New Framework To Diagnose the Direct Disposal of Prescribed Drugs in Wastewater – A Case Study of the Antidepressant Fluoxetine

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Supporting Information

ABSTRACT: Intentional or accidental release (direct disposal) of high loads of unused pharmaceuticals into wastewater can go unnoticed. Here, direct disposal of a pharmaceutical drug via the sewer network was identified for the first time using wastewater analysis. An irregularly high load of the antidepressant fluoxetine in raw wastewater ($10.5 \pm 2.4 \text{ g d}^{-1}$) was up to 11 times greater than any other day. National prescription data revealed a predicted daily fluoxetine load for the studied treatment works to be $0.4\text{ to } 1.6 \text{ g d}^{-1}$. Enantio-selective analysis showed the high load of fluoxetine was present as a racemic mixture, which is typical for fluoxetine in dispensed formulations. As fluoxetine undergoes stereoselective metabolism within the body, a racemic mixture in wastewater suggests a nonconsumed drug was the major contributor of the high load. This was confirmed by its major metabolite norfluoxetine whose load did not increase on this day. Considering the most commonly prescribed formulation of fluoxetine, this increased load accounts for the disposal of $\sim 915$ capsules. Furthermore, as fluoxetine is prescribed as one capsule per day, disposal is unlikely to be at the patient level. It is postulated that direct disposal was from a facility which handles larger quantities of the drug (e.g., a pharmacy).

1. INTRODUCTION

In 2001, wastewater analysis has been first proposed for community wide estimation of drug use. This approach has been applied in numerous studies to date, mainly for illicit drug use estimates throughout Europe. The same approach has been applied to pharmaceuticals. However, notable discrepancies between consumption estimates from wastewater analysis and prescription information have been observed for some compounds. For example, the study of 12 prescription drugs at a wastewater treatment works (WwTW) in the UK found consumption estimates from wastewater analysis can range from 12 to 514% of what is expected from prescription data. Possible reasons for inaccurate pharmaceutical drug consumption estimates from wastewater analysis includes the abuse of counterfeit drugs, the unavailability of information on drugs dispensed in hospitals, spatial differences in prescription-use (where national prescription information is used), prescribed drugs going unused, or drugs being directly disposed into the wastewater system.

Several studies have found direct disposal of unused pharmaceutical drugs to the sewer system as a viable route into wastewater at both the patient and pharmacy level. These studies rely on patients and pharmacies completing questionnaires on their disposal practices. However, to date there has been very little/no evidence of direct disposal of pharmaceutical drugs identified through wastewater analysis. This is because observing directly disposed drugs at the patient level is unlikely to provide a significant change to the composition of the wastewater itself. Observing direct disposal will be strongly dependent on the pharmacokinetics of the pharmaceutical in question, the extent of its usage within the population and the size of the receiving WwTW. On the other hand, direct disposal by a pharmacy will only be observed fortuitously as it is likely to occur infrequently. Furthermore, if observed by wastewater analysis, other than a high compound load there may not be further supporting evidence to adequately diagnose direct disposal. If the pharmaceutical is only available via prescription, national prescription information can be used to estimate the load of that drug in wastewater.

Enantio-selective analysis is an indispensable tool for resolving certain environmental problems. It can be used to identify the source of chiral drug loads found in wastewater. Directly disposed drug loads can be distinguished from consumed drug

Received: January 19, 2016
Revised: March 7, 2016
Accepted: March 14, 2016
Published: March 14, 2016
loads by determining the enantiomeric distribution of the chiral drug in question. This relies on the pharmaceutical drug in question being dispensed in a known enantiomeric form and it undergoing stereoselective changes within the body during metabolism. Vazquez-Roig et al. used enantioselective analysis to tentatively propose direct disposal of atenolol where a moderately higher average daily load was observed. Monitoring human metabolites of the compound in question can also be used to help distinguish between directly disposed and consumed drugs. The clearest case of direct disposal identified through wastewater analysis was by Emke et al. Here, high daily loads of the illicit stimulant 3,4-methylenedioxy-methamphetamine (MDMA) or ecstasy were found to be present in wastewater as a racemic mixture. This was observed following a police raid of an illegal production facility within the catchment. Nevertheless, to date there have been no cases with strong supportive evidence of the direct disposal of a pharmaceutical drug using wastewater analysis.

Directly disposed drugs can also have a significant impact upon the receiving environment if they are not sufficiently removed during wastewater treatment. The antidepressant fluoxetine has been identified as a compound of risk for the aquatic compartment in some studies. This is due to it being detected in river waters or predicted to be present in river waters at concentrations above predetermined predicted-no-effect-concentrations (PNECs). Oakes et al. proposed a fluoxetine PNEC of 0.012 μg L⁻¹. This was derived using a lowest observed effect concentration (LOEC) of ≤0.6 μg L⁻¹ for the chlorophyte Desmodesmus subspicatus and an assessment factor of 50. In freshwater ecosystems globally, fluoxetine has been reported at median concentrations of 0.020 μg L⁻¹. Considering the small differences between the proposed PNEC and concentrations observed in the environment, direct disposal of the drug can influence whether or not the PNEC of fluoxetine is exceeded.

In our study, during an eight day sampling period of raw wastewater at a municipal WwTW in South-West England, a high load of the antidepressant fluoxetine was observed. It was postulated that this was the result of direct disposal of the drug rather than increased consumption. Therefore, the aim of this study was to diagnose this high load of fluoxetine as direct disposal. This was assessed by investigating the following: i) UK prescription information for fluoxetine ii) The enantiomeric distribution of fluoxetine in raw wastewater iii) The relationship between fluoxetine and its major metabolite norfluoxetine

These findings were compared to a seven day sampling period at the same WwTW where no direct disposal was suspected. This study is the first to demonstrate with sufficient supporting evidence the direct disposal of a prescribed pharmaceutical drug using wastewater analysis. Using this information, we propose a new framework to distinguish between consumed and nonconsumed (directly disposed) drug loads in wastewater. Finally, the risk posed by direct disposal in the environment was evaluated by applying established environmental risk assessment calculations.

2. MATERIAL AND METHODS

2.1. Materials. R/S-(+)-Fluoxetine and norfluoxetine (Table S1) were purchased from Sigma-Aldrich (Gillingham, UK) and the internal standard R/S-(+)-fluoxetine-D5 from TRC (Toronto, Canada). Methanol (MeOH) was HPLC grade and purchased from Sigma-Aldrich (Gillingham, UK). Water (H₂O) was of 18.2 MΩ quality (Elga, Marlow, UK). All glassware was deactivated using 5% dimethylchlorosilane in toluene (Sigma-Aldrich, Gillingham, UK). Ammonium acetate (NH₄OAc), formic acid (HCOOH), and acetic acid (1.0 M) used in the preparation of mobile phases were also purchased from Sigma-Aldrich. Oasis HLB (60 mg, 3 mL) solid phase extraction (SPE) cartridges were purchased from Waters (Manchester, UK).

2.2. Analytical Methodologies. Briefly, samples for SPE were brought to room temperature and filtered (0.7 μm), and 50 mL aliquots were spiked with 50 ng of fluoxetine-D5. These were loaded onto preconditioned Oasis HLB cartridges, dried, and eluted using 4 mL of MeOH. Extracts were then dried under nitrogen and reconstituted in 500 μL of 80:20 H₂O:MeOH for the determination of whole drug concentrations. A fully validated method utilizing ultraperformance liquid chromatography tandem mass spectrometry using a Waters Acquity UPLC system (Manchester, UK) coupled to a Xevo TQD Triple Quadrupole Mass Spectrometer (Waters, Manchester, UK) was applied. A full description of the method is available in Petrie et al. Recoveries of fluoxetine and norfluoxetine ranged from 95 to 111%, with method quantitation limits of 2.1 to 2.5 ng L⁻¹ (Tables S2 and S3).

To investigate the enantiomeric fraction (EF) of fluoxetine, SPE cartridges were prepared in the same way. However, following elution and drying of MeOH extracts, reconstitution was in 500 μL of the appropriate mobile phase used for enantioselective separation (4 mM NH₄OAc in MeOH containing 0.005% HCOOH). For separation, a Chirobiotic V column (100 × 2 mm; 5 μm internal diameter) was used, as described in Evans et al. The EF of fluoxetine was calculated according to eq 1

\[
EF = \frac{S(+)}{[S(+) + R(\pm)]}
\]

where EF is the enantiomeric fraction, S(+) is the peak area of S-(+)-fluoxetine accounting for the response of S-(+)-fluoxetine-D₅, and R(–) is the peak area of R-(–)-fluoxetine accounting for R-(–)-fluoxetine-D₅. The uncertainty of EF measurement for fluoxetine in raw wastewater was <0.05.

2.3. Wastewater Treatment Works. A trickling filter WwTW in South-West England was studied. This receives mainly municipal wastewater with a population equivalent (PE) of 105,847. Raw wastewater was collected during an eight day monitoring campaign in December 2014 (08/12/14 to 15/12/14) and a seven day monitoring campaign in June 2015 (03/06/15 to 09/06/15). Samples were collected post primary screens but before primary sedimentation tanks. Volume paced composites were operated with a mean sampling frequency of 15 min (i.e., 96 subsamples throughout 24 h). This conservative sampling frequency was selected to ensure sampling error distributions were unbiased and <20%. The number of toilet flushes or “pulses” (p) per day estimated for fluoxetine in this WwTW was ∼6600 (>100 p d⁻¹ is required for representative information using volume-paced composites with a 15 min collection frequency). Subsamples were cooled on collection to <4 °C to limit biological activity. On the first Monday of the December sampling campaign (08/12/14), hourly grab samples were also collected between 8:00 and 0:00 (n = 17). Upon collection, all samples were filtered and subject to SPE immediately.
2.4. Environmental Risk Assessment. Environmental risk assessment of fluoxetine in receiving river water was undertaken with reference to the Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use. The ratio between the predicted environmental concentration (PEC) and PNEC was calculated as means of quantifying the risk posed. Ratios >1 require further evaluation of the fate of the drug and/or its metabolites in the aquatic environment. The PEC (μg L⁻¹) was calculated using the following equations with prescription information (2) and wastewater analysis (3), respectively

$$\text{PEC}_{\text{Prescription}} = \frac{\text{Load}_{\text{PRES}} \times S \times R}{Q \times PE \times DF} \quad (2)$$

$$\text{PEC}_{\text{Waste water analysis}} = \frac{\text{Load}_{\text{INF}} \times R}{Q \times PE \times DF} \quad (3)$$

Here Load_{PRES} is the maximum predicted load based on prescription information (accounting for the % dose excreted unchanged) (μg d⁻¹), Load_{INF} is the daily load of fluoxetine in the aqueous phase of influent wastewater (μg d⁻¹), S is the correction factor to account for the fraction of fluoxetine bound to suspended particulate matter (0.49)⁴ R is the correction factor to account for the known removal of fluoxetine during wastewater treatment at the site in question (0.673), Q is the total quantity of wastewater per inhabitant (L inh⁻¹ d⁻¹), PE is the population size contributing to the wastewater, and DF is the dilution factor of effluent into the river. The PNEC of fluoxetine was determined according to

$$\text{PNEC} = \frac{\text{Tox.}}{\text{AF}} \quad (4)$$

Here Tox. is the lowest available toxicity data (effect concentration (EC₅₀), lethal concentration (LC₅₀), LOEC, no observed effect concentration (NOEC)) considering at least three species type, and AF is the assessment factor (1000 for EC₅₀ and LC₅₀ and 10 for LOEC and NOEC).

3. RESULTS AND DISCUSSION

3.1. Daily Profile of Fluoxetine and Norfluoxetine in Raw Wastewater. 3.1.1. Predicted and Measured Daily Loads of Fluoxetine. On Wednesday of the December sampling campaign, a fluoxetine load of 10.5 ± 2.4 g d⁻¹ was observed (Figure 1). This was considerably greater than any other day (up to 11 times). The consumption of fluoxetine is not expected to vary greatly throughout the week as it is unlikely to be used as a drug of abuse which may result in recreational usage. Furthermore, no significant rainfall or increased wastewater flows were recorded on this day which could have resulted in an increased load of fluoxetine flushed from the sewer network (Table S4). Therefore, it was postulated that the high load of fluoxetine on Wednesday was a result of direct disposal. Using UK prescription information, the daily load of fluoxetine predicted to be observed at a 105,847 PE WwTW in England during December 2014 was 0.4–1.6 g d⁻¹ (Table 1). This was calculated according to eq 5

$$\text{Load}_{\text{PREDICTED}} = \frac{\text{PRES.} \times \left( \frac{\text{Excretion}}{100} \right) \times \left( \frac{100 - \text{part.}_1}{100} \right) \times \left( \frac{\text{WwTW PE}}{\text{Pop.}} \right)}{d} \quad (5)$$

Here PRES. is the quantity of drug prescribed nationally during a calendar month as the free base (g) (Table 1), Excretion is the quantity of drug excreted unchanged following consumption (%) (Table 1), part. is the sorption of drug to suspended particulate matter (%) (Table 1), WwTW PE is the population equivalent of the wastewater treatment works, Pop. is the population size to which the prescription information relates (57,000,000), and d is the number of days in the month studied. It should be noted that comparison of prescription information with measured drug loads in raw wastewater can have discrepancies. For example, a detailed study by Baker et al. at a 3,400,000 PE WwTW in England showed the difference between calculated fluoxetine loads and estimated loads from prescription data to be 57%. Furthermore, prescription data used in our study is at a national scale, and the WwTW studied here (105,847) represents <2% of the population. Therefore, there are uncertainties associated when comparing prescription data with wastewater analysis (e.g., possible spatial trends in prescription behavior). Nevertheless, a 10.5 g d⁻¹ observed on Wednesday of the December sampling campaign was +650% of the maximum predicted load (Figure 1, Table 1). On all other days, calculated loads ranged from 70 to 180% of the maximum predicted load.

During a seven day sampling period in June 2015 at the same WwTW where no significant contributions from direct disposal were suspected, daily loads ranged from 0.6 to 0.9 g d⁻¹ (Load_{PREDICTED} = 0.3–1.4 g d⁻¹) (Figure 1, Table 1). Here no significant contribution of the fluoxetine load is suspected from direct disposal as the predicted load was not exceeded. This uniformity of daily fluoxetine load is in good agreement with previous observations for prescription drugs, including fluoxetine. This supports the hypothesis that the irregularly high load of fluoxetine on Wednesday of the December sampling campaign was caused by direct disposal of a large quantity of the drug. To investigate this further, enantioselective analysis was undertaken to measure the enantiomeric distribution of fluoxetine in raw wastewater.

3.1.2. Enantio-Selective Analysis as a Tool To Distinguish between Consumed and Directly Disposed Unused Drugs. Enantio-selective analysis can be used to help distinguish between consumed drugs and those directly disposed. This is reliant on the drugs in question being chiral, dispensed in a known enantiomeric form, and subject to stereoselective changes to their composition during human metabolism. Fluoxetine satisfies these criteria as it is dispensed as a racemic mixture, and human metabolism results in the enrichment of S-(+)-fluoxetine. Consequently, an EF of >0.5 would be expected in raw wastewater containing the consumed drug. Between Thursday and Monday of the December sampling campaign (11/12/14 to 15/12/14), EFs ranged from 0.56 to 0.68 (Figure 1). Here fluoxetine loads were <1.8 g d⁻¹ (estimated load from prescription data = 0.4–1.6 g d⁻¹) demonstrating fluoxetine was consumed, and no notable direct disposal is suggested. This is in good agreement with data obtained in June where loads were ≤0.9 g d⁻¹ (Load_{PREDICTED} = 0.3–1.4 g d⁻¹) and EFs ranged from 0.66 to 0.70 (Figure 1).

On Monday (08/12/14), Tuesday, and Wednesday of the December sampling campaign where loads were >1.8 g d⁻¹, EFs were in the range 0.48 to 0.51 (Figure 1). Enantiomeric fractions close to racemic (0.50) here suggest a large contribution of the fluoxetine load observed in raw wastewater on these days is...
nonconsumed drugs. This provides further support that the high load of fluoxetine on Wednesday was caused by direct disposal. Furthermore, EFs close to 0.50 on Monday and Tuesday suggest that direct disposal also occurred on these days. Interestingly, due to inherent discrepancies in comparing prescription data

with measured drug loads,5,6 direct disposal would not have been suspected on either Monday (8/12/14) or Tuesday of the December sampling campaign without enantioselective analysis.

3.1.3. Distinguishing between Consumed and Non-consumed Drugs by Metabolite Profiling.

Figure 1. Daily fluoxetine load and EF (A) and norfluoxetine load and the fluoxetine:norfluoxetine ratio (B) during week long sampling campaigns in December 2014 and June 2015, respectively. The solid red line outlines a racemic EF (i.e., 0.5), and the broken red line shows the predicted fluoxetine:norfluoxetine ratio range for consumed fluoxetine (see Table 1). Days where direct disposal has been identified are highlighted with a blue background.

Figure 1. Daily fluoxetine load and EF (A) and norfluoxetine load and the fluoxetine:norfluoxetine ratio (B) during week long sampling campaigns in December 2014 and June 2015, respectively. The solid red line outlines a racemic EF (i.e., 0.5), and the broken red line shows the predicted fluoxetine:norfluoxetine ratio range for consumed fluoxetine (see Table 1). Days where direct disposal has been identified are highlighted with a blue background.

Table 1. Prescription, Metabolism, and Predicted Daily Loads of Fluoxetine and Norfluoxetine at a 105,847 Population Equivalent WWTW in the UK during December 2014 and June 2015

<table>
<thead>
<tr>
<th>compound</th>
<th>fluoxetine prescribed as free base (kg)</th>
<th>excretion of fluoxetine dose (%)</th>
<th>known metabolites</th>
<th>enantiomeric distribution in urine (human)</th>
<th>partitioning to influent solids (%)</th>
<th>predicted fluoxetine:norfluoxetine ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>fluoxetine</td>
<td>488a 430b</td>
<td>2.5–11c,d</td>
<td>norfluoxetine†</td>
<td>enriched with S(+)-enantiomer, EF &gt; 0.5f</td>
<td>0.4–1.6 0.3–1.4</td>
<td>0.3–1.9</td>
</tr>
<tr>
<td>norfluoxetine</td>
<td>7–10c,d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aNational Health Service.20 bNational Health Service.21 cCaccia et al.22 dTaylor et al.23 eBergstrom et al.24 fBaker et al.7 gAqueous phase only, see eq 5 for calculation. 7To calculate the predicted load of norfluoxetine, the difference in molecular weight between norfluoxetine and fluoxetine is considered as % excretion considers number of moles over mass.
3.2. Hourly Variations of Fluoxetine and Norfluoxetine in Raw Wastewater. During the first Monday (08/12/14) of the December sampling campaign, hourly grab samples were collected between 8:00 and 0:00 h (n = 17). This provided the opportunity to investigate the hourly variability in fluoxetine and norfluoxetine load during a day where direct disposal of the parent drug was suspected. Here the measured load was greater than the predicted daily load (2.9 g d\(^{-1}\) versus 0.4−1.6 g d\(^{-1}\)); an EF close to 0.50 and a fluoxetine:norfluoxetine ratio of 2.6 all indicated direct disposal had occurred within the catchment on this day (Figure 1). Grab sampling revealed that the hourly load of fluoxetine varied from 0.06 to 0.64 g h\(^{-1}\), with highest loads generally observed between 08:00 and 15:00 h (Figure 2A). During these times, an enrichment of S-(+)-fluoxetine was not observed, and the EF of fluoxetine was ∼0.50 ± 0.02 (Figure 2A). This suggests a large contribution of the fluoxetine load at these times was nonconsumed drugs. This was supported by the behavior of norfluoxetine which did not show such high variability in load indicating a constant (or unchanged) level of fluoxetine consumption in the studied population. Norfluoxetine loads varied from 0.04 to 0.07 g h\(^{-1}\) throughout the day (Figure 2B). Considering fluoxetine typically has a single daily dose and a half-life of between 2 and 3 days,\(^{37}\) this low variability in metabolite load is not surprising. Between 08:00 and 15:00 h the fluoxetine:norfluoxetine ratio was >1.9 supporting the proposal that fluoxetine was directly disposed on these times (Figure 2B). Most notably, the fluoxetine:norfluoxetine ratio on Wednesday was 8.3.

3.3. A New Framework To Distinguish between Consumed and Nonconsumed (Directly Disposed) Drugs in Wastewater. Only a few studies reported in the literature have proposed evidence for the direct disposal of drugs.\(^{3,7,11,12}\) There are a number of reasons for this: (i) direct disposal at the patient level (e.g., of a daily dose) is unlikely to have sufficient impact upon a 24 h composite sample collected from a medium to large sized WwTW, particularly if the drug in question is a high usage compound (and has a high excretion rate as the unchanged drug following consumption), (ii) the infrequent disposal of larger quantities of drugs by a patient (weekly or monthly dose), hospital, or pharmacy cannot be predicted and will only be observed fortuitously, and (iii) due to analytical or other limitations, the disposed drug may itself not be studied or (iv) further supporting evidence of direct disposal (e.g., enantiomeric distribution and determination of metabolites) may not be attainable.

Andrés-Costa et al.\(^{12}\) observed high loads of the illicit stimulant cocaine during a week-long sampling event at a WwTW in Spain. This corresponded with a higher than anticipated parent drug:metabolite ratio. Cocaine:benzoylcegonine ratios ranged from 1.6 to 2 and are considerably greater than the expected ratio of 0.2−0.5 (Table 2).\(^{12}\) This was supported by newspaper reports of police raiding an illegal production facility within the catchment. Similarly, higher than
Table 2. Suspected Direct Disposal of Drugs Reported in the Literature

<table>
<thead>
<tr>
<th>Drug</th>
<th>Class of Drug</th>
<th>Duration</th>
<th>Load (g d⁻¹)</th>
<th>EF</th>
<th>parent drug/metabolite</th>
<th>duration</th>
<th>Load (g d⁻¹)</th>
<th>EF</th>
<th>parent drug/metabolite</th>
<th>Further Information</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoxetine</td>
<td>Antidepressant</td>
<td>Mon-Mon</td>
<td>0.95–10.54</td>
<td>0.48–0.68</td>
<td>0.8–8.3</td>
<td>Wed-Tues</td>
<td>0.58–0.91</td>
<td>0.67–0.70</td>
<td>1.0–1.4</td>
<td>Predicted daily load using Dec 2014 prescription data = 0.4–1.6 g d⁻¹</td>
<td>This study</td>
</tr>
<tr>
<td>Atenolol</td>
<td>Beta-blocker</td>
<td>one week (2012)</td>
<td>1.3⁻</td>
<td>0.50</td>
<td></td>
<td></td>
<td>1.0⁻</td>
<td>&gt;0.50</td>
<td></td>
<td></td>
<td>Vasquez-Roig et al.¹¹</td>
</tr>
<tr>
<td>MDMA</td>
<td>Illicit stimulant</td>
<td>one week (2011)</td>
<td>184</td>
<td>0.50</td>
<td></td>
<td></td>
<td>9.3</td>
<td>&gt;0.57</td>
<td></td>
<td></td>
<td>Emke et al.⁷</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>Illicit stimulant</td>
<td>one week (2011)</td>
<td>1431</td>
<td>0.52</td>
<td></td>
<td></td>
<td>99</td>
<td>0.52</td>
<td></td>
<td></td>
<td>Emke et al.⁷</td>
</tr>
<tr>
<td>Cocaine</td>
<td>Illicit stimulant</td>
<td>Fri and Sun at two separate WwTWs (2013)</td>
<td>375–3292</td>
<td>1.6–2.0</td>
<td></td>
<td></td>
<td>110–464</td>
<td>0.2–0.5</td>
<td></td>
<td></td>
<td>Andrés-Costa et al.¹²</td>
</tr>
</tbody>
</table>

Abbreviations: EF, enantiomeric fraction; WwTW, wastewater treatment works. b g d⁻¹ 1000⁻¹ inhabitants.

Here Load is the calculated daily load at the WwTW (eq 6). Load_HIDDEN is the highest predicted daily load based on eq 5. Parent drug/metabolite concentration dependence is the sorption of drug to suspended particulate matter (Sorption). A WwTW wastewater analysis was used to help identify directly disposed drug in wastewater. Applying this framework in our study, direct disposal was confirmed on Monday (08/12/14) and Wednesday of the December sampling period. Direct disposal could not be confirmed on Tuesday as the fluoxetine:norfluoxetine ratio did not exceed the predicted ratio of 3:1.9 based on eq 1. The framework was applied to estimate the enantiomeric fraction of parent drug/metabolite expected in wastewater. On the other hand, the framework should act as a starting point for investigating the disposal of pharmaceutical drugs and adapted for the specific drug under investigation. Using the data obtained on Wednesday, the quantity of drug formulations estimated to be directly disposed was calculated using eq 6.
on 1 day is more likely to be by a facility which handles and dispenses a large quantity of pharmaceutical drugs (e.g., a pharmacy). In this catchment there are no registered pharmaceutical production companies. A study by Tong et al.\textsuperscript{10} found that 3.2% of 285 community pharmacies in New Zealand disposed of unused solid medications via the toilet or sink. Although this may not be directly comparable to current disposal practices in the UK, this is a possible route of entry for the relatively large quantity of unconsumed fluoxetine observed on Wednesday. Current EU directives only outline that member states shall ensure that appropriate collection systems are in place for medicinal products that are unused or have expired.\textsuperscript{28}

3.4. Environmental Risk Assessment. To assess the potential impact of fluoxetine in the environment, established environmental risk assessment protocols were applied.\textsuperscript{19} It should be noted that current environmental risk assessment can be inaccurate as it does not consider the impact of mixtures of a number of different compounds or the enantio-specific toxicity of chiral drugs. For example, enantio-specific toxicity has been observed for fluoxetine toward some aquatic species.\textsuperscript{29,30} Nevertheless, these established environmental risk assessment calculations have been applied here to compare the possible environmental impact of consumed and directly disposed drug loads. PECs were calculated taking into account site specific flow data (wastewater and receiving river). This was calculated using prescription data and wastewater analysis from December 2014 and June 2015. Using available toxicity data in the literature, the lowest derived PNEC was calculated to be 0.010 μg L\textsuperscript{−1} (Table S5). This was determined using toxicity data from studies using racemic fluoxetine. Therefore, there will be a degree of uncertainty here as directly disposed and consumed fluoxetine will have different enantiomeric distributions when entering the environment, and, as discussed previously, enantio-specific toxicity of fluoxetine is known to occur.\textsuperscript{29,30}

The PEC of fluoxetine for the load observed on Wednesday (December 2014) where direct disposal was identified was 0.0044 μg L\textsuperscript{−1} (Table 3). This corresponded to a PEC/PNEC of 0.44, and therefore low risk is assumed despite a high load of the drug observed. This is attributed to the high dilution in wastewater (354 L inh\textsuperscript{−1} d\textsuperscript{−1}) and in the receiving river (44 times) at this site during winter. No seasonal bias is expected for the direct disposal of fluoxetine. Therefore, if the directly disposed load (10.5 g d\textsuperscript{−1}) is applied to June conditions at the same WwTW (wastewater volume of 235 L inh\textsuperscript{−1} d\textsuperscript{−1} and a riverine dilution factor of 18), the PEC/PNEC is 1.59 (Table 3). The action limit of 1 is exceeded, and further investigation is needed. It should be noted that many WwTWs have similar or lower wastewater and river dilution ratios than those reported here. For example, seven of 16 WwTWs previously studied in the UK had river dilution factors of ≤7, with two of these sites having dilution factors of one (i.e., no dilution).\textsuperscript{31} Therefore, directly disposed drugs are expected to have an even greater environmental impact here. On the other hand, the PEC will be lower at sites which employ other wastewater treatment options (e.g., activated sludge) which are considered more effective in removing fluoxetine than trickling filters. However, greater stereoselective changes may be observed which need to be taken into account.

Finally, if the PEC of fluoxetine is calculated using UK prescription data and default conditions (wastewater volume of 200 L inh\textsuperscript{−1} d\textsuperscript{−1} and a riverine dilution factor of 10)\textsuperscript{19} as a means of prioritizing compounds for investigation, a concentration of 0.005 μg L\textsuperscript{−1} is derived (Table 3). Thus, the PEC/PNEC of fluoxetine is determined to be 0.50. This is similar to a study by Oakes et al.\textsuperscript{13} where a PEC/PNEC ratio of 0.83 was calculated from prescription data for Sweden. In both cases the PEC/PNEC action limit of 1 is not exceeded, and this compound may not be prioritized for further investigation here. However, if the directly disposed load is applied to default

Figure 3. Proposed framework to distinguish between consumed and directly disposed prescription drugs in wastewater.
Table 3. PEC/PNEC of Fluoxetine during December 2014 and June 2015 in the Receiving River at the Studied WwTW (105,847 Population Equivalent) and in Default Dilution Conditions under Normal and Direct Disposal Events

<table>
<thead>
<tr>
<th>conditions</th>
<th>influent wastewater load (g d⁻¹)</th>
<th>wastewater volume (L in h⁻¹ d⁻¹)</th>
<th>riverine dilution factor</th>
<th>PEC (µg L⁻¹)</th>
<th>PEC/PNEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dec 2014</td>
<td>prescription data</td>
<td>1.6 a</td>
<td>354</td>
<td>43</td>
<td>0.0007</td>
</tr>
<tr>
<td></td>
<td>wastewater analysis (no direct disposal)</td>
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Abbreviations: PEC, predicted environmental concentration; PNEC, predicted no effect concentration. a Aqueous phase. bMaximum load based on December 2014 prescription information. c Average daily load from 11/12/14 to 15/12/14 (n = 5). dDaily load from 10/12/14 (n = 1). eMaximum load based on June 2015 prescription information. fAverage daily load from 03/06/15 to 09/06/15 (n = 7). EMEA.19

**REFERENCES**


