

1 **Supporting information**

2 **A new framework to diagnose the direct disposal of prescribed drugs in wastewater – a**  
3 **case study of the antidepressant fluoxetine**

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9 The supporting information is eight pages in length and contains five tables:

10 **Table S1.** Physico-chemical properties of fluoxetine and norfluoxetine

11 **Table S2.** Mass spectrometry information for the determination of fluoxetine and  
12 norfluoxetine

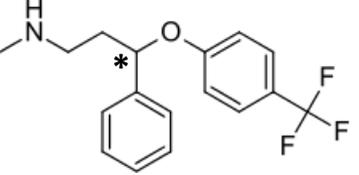
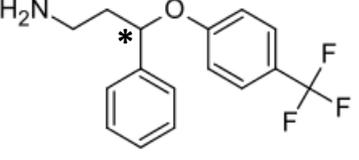
13 **Table S3.** Method validation parameters for fluoxetine and norfluoxetine in influent  
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15 **Table S4.** Wastewater flow data and rainfall during December 2014 and June 2015 sampling  
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**Table S1. Physico-chemical properties of fluoxetine and norfluoxetine**

| Drug/metabolite | Molecular weight (g mol <sup>-1</sup> ) | Chemical structure  | Water Solubility (mg L <sup>-1</sup> ) <sup>i</sup> | Log Kow <sup>ii</sup> | Log Koc <sup>iii</sup> | Log Dow <sup>iv</sup> | Henry's Law Constant (atm m <sup>3</sup> mol <sup>-1</sup> ) <sup>v</sup> | Vapour Pressure (Torr) <sup>vi</sup> | pKa (Most basic) <sup>vii</sup> |
|-----------------|---|---|---|-----------------------|------------------------|-----------------------|---|--------------------------------------|---------------------------------|
| Fluoxetine      | 309.33                                  |  | 60.3  | 4.65                  | 8.90E-08               | 1.92                  | 8.90E-08  | 1.88E-06                             | 10.05±0.10                      |
| Norfluoxetine   | 295.30                                  |  | -   | -                     | -                      | 1.54                  | -   | 5.21E-06                             | 9.05±0.13                       |

<sup>i</sup>As calculated by EPI Suite<sup>1</sup> at 25°C

<sup>ii</sup>As calculated by EPI Suite<sup>1</sup> (KOWWIN v1.68 estimate)

<sup>iii</sup>As calculated by EPI Suite<sup>1</sup> based on Log Kow

<sup>iv</sup>As calculated by Marvin Beans<sup>2</sup> at pH 7.5

<sup>v</sup>As calculated by EPI Suite<sup>1</sup> based on Bond SAR method

<sup>vi</sup>As stated on Scifinder calculated using Advanced Chemistry Development (ACD/Labs) Software v11.02 (©1994-2015 ACD/Labs)<sup>3</sup>

<sup>vii</sup>As stated on Scifinder calculated using Advanced Chemistry Development (ACD/Labs) Software v11.02 (©1994-2015 ACD/Labs) at 25°C<sup>3</sup>

\*Denotes chiral centre

**Table S2. Mass spectrometry information for the determination of fluoxetine and norfluoxetine**

| Drug/metabolite | Molecular ion (m/z) | Daughter 1 (m/z) | Cone voltage (V) | Collision energy (eV) | Daughter 2 (m/z) | Cone voltage (V) | Collision energy (eV) | Ion ratio | Internal standard |
|-----------------|---------------------|------------------|------------------|-----------------------|------------------|------------------|-----------------------|-----------|-------------------|
| Fluoxetine      | 310.2               | 44.1             | 34               | 10                    | 148.1            | 34               | 10                    | 14.9      | Fluoxetine D5     |
| Norfluoxetine   | 296.1               | 134.1            | 18               | 6                     | -                | -                | -                     | -         | Fluoxetine D5     |

**Table S3. Method validation parameters for fluoxetine and norfluoxetine in influent wastewater**

| <b>Drug/metabolite</b> | <b>IDL (ng L<sup>-1</sup>)</b> | <b>IQL (ng L<sup>-1</sup>)</b> | <b>Recovery (%)</b> | <b>Matrix suppression (%)</b> | <b>MDL (ng L<sup>-1</sup>)</b> | <b>MLQ (ng L<sup>-1</sup>)</b> |
|------------------------|--------------------------------|--------------------------------|---------------------|-------------------------------|--------------------------------|--------------------------------|
| Fluoxetine             | 0.01                           | 0.05                           | 111                 | 54                            | 0.50                           | 2.52                           |
| Norfluoxetine          | 0.01                           | 0.05                           | 95                  | 55                            | 0.42                           | 2.12                           |

**Table S4. Wastewater flow data and rainfall during December 2014 and June 2015 sampling campaigns**

| Measured variable                                 | Day of week          |        |        |        |        |        |        |        |        |
|---|----------------------|--------|--------|--------|--------|--------|--------|--------|--------|
|   | Mon                  | Tues   | Wed    | Thurs  | Fri    | Sat    | Sun    | Mon    | Tues   |
|   | <i>December 2014</i> |        |        |        |        |        |        |        |        |
| Wastewater flow (m <sup>3</sup> d <sup>-1</sup> ) | 32,444               | 36,843 | 34,363 | 42,651 | 38,857 | 40,247 | 38,266 | 36,369 | -      |
| Rainfall (mm)                                     | 0.0                  | 1.0    | 3.7    | 15.9   | 0.0    | 0.2    | 0.2    | 0.4    | -      |
|   | <i>June 2015</i>     |        |        |        |        |        |        |        |        |
| Wastewater flow (m <sup>3</sup> d <sup>-1</sup> ) | -                    | -      | 26,284 | 23,891 | 23,652 | 22,915 | 22,529 | 29,163 | 25,695 |
| Rainfall (mm)                                     | -                    | -      | 1.1    | 0.0    | 0.0    | 0.0    | 0.0    | 0.0    | 0.0    |

**Table S5. Toxicological information for fluoxetine towards aquatic test species**

| Trophic level | Test species                           | EC50 (mg L <sup>-1</sup> ) | LC50 (mg L <sup>-1</sup> ) | LOEC (µg L <sup>-1</sup> ) | NOEC (µg L <sup>-1</sup> ) | Reference                          | PNEC (µg L <sup>-1</sup> ) |
|---------------|--|----------------------------|----------------------------|----------------------------|----------------------------|------------------------------------|----------------------------|
| Algae         | <i>Dunaliella tertiolecta</i>          | 0.170                      | -                          | -                          | -                          | DeLorenzo and Fleming <sup>4</sup> | 0.170                      |
|               | <i>Pseudokirchneriella subcapitata</i> | 0.024                      | -                          | -                          | -                          | Brooks et al <sup>5</sup>          | 0.024                      |
|               | <i>Desmodesmus subspicatus</i>         | -                          | -                          | ≤0.6                       | <0.6                       | Oakes et al <sup>6</sup>           | 0.060                      |
| Fish          | <i>Gambusia affinis</i>                | -                          | 0.546                      | -                          | -                          | Henry and Black <sup>7</sup>       | 0.546                      |
|               | <i>Pimephales promelas</i>             | -                          | 0.705                      | -                          | -                          | Brooks et al <sup>5</sup>          | 0.705                      |
| Crustacean    | <i>Ceriodaphnia dubia</i>              | -                          | 0.234                      | -                          | -                          | Brooks et al <sup>5</sup>          | 0.234                      |
|               | <i>Ceriodaphnia dubia</i>              | -                          | 0.510                      | -                          | -                          | Henry et al <sup>8</sup>           | 0.510                      |
|               | <i>Daphnia magna</i>                   | -                          | 0.820                      | -                          | -                          | Brooks et al <sup>5</sup>          | 0.820                      |
|               | <i>Gammarus pulex</i>                  | -                          | -                          | 0.100                      | -                          | De Lange et al <sup>9</sup>        | 0.010                      |
|               | <i>Hyallea azteca</i>                  | -                          | -                          | 100                        | 33                         | Péry et al <sup>10</sup>           | 3.30                       |
| Mollusc       | <i>Daphnia magna</i>                   | -                          | -                          | 31                         | 8.9                        | Péry et al <sup>10</sup>           | 0.890                      |
|               | <i>Daphnia magna</i>                   | -                          | -                          | 135                        | <60                        | Oakes et al <sup>6</sup>           | 6.00                       |
|               | <i>Potamopyrgus antipodaram</i>        | -                          | -                          | 69                         | 13                         | Péry et al <sup>10</sup>           | 0.130                      |
|               | <i>Potamopyrgus antipodaram</i>        | -                          | -                          | -                          | 0.47                       | Nentwig <sup>11</sup>              | 0.047                      |
|               | <i>Potamopyrgus antipodaram</i>        | -                          | -                          | -                          | -                          |                                    |                            |

## References

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