for the high rates of hermaphroditic fish in English rivers (*9*, *10*) receiving wastes from urban sewage treatment plants. Antibacterials in water, soil, and sediments could cause alterations in microbial communities and affect the higher organisms in the food chain (*11*).

Directives exist on environmental risk assessment for veterinary drugs in the European Union (*12*, *13*), and methods for assessing the environmental risk for human pharmaceuticals are suggested in a recent European Agency for the Evaluation of Medicinal Products (EMEA) draft document (*14*). As few data are available on concentrations in water, exposure can hardly be evaluated, so risk assessment is not feasible, and more basic data are necessary. The problem is now arousing worldwide interest, and in several European countries and in the United States monitoring programs have been started (*4*, *15*) or are being designed. The numbers of studies on these issues are increasing fast, and suitable analytical methods and different strategies have been developed for selecting the molecules. Some scientists have concentrated on selected classes of chemicals (*16*), whereas others have studied long lists of different molecules (*4*).

In Italy a preliminary monitoring program has been set up in the past few years, but data are still scant. On the basis of a previous investigation on drug consumption and analysis in river water and sediments (*3*, *17*), a wider campaign was organized in the Po and Lambro rivers, and the results are presented in this paper.

An attempt to predict pharmaceutical concentrations in the River Lee (the source of drinking water for North London, England) was made by Richardson and Bowron (*18*) in a worst-case scenario based on the amounts of drugs dispensed or sold to the public. The calculation was done from a theoretical point of view with a very small basis of analytical results. The aims of the present study were first, to check for pharmaceuticals in the Po and Lambro rivers in the north of Italy, and second, to find out if there are any correlations between the concentrations of pharmaceuticals detected in

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TABLE 1. Estimated Sales and Theoretical Environmental Loads[a] of Active Substances for Human Use in Italy in 2001 and 1997, and their Therapeutic Categories

pharmaceutical (therapeutic category)	sales data in 2001 (tons)	% excretion as parent compound (reference)	theoretical environmental load in 2001 (tons)	theoretical environmental load in 1997 (ton)
		Human Use		
amoxycillin (antibacterial)	209.58	60% (19)	125.75	59.64
atenolol (β-blocker)	22.07	90% (20)	19.86	7.54
bezafibrate (lipid regulating)	7.60	50% (19)	3.80	-
ceftriaxone (antibacterial)	8.47	70% (19)	5.93	11.42
ciprofloxacin (antibacterial)	14.82	20% (21)	2.96	-
clarithromycin (antibacterial)	33.87	25% (19)	8.47	-
clofibric acid (lipid regulating)	-	-	-	-
cyclophosphamide (cytotoxic)	-	-	-	-
diazepam (anxiolytic)	-	-	-	-
enalapril (antihypertensive)	4.91	30% (22,23)	1.47	-
erythromycin (antibacterial)	3.92	10% (20)	0.39	1.00
furosemide (diuretic)	6.40	90% (19)	5.76	3.49
hydrochlorothiazide (diuretic)	14.66	95% (24)	13.93	1.37
ibuprofen (antiinflammatory)	1.90	10% (20)	0.19	1.00
omeprazole (ulcer healing)	3.34	20% (20)	0.67	-
ranitidine (ulcer healing)	26.67	40% (19)	10.67	10.46
spiramycin (antibacterial)	5.11	20% (20)	1.02	-
		Veterinary Use		
lincomycin (antibacterial)	7.19	50% (20)	3.60	5.11
oleandomycin (antibacterial)	-	-	-	-
oxytetracycline (antibacterial)	-	-	-	-
salbutamol (bronchodilator)	0.42	30% (20)	0.126	0.034
tilmicosin (antibacterial)	-	-	-	-
tylosin (antibacterial)	-	-	-	-

[a] Theoretical environmental loads were obtained by correction of the sales by the excretion rate as parent compound in humans.

the rivers and those calculated from theoretical loads. This correlation could be useful to predict pharmaceutical concentrations in water in given catchment areas or in wider basins using theoretical loads and a solid analytical base.

Experimental Section

Pharmaceuticals. The list of pharmaceuticals considered in the present study was selected on the basis of a "leading medicinal products list" drawn up in 1997 (*3*), which identified the main active substances sold in tons per year in Italy and excreted in the environment as parent compounds (amoxycillin, atenolol, ranitidine, lincomycin, erythromycin, ceftriaxone, furosemide, salbutamol, and spiramycin). Other drugs, detected in Europe during occasional surveys, were also included (ibuprofen, diazepam, clofibric acid, cyclophosphamide, and bezafibrate); finally, a group of widely used veterinary drugs (tylosin, tilmicosin, oleandomycin, and oxytetracycline) was considered, despite much more limited information on sales and use. The list adopted for this study was a revision of the previous list, updated to 2001 in Italy, including clarithromycin, omeprazole, enalapril, ciprofloxacin, and hydrochlorothiazide. Sales in 2001 were calculated using Ministry of Health drug prescription figures and the list is shown in Table 1. The theoretical environmental loads were estimated by correcting the sales figures for the percentage of each drug excreted as parent compound. Values are reported in the third column of Table 1 (*19*−*24*). Table 1 also gives the environmental loads estimated for 1997. Present knowledge of the behavior of pharmaceuticals in treatment plants is summarized in a recent paper (*25*). Because information is still inadequate, in this preliminary study we did not distinguish removal during treatment from the other degradation processes taking place in the environment. Pharmaceuticals considered in the present list belong to different therapeutic categories and chemical classes. They can be grossly divided into two major groups according to their use: human medicine, or veterinary medicine, as shown in Table 1. Some, including erythromycin, amoxycillin, spiramycin, salbutamol, and lincomycin, are used in both human and veterinary medicine. Because for research purposes we had to attribute such pharmaceuticals to one group only, we made the assumption, supported by market data, that erythromycin, amoxycillin, and spiramycin are used more in human medicine, while lincomycin is now used mainly in veterinary medicine. Salbutamol is theoretically used only in human medicine, but unofficial information indicates its illegal use in veterinary medicine (for its anabolic activity) as quantitatively more substantial than in human medicine.

Sampling Sites. The sampling sites were selected on the Rivers Po and Lambro in the north of Italy, mainly close to the inputs of wastewater from cities or other rivers (Figure 1). The Po is the main Italian river, with a length of 652 km (from the Alps to the Adriatic sea) and an average and a maximum flow rate at the mouth, in Pontelagoscuro, of respectively 1500 and 10 300 m^3/s. It collects wastewater from a catchment area of about 71 000 km^2 in the most densely inhabited and industrialized areas of Italy. The River Lambro collects wastewater from Milan, a city with more than a million inhabitants, which has no wastewater treatment plant. All the other major towns and animal farms along the river Po are equipped with secondary sewage treatment plants. The river Po drains sewage from about half of all the animal settlements in Italy.

Sampling stations were located after the inlets of the main affluents, and downstream of the major towns. The sampling sites on the Po were at Chivasso (sampling site 1, distance from the source 136 km, flow rate 140 m^3/s, 2.1 million inhabitants in the drainage basin), close to the city of Turin; then at Mezzano (site 2, distance from the source 298 km, flow rate 932 m^3/s, 5.4 million inhabitants in the drainage basin); and then at Boscone (site 3, distance from the source 320 km, flow rate 932 m^3/s, 10.0 million inhabitants in the drainage basin) which are, respectively, before and after the mouth of the Lambro. They were selected to detect the contribution of the Lambro's flow into the Po. Piacenza

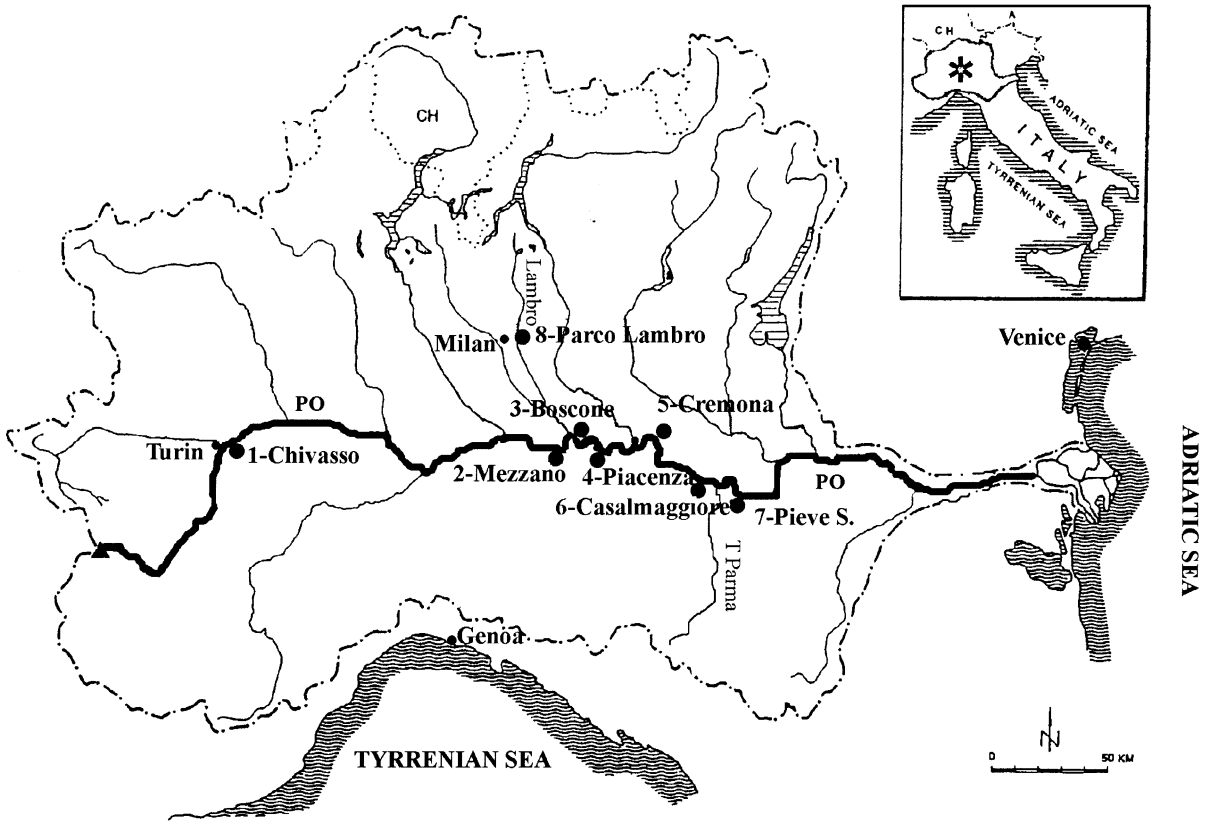

FIGURE 1. Map of the sampling stations along the rivers Po and Lambro in the north of Italy.

(sampling site 4, distance from the source 337 km, flow rate 932 m^3/s, 10.0 million inhabitants in the drainage basin) and Cremona (site 5, distance from the source 386 km, flow rate 1150 m^3/s, 11.7 million inhabitants in the drainage basin) were two sampling sites from the previous investigation (*3*) and receive loads from the two towns and from animal farms, which are very frequent in this area. Casalmaggiore (site 6, distance from the source 440 km, flow rate 1150 m^3/s, 12.3 million inhabitants in the drainage basin) and Pieve Saliceto (site 7, distance from the source 460 km, flow rate 1085 m^3/s, 12.3 million inhabitants in the drainage basin) are the terminal sites. They were selected to measure the load from the whole drainage basin as they can be approximately considered the points with the highest contamination. The sampling site selected on the River Lambro was Parco Lambro (site 8, flow rate 5 m^3/s, 1.5 million inhabitants in the drainage basin), that collects most of the untreated wastewaters from Milan.

A single 5-L sample was collected at each sampling site and immediately transferred into a glass flask. All samples were collected the same day in the late morning. The sampling period was October 2001, and the average flow rate was about 1000 m^3/s. This represented a medium flow for the Po river, and made the results of the present study comparable with those of the previous investigation, carried out in October 1997, with an average flow rate of 1100 m^3/s.

Distances from the source and number of inhabitants were obtained from the Istituto di Ricerca sulle Acque (IRSA (*26*)). The annual flow rates were kindly provided by the Ufficio Mareografico ed Idrografico del Po. Other data are from Idrografia e Idrologia del Po (*27*).

Sample Preparation and Analysis. To analyze pharmaceuticals in river water samples a specific multiresidue method was developed. Pharmaceuticals selected for analysis were divided into three groups (see below) according to the conditions used for their extraction, and were then extracted from water using various solid-phase extraction (SPE) cartridges and pH conditions. For this, aqueous samples were divided into three 500-mL aliquots, each processed through a different SPE cartridge. Cartridges used were as follows: 3-mL disposable Oasis MCX cartridges (60 mg, Waters Corp, Milford, MA), 3-mL disposable LiChrolutEN cartridges (200 mg, Merck, Darmstadt, Germany), and 3-mL disposable Bakerbond C_{18} cartridges (500 mg, Baker, Phillipsburg NJ).

Before extraction, water samples were filtered on a glass microfiber filter GF/D 2.7 μm (Whatman, Kent, U.K.) and spiked with 100 μL (0.1 ng/μL) of internal standards: salbutamol-D6 for analysis in positive ionization mode, or warfarin for analysis in negative ionization mode. The two internal standards were chosen mainly on the basis of their good mass spectrometric response in positive and negative ionization mode, their HPLC retention times (which were in the same range as those of the drugs under analysis), and their good recovery in the experimental conditions. The use of only two internal standards to analyze a variety of molecules with different chemical structures and properties is an obvious limitation of the method. However, this choice proved to be a good compromise between the need to obtain concentration data on a wide array of drugs and acceptable data quality. The drugs selected for analysis, their groupings, and the experimental conditions which were used are described below.

Group 1. Salbutamol, atenolol, ranitidine, diazepam, enalapril, lincomycin, oxytetracycline, ciprofloxacin, tilmicosin, oleandomycin, clofibric acid, bezafibrate and ceftriaxone were extracted with Oasis MCX cartridges. Samples were prepared by dissolving 100 mg of disodium ethylenediaminetetraacetate (Na_2-EDTA) in 500 mL of water to prevent tetracyclines from complexing with Ca^{2+} and Mg^{2+} ions and residual metals on the SPE cartridges. The pH was adjusted to 2.0 with 37% HCl. The cartridges were conditioned before use by washing with 2 mL of methanol and 2 mL of MilliQ

TABLE 2. Concentrations of Pharmaceuticals Measured at Sampling Sites in the Po and Lambro Rivers[a]

Pharmaceutical	1 (140)	2 (932)	3 (932)	4 (932)	5 (1150)	6 (1150)	7 (1085)	8 (5)
amoxycillin	nd	nd	nd	nd	nd	nd	nd	nd
atenolol	38.13	3.44	5.79	41.7	39.43	15.13	17.23	241.00
bezafibrate	1.58	1.09	0.79	2.75	2.66	1.92	2.30	57.15
ceftriaxone	nd	nd	nd	nd	nd	nd	nd	nd
ciprofloxacin	26.15	nd	nd	nd	nd	nd	nd	14.36
clarithromycin	20.30	1.24	1.56	3.38	0.49	0.80	1.67	8.31
clofibric acid	5.77	0.80	0.41	1.08	0.80	1.05	1.30	nd
cyclophosphamide	nd	nd	nd	nd	nd	nd	nd	nd
diazepam	0.13	0.23	nd	0.21	2.13	1.16	0.83	0.29
enalapril	nd	nd	nd	0.12	0.07	nd	0.05	0.54
erythromycin	15.90	3.92	3.24	4.56	1.41	1.40	2.75	4.50
furosemide	67.20	4.81	2.30	5.30	1.72	4.80	3.48	254.70
ibuprofen	nd	nd	nd	9.76	5.07	4.46	7.23	78.50
hydrochlorothiazide	24.40	0.53	nd	3.98	21.94	4.60	9.73	255.80
omeprazole	nd	nd	nd	nd	nd	nd	nd	nd
ranitidine	3.97	nd	1.17	1.57	1.27	0.86	1.60	38.50
spiramycin	43.80	9.79	nd	nd	nd	nd	nd	74.20
lincomycin	3.13	5.54	32.56	20.80	248.90	107.20	139.40	24.40
oleandomycin	nd	nd	0.09	0.07	0.13	nd	0.08	2.79
oxytetracycline	19.20	4.5	nd	nd	nd	nd	0.19	14.35
salbutamol	nd	nd	nd	nd	1.68	1.14	1.56	2.48
tilmicosin	nd	0.43	nd	nd	nd	nd	nd	nd
tylosin	nd	nd	nd	0.29	nd	nd	0.30	2.77

[a]Sampling sites: 1, Chivasso; 2, Mezzano; 3, Boscone; 4, Piacenza; 5, Cremona; 6, Casalmaggiore; 7, Pieve Saliceto; 8, Parco Lambro. River flow rates (m^3/s) are given in parentheses after each site. Concentrations of pharmaceuticals are expressed in ng/L.

purified water, and the water samples were then passed over the cartridges under vacuum. After the cartridges were dried under vacuum, they were washed with 1 mL of HCl 0.1 N and eluted with 2 mL of methanol and 2 mL of 5% ammonium hydroxide in methanol.

Group 2. Spiramycin, erythromycin, tylosin, clarithromycin, ibuprofen, furosemide, cyclophosphamide, and hydrochlorothiazide were extracted with LiChrolutEN cartridges. Water samples were adjusted to pH 7.0 with ammonium acetate (50 mM) buffer solution. Cartridges were conditioned before use by washing them with 6 mL of methanol and 6 mL of MilliQ water. The water samples were then passed over the cartridges under vacuum; the cartridges were then dried and eluted with 3 mL of methanol and 3 mL of ethyl acetate.

Group 3. Amoxycillin and omeprazole were extracted with Bakerbond C_{18} cartridges. Water samples were adjusted to pH 8.0 with ammonium acetate (50 mM) buffer solution. The cartridges were conditioned by washing with 4 mL of methanol, 4 mL of MilliQ water, and 2 mL of MilliQ water/NaCl 2% solution. The water samples were then passed over the cartridges under vacuum; the cartridges were then dried and eluted with 2 mL of acetonitrile.

The eluates were dried under nitrogen and dissolved in 200 μL of formic acid 0.1% and acetonitrile (9:1) for analysis in HPLC−MS-MS.

The HPLC system consisted of two Perkin-Elmer Series 200 pumps and a Perkin-Elmer Series 200 autosampler. A LUNA C8 column 50 mm × 2 mm i.d., 3 μm particle size (Phenomenex, Torrance, CA) was used for the chromatographic separation. An Applied Biosystem-SCIEX API 3000 triple quadrupole mass spectrometer equipped with a turbo ion spray ionization system (Perkin-Elmer, Sciex Instruments, Foster City, CA) was used to detect the compounds. The MS detection was done in the negative ionization mode for clofibric acid, bezafibrate, ceftriaxone, ibuprofen, furosemide, and hydrochlorothiazide, and in the positive ionization mode for the other pharmaceuticals. Mass spectrometry analyses were done in the multiple reaction monitoring mode (MRM). (See Supporting Information for analytical details on HPLC and MS detection).

The recoveries of the pharmaceuticals from water (10 ng/L in 100 mL of MilliQ water) were mostly higher than 70%, with some exceptions (amoxicillin 49%, clarithromycin 61%, erythromycin 26%, hydrochlorothiazide 65%, omeprazole < 10%, oleandomycin 58%, spiramycin 28%, and tylosin 45%). The correlation coefficients (calculated in the range between 0.1 and 0.01 ng/μL) were 0.99 in almost all cases. The limits of quantification (LOQ) were below 0.3 ng/L with few exceptions (ceftriaxone 4 ng/L, ibuprofen 4.2 ng/L, amoxycillin 6 ng/L, and omeprazole 10 ng/L). Detailed results with means and standard deviations of recovery and LOQ values are provided in the Supporting Information.

Results and Discussion

Pharmaceuticals in Po and Lambro Rivers. We analyzed samples from seven locations on the River Po and one on the Lambro; we could therefore assess the patterns of contamination in a highly populated region with a large number of animal farms. Atenolol, lincomycin, erythromycin, clarithromycin, bezafibrate, and furosemide were present at all the sampling sites; ranitidine, clofibric acid, diazepam, and hydrochlorothiazide were measurable in several sites on both rivers. Omeprazole, amoxycillin, cyclophosphamide, and ceftriaxone were never detectable. There are several reasons for this. Amoxycillin has a heavy environmental load, as it is one of the most widely used antibiotics, but it is easily degraded in the environment, its t_{90} being <2 days at environmental temperature (*28*). Cyclophosphamide is quite stable in the environment, with a half-life longer than one year (*29*). However, its environmental loads are probably too low for a reliable detection. Omeprazole and ceftriaxone were not measurable probably because of their low environmental stability: the shelf life (t_{90}) of ceftriaxone in intravenous solutions is less than 250 h (*30*). Another possibility is that omeprazole, ceftriaxone, and amoxicillin were not detected because of analytical sensitivity that was too low (LOQ > 1 ng/L). The concentrations of the selected pharmaceuticals in Po and Lambro samples, reported in Table 2, are in the range 0.1−250 ng/L. The results of this sampling campaign were compared with those of a previous investigation, in 1997, as the analytical method used was the same (*3*). Three sites were sampled in both the campaigns and can there-

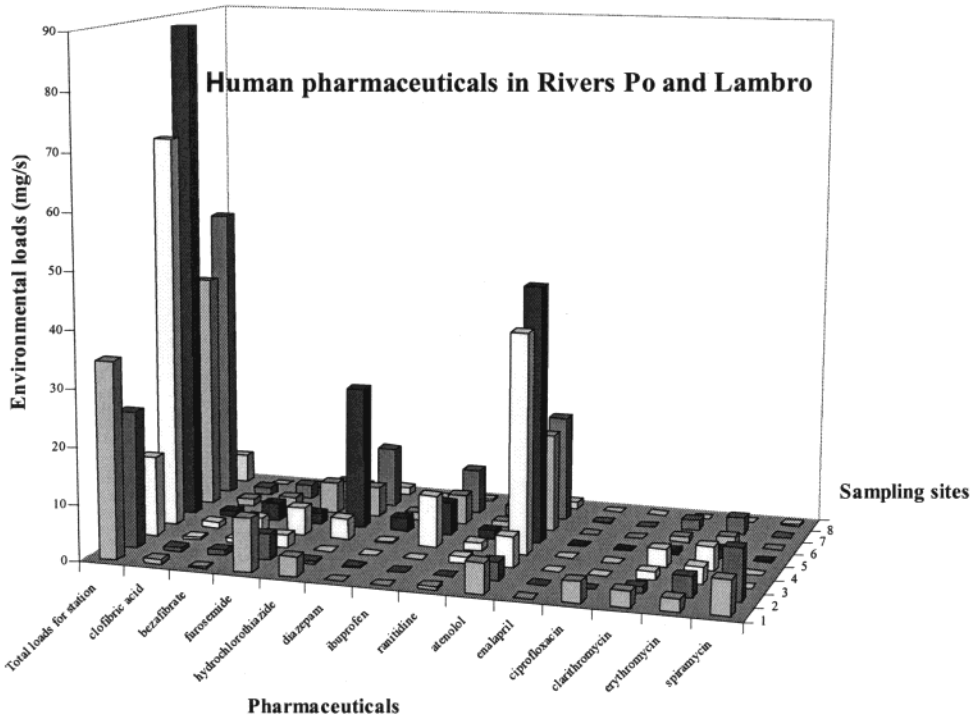

FIGURE 2. Environmental loads in the River Po due to human pharmaceuticals: 1, Chivasso; 2, Mezzano; 3, Boscone; 4, Piacenza; 5, Cremona; 6, Casalmaggiore; 7, Pieve Saliceto; 8, Parco Iambro.

fore be compared: Parco Lambro on the River Lambro (sampling site 8), and Piacenza and Cremona (sampling sites 4 and 5, respectively) on the Po. Results show good consistency in general. Atenolol, erythromycin, ibuprofen, diazepam, oleandomycin, salbutamol, spiramycin, and tylosin were in the same range of concentrations in both campaigns, but furosemide, ranitidine, and lincomycin concentrations were higher in the recent campaign. Bezafibrate was detected in lower concentrations in 2001, ceftriaxone was never detected, and cyclophosphamide was detected only in the River Lambro in 1997.

Environmental Loads in the River Po. The concentrations of human and veterinary drugs detected in water were multiplied by the average annual flow rate at each sampling site to obtain environmental loads, expressed in mg of pharmaceuticals/second. Figures 2 and 3 show the loads for human and veterinary pharmaceuticals in the different sampling sites. Lincomycin had the highest load and is presented alone in Figure 4; the increasing loads observed in Cremona, Casalmaggiore, and Pieve Saliceto are most probably due to the large number of animal farms in the sub-basin. The general pattern of contamination from human pharmaceuticals is described in Figure 2. There are peaks in correspondence to inputs from the main cities. The total loads show four peaks, at Chivasso (about 40 mg/s), where there is the influence of Turin (977 000 inhabitants), Piacenza and Cremona (between 70 and 90 mg/s, with 95 100 and 73 680 inhabitants, respectively) and Pieve Saliceto, which collects the wastewaters from Parma (60 mg/s, with 156 170 inhabitants). The role of the loads from Milan, carried by the Lambro, is not particularly evident in the contamination of the River Po, probably because the flow rate and the load of the Lambro (5 m^3/s) are small compared with the Po (1000 m^3/s). The general pattern shows therefore a peak of input in Chivasso, a decrease of the load in Mezzano and Boscone, a second major input in Piacenza and Cremona, another decrease of the load in Casalmaggiore, and a further input in Pieve Saliceto.

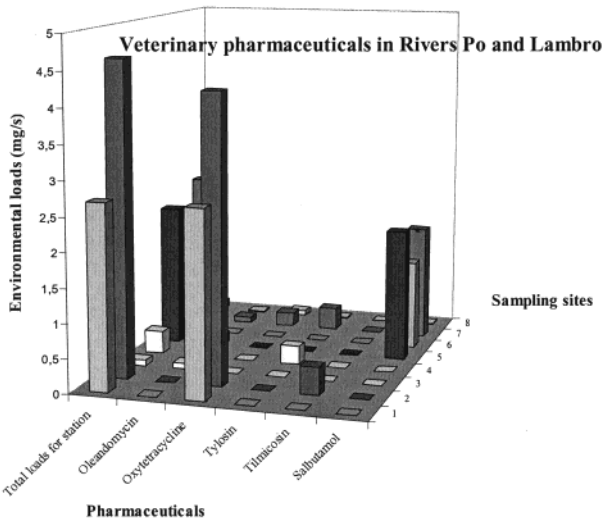

FIGURE 3. Environmental loads in the River Po due to veterinary pharmaceuticals: 1, Chivasso; 2, Mezzano; 3, Boscone; 4, Piacenza; 5, Cremona; 6, Casalmaggiore; 7, Pieve Saliceto; 8, Parco Iambro.

Veterinary pharmaceuticals had lower loads in general (Figure 3), with two exceptions: oxytetracycline had a peak of 4 mg/s in Chivasso and Mezzano, and salbutamol was detected in Cremona, Casalmaggiore, and Pieve, between 1.3 and 1.9 mg/s. Salbutamol is used as a bronchodilator in humans but it may also be used illegally in animals as an anabolic agent. Considering the anabolic activity is exerted at higher doses than the bronchodilator effect, this might explain the large amount of this drug detected in areas with a high density of animal farming. Oleandomycin, tylosin, and tilmicosin had loads lower than 0.4 mg/s. Lincomycin is used to treat humans and animals. However, since environmental concentrations rose in 2001 compared with those in 1997, despite a drop in sales of this pharmaceutical for human use (Figure 4 and Table 1), we assumed that at present

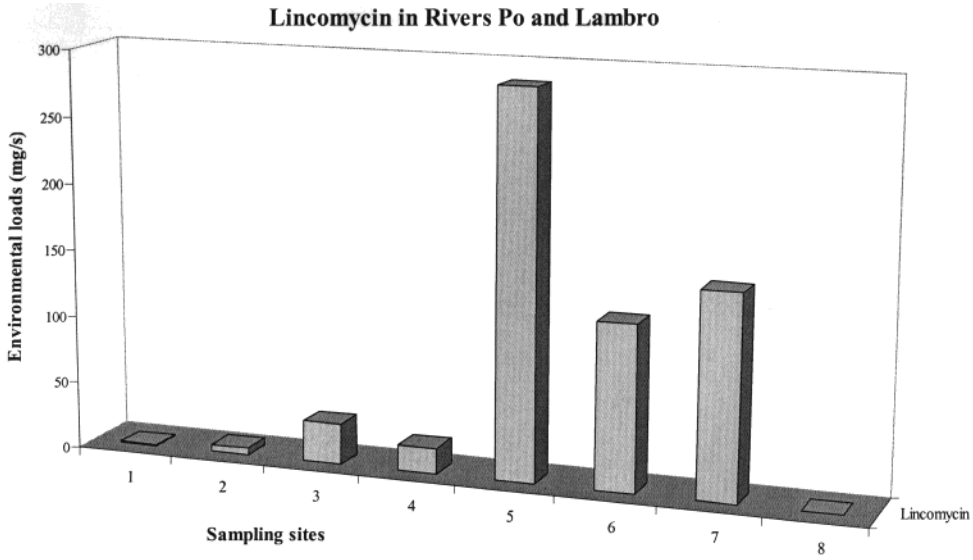

FIGURE 4. Lincomycin environmental loads in the River Po: 1, Chivasso; 2, Mezzano; 3, Boscone; 4, Piacenza; 5, Cremona; 6, Casalmaggiore; 7, Pieve Saliceto; 8, Parco Iambro.

it is mainly used in veterinary medicine. This might explain the loads detected at Cremona (286 mg/s), Casalmaggiore (123 mg/s), and Pieve Saliceto (151 mg/s), which are all areas with a high density of cattle breeding.

A principal component analysis (PCA) was done introducing the environmental loads into the matrix of Table 2. The environmental loads were calculated by multiplying the concentrations by the annual average flow rates. PCA was done to provide a description of the relationships between pharmaceutical environmental loads and sampling station characteristics (Figure 5). For both the human and the veterinary compounds, the sampling sites are distributed along the first axis according to a gradient of increasing overall contamination, and increasing number of molecules detected, from upstream to downstream sites. This explains more then 50% of the variability of the three sources listed. The second axis, which estimates other causes of variability, is only 20%, so it can be assumed there are no major hidden sources of variability.

Mass Balance. A mass balance study was done to compare predicted (input) and measured (output) concentrations of the pharmaceuticals, to assess the reliability of the approach we used to predict concentrations, and to assess the utility of predictive approaches for studying environmental contamination by pharmaceuticals. The differences in the measured-to-predicted ratios for pharmaceuticals are probably ascribable to their different environmental behavior along the river, where various chemical and biological processes of degradation are supposed to occur. However, data are scant and at the moment it is not possible to quantify the specific role of each of these processes.

The theoretical environmental loads for each sampling site were estimated by normalizing the nationwide loads to the number of inhabitants of the drainage basin at the sampling stations. The predicted concentrations were then obtained by dividing the theoretical loads (annual sales loads corrected for the metabolic rates, Table 1) by the average annual flow rate of the rivers at each sampling site. Table 3 reports the ratios between measured (MEC) and predicted environmental concentrations (PEC). The MEC values used were the means of all the measured concentrations in the various sampling stations along the Po.

MEC values for pharmaceuticals for human use, in the River Po, were generally lower than the PEC values. The highest MEC/PEC ratios were for furosemide, atenolol, and hydrochlorothiazide (respectively 0.19, 0.12, and 0.31). A

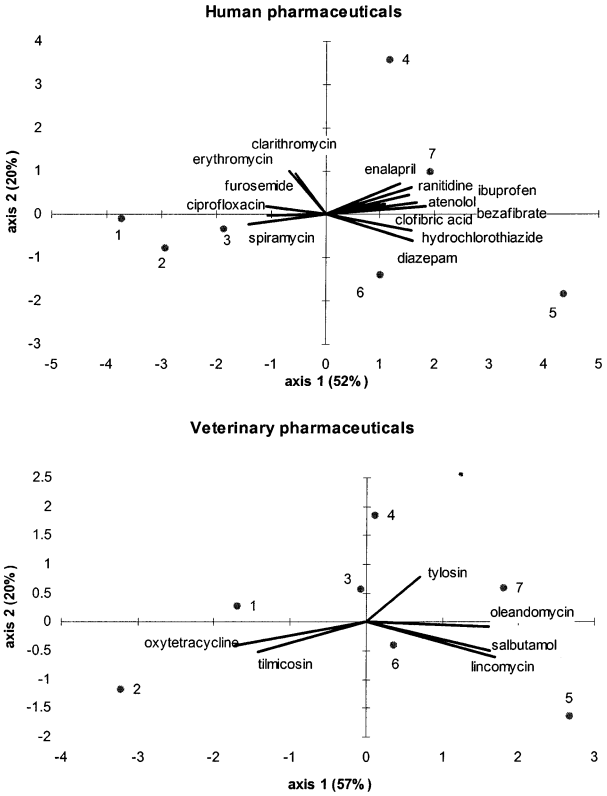

FIGURE 5. Sites ordination for human and veterinary pharmaceuticals resulting from principal component analysis; names of pharmaceuticals and numbers for sampling stations are shown in the figure: 1, Chivasso; 2, Mezzano; 3, Boscone; 4, Piacenza; 5, Cremona; 6, Casalmaggiore; 7, Pieve Saliceto.

characteristic of this group of molecules is that they are poorly metabolized in humans, and are therefore mainly excreted as parent compounds (90−95%). On the other hand, ranitidine, clarithromycin, enalapril, and bezafibrate, which had lower MEC/PEC ratios (0.01−0.09), are more subject to metabolism in humans, and 25−50% is excreted as the parent compound. The difference in the MEC/PEC ratios is not a simple effect of the differences in the metabolic rates, because PEC values are already corrected for metabolism. Therefore, even after correction there remains a gross relationship

TABLE 3. Mass Balance of Pharmaceuticals in the Po and Lambro Rivers by Comparison of Predicted and Measured Concentrations in Water

pharmaceutical	MEC/PEC ratio[a] in Po river	MEC/PEC ratio[a] in Lambro river
Human Use		
atenolol	0.19	0.07
bezafibrate	0.09	0.09
clarithromycin	0.07	0.006
enalapril	0.01	0.002
erythromycin	2	0.07
furosemide	0.31	0.28
hydrochlorothiazide	0.12	0.11
ibuprofen	5.86	2.53
ranitidine	0.02	0.02
Human and Veterinary Use[b]		
lincomycin	3.84	0.04
salbutamol	1.96	0.12
spiramycin	4.12	0.45

[a] MEC, measured environmental concentration; PEC, predicted environmental concentration. [b] PECs for pharmaceuticals used in veterinary and human medicine were estimated from the sales figures for human use only.

between the percentage of excretion of the pharmaceutical as parent compound and its MEC/PEC ratio, and we can assume that a drug that is highly metabolized in humans is also subject to extensive degradations in the environment. In this process, environmental behavior and fate of the pharmaceutical might well play significant roles, which still need to be clarified.

Ratios for erythromycin and ibuprofen were respectively 2 and 5.8. MEC/PEC ratios were higher than expected very likely because of an underestimate of the environmental burdens, and therefore of the PEC values, probably linked to the fact that these drugs can be bought without a prescription (self-prescription or over-the-counter drugs) whereas our PEC calculations are based on drug prescription figures only.

The ratios MEC/PEC were also greater than 1 for salbutamol, spiramycin, and lincomycin, probably because of an underestimate of the theoretical loads, as these compounds can be used in either human or veterinary medicine, but sales figures are for human use only because information on sales for veterinary use is not available.

Results in the River Lambro confirm the data obtained in the Po, except for salbutamol, spiramycin, lincomycin, and erythromycin which in the Lambro had a ratio below unity. Considering these pharmaceuticals can be used in either humans or animals and the Lambro drains wastewaters from Milan, an urban area with no animal farms, these differences can be ascribed to the lack of the veterinary pharmaceuticals.

We tried to find a correlation between the MEC/PEC ratios and the physicochemical properties of the pharmaceuticals. Atenolol, hydrochlorothiazide, and furosemide, which gave high ratios, have low log K_{ow} values, respectively 0.16, −0.07, and −0.92 (*31*), and high or medium solubility in water, respectively 26.5 g/L (*32*), 0.1 g/L (*20*), and 0.01 g/L (*33*). Enalapril, clarithromycin, and bezafibrate, which gave low ratios, have comparable solubility, respectively 0.025 g/L (*32*), 3.42×10^{-4} g/L (*34*), and 0.016 g/L (*32*), but higher log K_{ow} values, respectively 2.45 (*35*), 3.16 (*36*), and 4.25 (*35*). This could explain their low ratios in water because these compounds have greater affinity for sediments and suspended solids, but other factors must be considered too (*5*).

For the other pharmaceuticals it was impossible to find any simple relationships probably because other unknown factors must be taken into account for their mass balance studies, such as their environmental partitioning, degradation, and different types of interaction with other environmental media. For example, some pharmaceuticals are polar and can dissociate to their ionic form according to their pK_a values. In this case a number of hydrophobicity-independent mechanisms, such as cation exchange and hydrogen bonding, appear to be involved in the sorption on soils and sediments (*5*). Data about the environmental stability of pharmaceuticals and their removal by sewage treatment plants are still lacking (*25*).

In conclusion, in this investigation we detected most of the drugs selected on the basis of high consumption and a low metabolic rate (high excretion as parent compound) in humans. Therefore, this may be a good approach for defining a priority list for a wider monitoring campaign. Factors such as the environmental behavior of pharmaceuticals and removal during wastewater treatment should be included as well, though at the moment this is mostly not available.

Recent EU documents set out some requirements for the ecotoxicological environmental risk assessment of human pharmaceuticals (*14*). The PEC is used as a trigger value to establish the level of concern for the environment. A recent document suggests a trigger value of 10 ng/L for the aquatic environment (*14*, *37*). In the largest Italian river the level of contamination by pharmaceuticals exceeded this trigger value in a number of cases, and could therefore be a cause for concern. The results of the analyses made in 1997 and in 2001, in the same season, are generally comparable and give a relatively consistent picture of the situation.

By transforming concentrations (useful for exposure assessment) to environmental loads, one can obtain a good picture of the patterns of contamination along the River Po, and PCA showed that, despite the short distances between the sampling sites and the relatively high flow of the river, the fingerprint of contamination from pharmaceuticals was clearly different in the sub-basins, and correlated to the types of human settlement and the presence of animal farms.

The mass balance calculation permits a comparison of the measured and predicted concentrations, and the MEC/PEC ratio serves to test the consistency of the findings (to identify outliers and anomalous situations) and to evaluate methods to calculate PECs, for testing the reliability of predictive approaches for pharmaceuticals in the environment. The ratios also serve to divide the drugs grossly into two groups. The first group consists of pharmaceuticals with MEC/PEC ratios in the range 0.01−0.3, where the ratio is possibly affected by the behavior of the molecules and extent of their degradation in the environment. The other group consists of pharmaceuticals detected at concentrations higher than expected (MEC/PEC >1). In this group, consisting of drugs sold without prescription or for veterinary use, market justifications (sales load uncertainty) have more role than chemical properties and environmental fate to explain differences between measured and predicted environmental concentrations.

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Supporting Information Available

Tables of conditions for the HPLC separation of the pharmaceuticals, their MS−MS determinations, and analytical quality assurance data. This material is available free of charge via the Internet at http://pubs.acs.org.

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