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Short communication

Effluent from drug manufactures contains extremely high levels of pharmaceuticals

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Abstract

It is generally accepted that the main route for human pharmaceuticals to the aquatic environment is via sewage treatment plants receiving wastewater from households and hospitals. We have analysed pharmaceuticals in the effluent from a wastewater treatment plant serving about 90 bulk drug manufacturers in Patancheru, near Hyderabad, India—a major production site of generic drugs for the world market. The samples contained by far the highest levels of pharmaceuticals reported in any effluent. The high levels of several broad-spectrum antibiotics raise concerns about resistance development. The concentration of the most abundant drug, ciprofloxacin (up to 31,000 μg/L) exceeds levels toxic to some bacteria by over 1000-fold. The results from the present study call for an increased focus on the potential release of active pharmaceutical ingredients from production facilities in different regions.

Keywords: Pharmaceuticals; Antibiotics; Environment; Effluent; Toxicity

1. Introduction

The release of pharmaceuticals from sewage effluents to rivers and lakes is an issue of growing concern. Drugs are frequently detected in effluents at levels from below 1 ng/L up to a few μg/L. Ethinylestradiol, the estrogen in many hormonal contraceptives, is at least in part responsible for the feminization of fish downstream from sewage treatment plants [1–3]. Propranolol [4], diclofenac [5], gemfibrozil [6], ibuprofen and fluoxetine [7] are other examples of pharmaceuticals reported to affect aquatic organisms at or around environmentally relevant levels in laboratory experiments, but causal links between the exposure to these drugs and any observed environmental effects in the field have so far not been established. Antibiotic-resistant bacteria are found in the aquatic environment, but to what extent the antibiotics in the sewage effluents contribute to this development is also not clear [8].

Current environmental risk assessment procedures in different regions focus on the release of active ingredients from municipal sewage treatment plants [9,10]. Production facilities represent another potential way of entry of drugs to the environment [11]. The environmental standards of production facilities is generally covered by a different set of regulations, although without a similar focus on the potential release on active substances as for the registration and use of the final products [9,10]. Indeed, arguments have been raised that highly controlled production processes, as well as the great value of the drugs, would assure that only minor amounts of active substances would escape [12]. Interestingly, there is to our best knowledge no publicly available peer-reviewed information available that can confirm or reject this claim. In this study we therefore hypothesized that discharges of active ingredients during production could be of substantial environmental concern. We began to address this hypothesis by analysing active ingredients in a common effluent from a large group of production facilities in south-central India.

2. Materials and methods

2.1. Description of the treatment plant and sampling of effluent

The investigated plant (Patancheru Enviro Tech Ltd.; PETL) is situated in Patancheru, near Hyderabad. PETL receives

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approximately 1500 m^3 of wastewater per day, primarily from about 90 bulk drug manufacturers. These industries comprise examples of the entire production chain, via synthesis of intermediates to active ingredients. The wastewater is transported on trucks to PETL, where it is collected in a buffer cistern with a retention time of approximately 2 days thereby ensuring a less variable influent. After chemically assisted removal of solids, about 20% raw domestic sewage is added to improve biological treatment efficiency. The retention time in the aerated/oxygenated biological treatment is about 4 days, followed by settling in tanks and centrifugation of sludge. Some sludge is fed back into the process. The content of organic material measured as BOD and COD is reported to be reduced from typically 1300 and 6000 mg/L, respectively in the mixed influent to 270 and 1400 mg/L in the treated effluent. Similarly, the amount of total dissolved solids (TDS) and total suspended solids (TSS) is reduced from about 9000 and 500 mg/L to 5000 and 300 mg/L, respectively. The pH of both the influent and the treated effluent is around 7.5. The estimated effluent volume of 1500 m^3/day is based on the reported incoming volumes and assuming that the 20% added domestic sewage roughly equals the evaporation and sludge removal. The clarified effluent is discharged in the Isakavagu stream feeding the Nakkavagu, Manjira and eventually Godawari rivers. Solid waste is transported to a land fill unit.

With valuable assistance from local authorities and organizations effluent was sampled from the treatment plant on two consecutive days in November 2006 during normal operation under the supervision of the Andhra Pradesh Pollution Control Board and PETL. The samples were frozen on dry ice and shipped to Sweden for further analyses. The extensive mixing of various influents (about 150 trucks per day) and long retention time in the plant (approximately 6.5 days) suggest that grab samples will represent normal operation conditions reasonably well.

2.2. Chemical analyses of pharmaceuticals in effluent

A contract lab (Analycen AB) first screened the samples for the presence of 59 pharmaceuticals (Supplementary Table S1). Based on these preliminary data we made a more precise quantification of the nine most abundant drugs from the screening plus two additional fluoroquinolones. The analysis was performed using Surveyor HPLC and LCQ-Duo MS (ThermoFinnigan Inc., USA) acquiring MS/MS data in ESI+ mode. The reference compounds (purity > 97% by weight), LC–MS-grade solvents and other chemicals used for analysis were purchased from Sigma–Aldrich Sweden AB (Stockholm, Sweden), LGC Promochem AB (Borås, Sweden) and Riedel-de Haen (Seelze, Germany). Chromatographic separations were performed on two columns purchased from Thermo Scientific (Waltham, MA, USA), namely Hypersil Fluorophase RP (100 mm × 2.1 mm ID, packed with 5 μm perfluorinated RP-C6; method 1; used for all fluoroquinolones except ciprofloxacin) and Hypersil Gold (150 mm × 2.1 mm ID, packed with 5 μm end-capped, base-deactivated RP-C18; method 2; used for the rest of drugs and ciprofloxacin). In both cases the column temperature was kept at 25 °C and the flow rate at 200 μL min^{-1}. Method 1: using solvents A (water, 0.1% formic acid) and B (methanol) the gradient program was run as follows—isocratic 90% A and 10% B for 10 min, then to 25% B in 5 min, then to 40% B in 15 min, then to 55% B in 10 min, then to 70% B in 10 min and finally to 100% B in 5 min. Method 2: using solvents A (water, 15 mmol ammonium formate) and B (acetonitrile) the gradient program was run as follows—isocratic 96% A and 4% B for 8 min, then to 15% B in 7 min, then to 55% B in 25 min and finally to 98% B in 5 min. MS/MS data were acquired in ESI+ mode (capillary temperature 240 °C; sheath and auxiliary nitrogen gas flows set to respectively 70 and 4; source voltage 4.50 kV; source current 80 μA; capillary voltage 29 V). The collision energy required to produce the desired quantity of daughter ions was individually optimized for each analyte. Detection by a selective monitoring of daughter ions (parent ion $MH^+ \rightarrow$ daughter ions monitored, with the underlined *m/z* being used for quantification) is indicated as follows: cetirizin (389.1 → 201.0), citalopram (325.1 → 262.1, 279.9 and 307.2), ciprofloxacin (332.1 → 288.2 and 314.2), enoxacin (321.1 → 257.3, 277.2 and 303.2), enrofloxacin (360.1 → 316.2, 245.1 and 217.1), lomefloxacin (352.1 → 308.2, 288.3 and 265.2), losartan (423.1 → 207.2, 377.0 and 405.0), metoprolol (268.2 → 191.0 and 218.1), norfloxacin (320.1 → 276.2 and 302.2), ofloxacin (362.1 → 318.2 and 261.3) and ranitidine (315.0 → 270.0, 224.0 and 175.9). Although the concentrations of the 11 analysed drugs were sufficiently high not to justify any pre-concentration step, the removal of debris and particles in order to protect the analytical equipment was found to be necessary. Additionally, to diminish possible ion suppression effects and assess correct quantification, a standard addition method with parallel spiked samples and four-point (unknown plus three spikes) calibration curve was used. Thus the pre-treatment of the samples (native and spiked with known concentrations of the pharmaceuticals) included acidification to pH 2 (phosphoric acid), centrifugation at 13,500 rpm for 3 min (MiniSpin from Eppendorf Nordic ApS, Hørsholm, Denmark) and filtration (20 μm, glass-fibre filter from Millipore AB (Solna, Sweden)) before injection and analysis.

2.3. Toxicity tests

Standard toxicity tests were performed on thawed effluent samples in Sweden. The acute effects on bioluminescence of the bacteria *Vibro fisheri* were carried out using a Microtox M500 toxicity analyzer according to the manufacturer's instructions (Azur Environmental, Newark, Delaware, USA). Each sample/concentration was analysed in duplicate at 0, 1.25, 2.5, 5 and 10% dilutions. Immobilization of the water flea *Daphnia magna* was performed according to EN ISO 6341:1996. Each sample/concentration was analysed in quadruplicates (0, 0.6, 1.3, 2.5, 5, and 10%). Germination tests with salad seeds (*Lactuca sativa*) were performed in Petri dishes according to [13] at 0, 1, 2, 5, 10, 20 and 50% dilutions using 120 seeds per concentration. After 5 days the number of seedlings penetrating the cover sand was counted as well as the number of seedlings developing cotyledons.

Table 1
Top 11 active pharmaceutical ingredients analysed in effluent samples from PETL, a common effluent treatment plant near Hyderabad serving about 90 bulk drug manufacturers

Active ingredient	Type of drug	Range (μg/L)
Ciprofloxacin	Antibiotic-fluoroquinolone	28,000–31,000
Losartan	Angiotensin II receptor antagonist	2,400–2,500
Cetirizine	H_1-receptor antagonist	1,300–1,400
Metoprolol	β_1-adrenoreceptor antagonist	800–950
Enrofloxacin	Antibiotic-fluoroquinolone (veterinary use)	780–900
Citalopram	Serotonin reuptake inhibitor	770–840
Norfloxacin	Antibiotic-fluoroquinolone	390–420
Lomefloxacin	Antibiotic-fluoroquinolone	150–300
Enoxacin	Antibiotic-fluoroquinolone	150–300
Ofloxacin	Antibiotic-fluoroquinolone	150–160
Ranitidin	H_2-receptor antagonist	90–160

Drugs were analysed using LC–MS/MS monitoring at least two specific fragment ions per substance when possible and quantified using a four-point calibration. Data from two samples taken on consecutive days are presented.

3. Results and discussion

The initial screening of 59 pharmaceuticals suggested that 21 of these were present at concentrations above 1 μg/L (Table S1). An independent, quantitative analysis in our laboratory of the nine tentatively most abundant drugs and two additional antibiotics confirmed the findings of the screening. All 11 drugs were detected at levels >100 μg/L (Table 1). To the best of our knowledge, the concentrations of these 11 drugs were all above the previously highest values reported in any sewage effluent.

We would like to highlight the exceptional concentrations of fluoroquinolones found here, particularly ciprofloxacin—an antibiotic produced by several companies in the area. Normally, all of the drugs, including ciprofloxacin, are found in sewage effluents at concentrations around or below 1 μg/L and occasionally at somewhat higher levels in discharges from hospitals [14–20]. The concentrations of ciprofloxacin (up to 31,000 μg/L) were higher than the maximal therapeutic human plasma levels. In an ecotoxicological context, the levels of ciprofloxacin were orders of magnitude above the published EC_{50} toxicity values for *Microcystis aeruginosa* (17 μg/L) and *Lemna minor* (203 μg/L) [21]. The concentrations of lomefloxacin, norfloxacin, ofloxacin, enrofloxacin and enoxacin also exceed levels toxic to plants, diatoms, blue green algae and/or other bacteria [21–24]. The discharge load of ciprofloxacin corresponds to approximately 45 kg of active pharmaceutical ingredient per day, which is equivalent to the total amount consumed in Sweden (population nine million) over an average 5-day period [12].

Of further concern is that the industrial effluent is mixed with human sewage within the plant to improve biological treatment efficiency. Hence, there is a risk that pathogens will be exposed to antibiotics for prolonged periods. Ciprofloxacin is genotoxic and induces horizontal transfer of resistance between different species of bacteria, effects that may be observed at concentrations as low as 5–10 μg/L [14,15,25]. Therefore, the recipient waters and the treatment plant itself may be spawning grounds for resistant bacteria. One may also anticipate a reduced overall performance of the plant due to the expected toxicity of the pharmaceuticals to the microorganisms within the plant. Moreover, the microbial flora downstream from the plant is likely to be severely affected by the mixture of residual fluoroquinolones [22]. Thus, there are multiple reasons to consider alternatives to normal biological treatment for the removal of high levels of antibiotic residues from wastewater.

In addition to several broad-spectrum antibiotics being present, the list contained well-known drugs of different classes of diverse chemical structure, frequently used to treat allergies, ulcers, hypertension, migraine, depression and other common disorders. For most of these non-antibiotic drugs, there is yet insufficient chronic effects data on organisms likely to have highly conserved target molecules with humans (i.e. fish) to make adequate risk assessments. For example, citalopram is known to affect the behaviour of fish [26] but the dose–response relationship remains to be established. Citalopram has previously been reported in sewage effluents up to 612 ng/L [27].

The amounts of pharmaceuticals detected could be expressed in economical terms: if the equivalent amount of the 11 most abundant active substances released during 24 h were to be purchased as final products in a Swedish pharmacy, they would cost over €100,000 even if generic brands were selected (data not shown). However, the production cost of the bulk drugs would apparently be much lower than the price paid by the final consumer. Since measures to minimize the release of certain drugs during production may require significant investments, a high value of the final product does not necessarily guarantee that only trace amounts would be present in the waste [12].

A limitation of the study is that samples were collected on 2 days only. It is quite possible that the highest concentrations found reflect individual deliveries of waste to the treatment plant containing extreme quantities of drugs. However, the high concentrations of active ingredients for many different types of drugs strongly suggest that several industries are contributing. Thus, deliveries with high contents of drugs to PETL are not isolated, unique events. The addition of raw sewage (20%) to the process has likely contributed with some pharmaceutical residues but these amounts would be low compared to the highest values analysed here.

Despite the fact that the applied toxicity tests may be considered crude, they were sufficiently sensitive to identify high toxicities of the effluent to different types of organisms (Table 2).

Table 2
Toxicities of effluent samples from PETL sampled on two consecutive days in November 2006

Test organism	Duration of test	Endpoint	EC_{50} range (%)
Vibrio fisheri	15 min	Luminescence	3
Daphnia magna	48 h	Immobility	6.7–7.2
Lactuca sativa	120 h	Emerging seedlings	17–35
L. sativa	120 h	Developed cotyledons	1.6–3.2

Data are expressed as EC_{50}-values, i.e. the concentrations of effluent required to reduce the measured endpoint to 50% (*Vibrio*, *Lactuca*) or to immobilize 50% of the *Daphnia*.

The toxicity to *L. sativa* is in agreement with reports that irrigated fields in Patancheru are no longer productive [28]. Based on these toxicity tests alone, it is not possible to assign the toxicity to a particular drug(s) or other constituents of the effluent. For example, the fluoroquinolone antibiotics, including ciprofloxacin, are not very potent in the short-term Microtox test despite that it is a bacterial toxicity test [29]. Nevertheless, from the chemical analyses together with published toxicity data it is undisputable that the levels of fluoroquinolones found in the effluent are very toxic. It should be stressed that most drugs have human target proteins. Other tests could reveal even higher toxicities than the tests applied in this study.

The treated effluent studied does not constitute the only, or even worst, contribution to environmental pollution by local industries in Patancheru. Dumping of untreated industrial waste is a recognized problem [30]. There are reports in the non-peer-reviewed literature on severe environmental problems in nearby villages, including deaths of cattle as well as a number of human health issues [28]. This urgently motivates a thorough search for responsible causes and appropriate mitigation measures. Drugs produced in Patancheru are to a large extent globally distributed and incorporated into products marketed by other pharmaceutical companies. This implies that the environmental impact of the production is not only a matter of local concern.

The present study demonstrates that there are production facilities that release substantial amounts of drugs to the aquatic environment. To the best of our knowledge such information has previously not been reported in the peer-reviewed literature and at present it is not possible to say how widespread the problem is. It is plausible that the overall amount of drugs reaching the environment via excretion from humans and via incorrect disposal is larger than the amount released from production facilities on the global scale. However, the present study demonstrates that production facilities may be the most important point sources in specific locations and the source for the highest environmental concentrations. The data presented here call for extended investigations on the effluent quality, including the release of active pharmaceutical ingredients, from production facilities in different regions of the world.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2007.07.008.

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