Assessing exposure and risks of pharmaceuticals in an urban river system

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Abstract

Pharmaceuticals are ubiquitous in the freshwater environment, a result of an increasingly urbanised water cycle. Environmental risk assessments are available for a small proportion of the over 1900 pharmaceuticals in use, raising concern over the potential risks posed by pharmaceuticals with limited data, as effects on non-target organisms have been observed. Experimentally filling these gaps is a large, costly and likely unnecessary task. Risk-based prioritisation is a potential tool for addressing this challenge by identifying which pharmaceuticals may pose risks and are therefore a priority for study. Simple exposure models are commonly used to predict environmental concentrations (PECs), however the suitability of these models for prioritisation is unknown.

A scoping study targeted 95 pharmaceuticals in samples from the Rivers Ouse and Foss in York, UK, 25 were quantified. Measured environmental concentrations (MECs) were compared with simple PECs based on local usage data and dilution factors. MECs and simple PECs were used to prioritise pharmaceuticals and, for the larger River Ouse, different priority lists using the two approaches emerged. This conclusion was based on limited monitoring data, therefore an HPLC-MS/MS quantification method for 33 pharmaceuticals was developed, validated and applied to a year-long monitoring campaign to build a robust monitoring dataset. Significant spatial and temporal trends were observed in both rivers apparently driven by flow, pharmaceutical usage, wastewater treatment removal, and in-stream attenuation. These drivers differently influenced concentrations in either river. The simple PECs and PECs derived from a higher-tier spatial exposure model (LF2000-WQX) were validated against annual average MECs. LF2000-WQX outperformed the simple PEC in both rivers. A re-prioritisation using LF2000-WQX demonstrated that improved predictive power translated into better agreement of prioritisation outcomes with MECs. The use of simple PECs for the prioritisation and risk assessment of pharmaceuticals should be avoided and the use of higher-tier spatial exposure models encouraged.

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Declaration

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Chapter	Title	Journal	Status and DOI
2	Application of prioritization approaches to optimize environmental monitoring and testing of pharmaceuticals	Journal of Toxicology and Environmental Health, Part B: Critical Reviews	Published 10.1080/10937404.2018.1465873
3	Are exposure predictions, used for the prioritization of pharmaceuticals in the environment, fit for purpose?	Environmental Toxicology and Chemistry	Published 10.1002/etc.3842
4,5	Temporal and spatial variation of pharmaceutical concentrations in an urban river system	Water Research	Published 10.1016/J.WATRES.2018.02.066
6	Validation of LF2000-WQX pharmaceutical exposure predictions in an urban river system	Water Research	In preparation

I hereby declare that this thesis is my original work, except where otherwise acknowledged, and that it has not been submitted previously for a degree at this or any other University. All sources are acknowledged as References.

Emily Burns

Chapter 1

Introduction

1.0 Introduction

Pharmaceuticals are an invaluable commodity to society; their use enables greater quality and longevity of life. Extensive and continuous patient and veterinary use has resulted in their ubiquitous presence in the aquatic environment.[1,2] This presence, while generally low (sub-µg/L), is of concern due to the intended biological activity of pharmaceuticals at low concentrations, highlighting a potential risk of unintended effects in non-target organisms.[3] There are over 1900 pharmaceuticals currently authorised for use in the United Kingdom (UK),[4] few of which have information pertaining to their ecotoxicity, behaviour and fate in the environment.[5] To fill these gaps experimentally would require a substantial effort in terms of time and cost. Risk-based prioritisation methodologies are a useful tool for identifying which of the thousands of pharmaceuticals in use have the greatest potential to cause unintended effects to non-target organisms in the environment and thus should be afforded research resources.[6] Pharmaceuticals potentially posing the greatest risk are identified by comparing modelled exposure concentrations with modelled effect concentrations, although the validity of these models is not well understood. The exposure models that are typically used for prioritisation are simple and rely on assumptions which may not make them suitable for predicting environmental concentrations.[7] It is therefore important to understand how accurate these exposure models are, if they impact prioritisation outcomes, and whether in their current form they can ensure that all potential risks are accounted for.

1.1 Pharmaceutical pathways to the environment

Pharmaceuticals can enter the environment through their manufacture, use (human and veterinary) or disposal (Figure 1). During the manufacturing process pharmaceutical laden effluents are either directly released to the environment, treated on-site and subsequently released to the environment or diverted to municipal wastewater treatment.[8] In regions such as India and China, where much of the world's supply of pharmaceuticals is manufactured,[9] less stringent emission regulations than in the European Union or North America, paired with generally poorer wastewater treatment has resulted in extraordinarily high pharmaceutical concentrations in effluent-receiving

surface waters (up to 237 mg/L).[8,10] Pharmaceutical manufacturing is less prolific in the European Union and North America and is also accompanied by regulations, for example the European Union's Industrial Emission Directive,[11] requiring effluents be treated on-site or diverted to a wastewater treatment plant (WWTP). Despite these requirements, concentrations of 10 up to 1000 times that observed in non-manufacturing areas have been documented in discharge from WWTPs which receive manufacturing effluent in the European Union[12,13] and the United States[14].

Human usage is a significant environmental source of pharmaceuticals.[15] After patient administration, the pharmaceutical is metabolised to varying degrees and excreted as a mixture of parent pharmaceutical and metabolites principally in the urine and faeces.[16] The extent of metabolism is highly variable between pharmaceuticals, for example the type II diabetes drug, metformin, is excreted >90% unchanged,[17] while the antidepressant amitriptyline is heavily metabolised and approximately only 5% excreted unchanged.[18] Metabolism may also vary depending on age, gender, ethnicity or pre-existing health conditions.[19]

In developed and relatively urban areas, pharmaceuticals enter the municipal waste stream, from the home or hospital, and are subject to a wastewater treatment process prior to release to the environment. In general, pharmaceuticals are poorly removed during wastewater treatment. Conventional WWTPs were designed to reduce nutrient loads, organic matter and harmful microorganisms, not small organic contaminants (e.g. pharmaceuticals) therefore many pharmaceuticals are incompletely removed.[20] The fraction of pharmaceuticals removed from influent during wastewater treatment are either biodegraded or sorbed to sludge.[21] Persistent pharmaceuticals which are sorbed to sludge can reach the terrestrial environment *via* the spreading of biosolids for agricultural purposes.[22] The fraction of pharmaceuticals remaining in the water column reaches the aquatic environment with effluent released from the WWTP. Direct emission of untreated wastewater can also occur during episodic combined sewer overflow releases[23] or continuously *via* sewer connectivity leakage.[24] Alternatively, wastewater can be used directly for irrigation purposes thereby entering the terrestrial environment and then potentially running off into nearby water.[25] In areas without municipal sewerage

connectivity, pharmaceuticals excreted may be released to domestic septic systems, eventually entering groundwater[26] or be released to the environment directly.

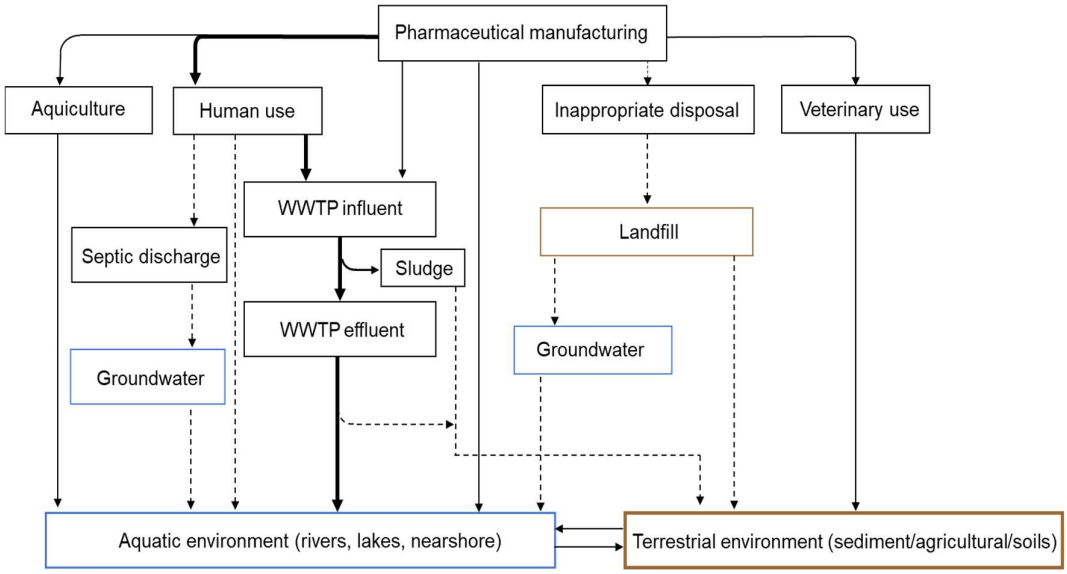


Figure 1. Pathways of pharmaceuticals to the environment. The thick arrows represent the pathways mainly considered in this study. Solid arrows represent major emission pathways, while lesser emission pathways are depicted by dotted arrows.

Pharmaceuticals are administered for aquaculture, livestock rearing or for the treatment of domestic animals. This results in a complex set of interacting environmental pathways, the details of which are beyond the scope of this thesis. A summary of routes is included in Figure 1 and includes direct terrestrial emissions *via* animal excretion,[27] while aquaculture application results in a direct release to the aquatic environment.[28]

Pharmaceuticals can also enter the environment through disposal to solid waste. Unfinished or unwanted pharmaceuticals can thus reach landfill and then be transported in the leachate, resulting in groundwater contamination if landfill leachate is not diverted to a WWTP.[29] Inappropriate disposal to wastewater can occur (e.g. flushing medicines down the toilet), therefore by-passing human metabolism, which could increase pharmaceutical wastewater emissions; however experimental evidence suggests this pathway is limited.[30] Pathways to and the interactions between receiving environments are complex, although in terms of human therapeutic use, WWTP discharge to the aquatic environment is the most significant[31,32] and consequently the focus of the following sections.

1.2 Occurrence of pharmaceuticals in surface water

The identification and quantification of pharmaceuticals in the aquatic environment has become a substantial area of environmental research since the 1990s.[33] A recent comprehensive review of pharmaceutical monitoring studies conducted throughout the world identified that 631 different pharmaceuticals have been detected (including transformation products and metabolites) in 71 countries covering all continents.[31] The vast majority of these studies have taken place in Western Europe and the United States (US) and concentrations reported were typically in the ng/L to µg/L range. The authors sorted existing monitoring data into United Nations- designated regional groups, which have been plotted for selected pharmaceuticals in Figure 2. Regional trends in surface water occurrence emerge; for example, higher concentrations of antibiotics (e.g. ciprofloxacin, norfloxacin, ofloxacin and trimethoprim) are observed in Asia. This is consistent with higher per capita usage (six times that of the UK) in that region.[34] The highest concentrations of analgesics (e.g. ibuprofen and paracetamol) in surface water were reported in the Western Europe and others group, again in line with higher over the counter analgesic usage in this region (USA alone accounted for 40% of the global paracetamol market share in 2014).[35,36] These comparisons demonstrate that the global surface water distribution of pharmaceuticals is not uniform, regional disease pressures, population size and prescribing practice influence observed trends at this scale.[37] Furthermore, global pharmaceutical usage is expected to rise coinciding with population growth, ageing demographics, increased access to healthcare and reduced pharmaceutical costs. Recent projections suggest that pharmaceutical usage in the UK alone will double by 2052,[38] so that future increases in environmental concentrations can be expected.

In the UK specifically, several pharmaceutical monitoring campaigns have been reported, covering approximately 70 compounds and belonging mainly to high-use therapeutic classes including: anti-inflammatories, β-blockers, antidepressants and antiepileptics.[32] Compounds selected for these monitoring campaigns have been based on those expected to be present in surface water owing to their high usage and patient metabolism[39] and expected passage though water treatment.[40] The majority of UK surface water monitoring data has been focused in Wales and the south of England, and

reported by a single research group.[39,41–46] However, more recently other studies have focused near London[47,48] and in the north of England.[49] Boxall et al.[50] undertook a targeted monitoring campaign of 17 pharmaceuticals from various therapeutic classes predicted to be of greatest risk to humans in vulnerable surface waters (e.g. near drinking water abstraction points, points of limited dilution or large population). In these vulnerable rivers, ten of the 17 pharmaceuticals were detected and all at sub-µg/L concentrations. Overall, many concentrations reported are in the tens of ng/L range, while the highest surface water concentration reported in the UK was for the analgesic tramadol (7731 ng/L).[39] Kay et al.[49] reported the second highest concentration, 4838 ng/L, for the anti-inflammatory ibuprofen. The third highest concentration was the analgesic paracetamol (2382 ng/L)[39] and forth was the antidiabetic, metformin (2318 ng/L).[44] This prevalence of anti-inflammatories/analgesics is consistent with surface water occurrence trends and prescribing practices for the Western Europe and others region (Figure 2).[31]

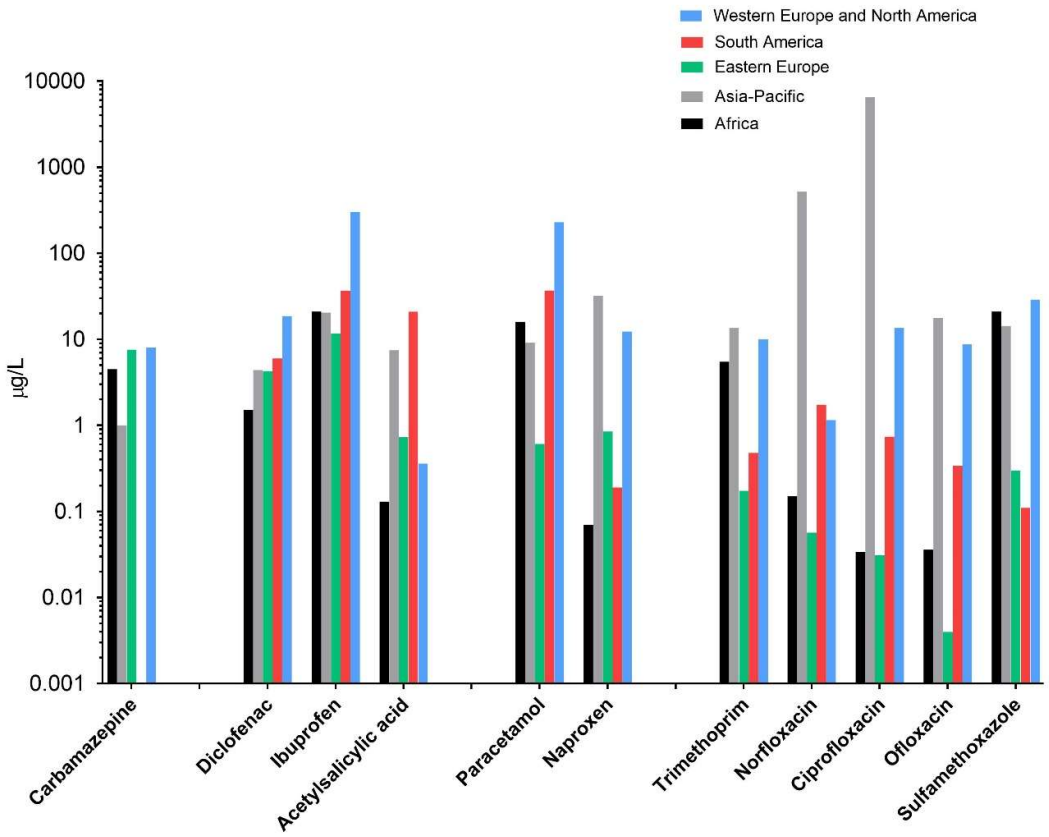


Figure 2. Global pharmaceutical surface water maximum concentrations available in all five of the United Nations regional areas compiled by aus der Beek et al.[31] Compounds are grouped by therapeutic class: anticonvulsant (carbamazepine), analgesics, anti-inflammatory and antibiotics.

1.2.1 Factors influencing concentrations in surface waters

Pharmaceutical concentrations reported in the UK aquatic environment indicate that concentrations range over several orders of magnitude from not-detected to >1000 ng/L. The concentration of a pharmaceutical at any one point in the environment is the consequence of many interacting factors. The first factor to consider is pharmaceutical usage and subsequent metabolism, which varies based on population size, demographics and disease pressures. The following sections detail factors that could influence pharmaceutical occurrence after excretion to municipal wastewater streams.

1.2.1.1 Wastewater treatment

Wastewater treatment does reduce pharmaceutical concentrations from influent to effluent; however for many compounds this reduction is limited and highly variable, while others can actually increase from influent to effluent.[20] In an extensive global review, Verlicchi et al.,[51] compiled removal rates for 118 pharmaceuticals. Based on those data, an average removal of 57% was calculated, ranging from not removed to 99% removed.[51]

Pharmaceuticals are removed from wastewater via biotic degradation or sorption to sludge; the effectiveness of these processes is related to a compound's physico-chemical characteristics which for pharmaceuticals, are highly variable. Sorption to sludge is influenced by the hydrophobicity of a compound, usually expressed in terms of the octanol-water partition coefficient (Kow). Stevens-Garmon et al.[52] correlated the logKow with sludge sorption, although many pharmaceuticals are hydrophilic (logKow<3), suggesting sorption to sludge is limited for many compounds.[51] Also important to consider is that many pharmaceuticals are weak acids or bases, which can be ionised at a pH relevant to WWTP conditions, depending on the negative log of their acid dissociation constant (pKa).[53] Ionisable chemicals can partition to the sludge, however unlike neutral compounds, hydrophobicity is not the driving mechanism of this process. The mechanisms of ionisable sludge sorption are not yet fully understood,[53,54] however processes including cation exchange, ion bridging and electrostatic interactions have been suggested to play a key role.[52,55,56]

In the WWTP, after sedimentation and filtration (primary treatment, during which large particles are removed) a secondary biological treatment step is employed. Influent is sprayed over rocks saturated with microbial organisms and trickles through (trickling

filter) or is filtered into an aerated tank containing suspended sludge laden with microbial organisms (activated sludge). These microbial organisms are intended to break down organic matter and in the process degradation of pharmaceuticals can also occur to varying degrees.[20] Microbial degradation is thought to be the major WWTP removal mechanism for several therapeutic classes including analgesics, antibiotics, β-blockers and hormones.[51] However, the biotransformation mechanism for many pharmaceuticals is not known and nor is whether this is facilitated by specific species or a community interaction.[57] Biodegradation has been linked to molecular structure, for example the presence of electron donating functional groups such as hydroxyl groups[58] and a primary amine.[59]

While physico-chemical parameters can be used to help understand WWTP removals, there is still a great deal of uncertainty associated with removal estimates. This can stem from inadequate sampling strategies used to quantify removal in a particular WWTP,[60] but also the variability in operating conditions within and among treatment facilities.[61] Parameters which affect removal are thought to be: temperature, pH, organic carbon content, biological oxygen demand, sludge age and the structure of the microbial community.[51] For example, seasonal fluctuations in temperature have been linked to removal efficiency. Poorer removals were observed in colder seasons, likely due to decreased microbial activity or decreased hydraulic retention time resulting from higher flows during this season.[62,63] Treatment plant type can also affect removal rates. In the UK, the majority of WWTPs have secondary (biological) treatment steps, employing activated sludge or trickling filter treatment.[64] It has been reported that trickling filter plants are generally less efficient at removing pharmaceuticals than activated sludge treatments,[43] however in general, fewer data are available for this type of plant. Pharmaceutical removal rate uncertainty is further complicated by the potential for the conversion of conjugated metabolites back to the parent compound during treatment, potentially resulting in increased concentrations of the parent compound in effluent compared to the influent.[5]

1.2.1.2 Environmental fate

Once in the environment, WWTP effluent is diluted based on the size of the receiving water body. In the UK specifically, effluent dilution can be highly variable

spatially and seasonally, with smaller rivers composed of 60 to 85% effluent in summer months.[40,65] Dilution is therefore an important driver in the concentrations observed in the environment.[66] Environmental persistence is another important factor and is highly variable, with certain pharmaceuticals not persistent (environmental half-life less than a day, e.g. paracetamol) and others highly persistent (half-lives over 100 days, e.g. carbamazepine).[67]

Similar attenuation processes to those present in the WWTP operate in the aquatic environment. The impact these processes have on reducing concentrations in the water column is variable and again is related to the physico-chemical properties of the pharmaceutical and various water quality parameters. Sorption to sediment directly or to colloids suspended in the water column can also be an in-stream attenuation mechanism for certain pharmaceuticals.[68] This sorption is again related to the hydrophobicity of the compound; however if ionised at an environmental pH this relationship is more complex, similarly to in the WWTP. Processes such as cation exchange, cation bridging or hydrogen bonding can be important for pharmaceutical sorption to sediment.[69] The conditions of the sediment/colloidal fraction are also important as higher cation exchange capacity or organic matter content can influence sorption.[70]

Several transformation processes are also operating alongside sorption to attenuate pharmaceuticals in surface water, such as hydrolysis, photodegradation and microbial degradation resulting in partial or full losses (mineralisation) of susceptible pharmaceuticals.[71] For example, tetracycline antibiotics can be hydrolysed and the efficiency of this process is influenced by temperature and pH.[72] Photodegradation is an important natural attenuation process for many pharmaceuticals and is influenced by solar irradiation, water depth, turbidity and pH.[73,74] Chemical structure is important as it can influence the absorption of solar radiation, permitting direct photolysis (e.g. aromatic rings).[73] Indirect photodegradation can also occur, where pharmaceuticals react with other components present in the water column that have been photolytically excited, such as organic matter, iron or nitrates.[75] Similarly to wastewater treatment, microbial degradation also occurs in the environment. Again the extent (partial transformation or mineralisation) and rapidity of this process varies with environmental conditions such as redox potential, temperature and pH.[69]

The transport and fate of a pharmaceutical in surface water is a result of the interactions between these in-stream attenuation processes. Moreover, each of these processes can be enhanced/limited by water quality parameters which are variable spatially and temporally (seasonally). This is further complicated by the variability in pharmaceutical emissions to the environment (usage and WWTP removal), which can also be temporally and spatially influenced. Many monitoring studies have reported pharmaceutical concentrations in the environment; however two recent review articles identified that the spatial and temporal variability of pharmaceutical occurrence in surface water is an understudied area.[32,71] Understanding this occurrence is important as it helps characterise the magnitude and duration of pharmaceutical exposure, which is critical for determining risks to the environment.

1.3 Determination of pharmaceuticals in aqueous samples

The quantification of pharmaceuticals in environmental samples provides a significant analytical challenge. This is because pharmaceuticals, even within the same therapeutic class, are physico-chemically diverse and are usually present in trace amounts (sub-ng/L to µg/L) within a highly complex sample matrix (e.g. surface water).[76] Relatively recent advances in trace analysis have enabled the emergence of multi-residue methods capable of simultaneous detection of over 100 pharmaceuticals at ng/L levels in aqueous environmental samples.[77] This has been achieved largely through the improvement of mass spectrometers, especially for tandem applications[78] and the coupling with chromatographic separation techniques. A large number of pharmaceutical quantification methods have been reported which utilise various sample preparation and instrumentation configurations.[79] Mass spectrometry (MS) is the core analytical technique applied in pharmaceutical quantification. Specific instrumentation varies between research groups, but one of the most successful and commonly used is the triple quadrupole mass spectrometer (QqQ) with an electrospray source (ESI) coupled to high-performance liquid chromatography (HPLC).[80] These techniques have been described in detailed elsewhere (e.g. de Hoffmann and Stroobant[81]), therefore only a brief description is provided.

1.3.1 Electrospray ionisation

Ionisation of analytes prior to introduction into the MS is critical as the quadrupole mass analyser uses electric potentials to manipulate analytes. ESI is a soft ionisation technique (analytes do not fragment),[82] which gained initial success as a technique to apply multiple charges to large biomolecules (e.g. proteins) enabling their detection in MS instruments with limited *m/z* ranges.[83,84] Since the 1990s, ESI has become a popular technique for many biological applications, including analysis of small polar molecules, such as pharmaceuticals.[81,85] Furthermore, ESI is highly advantageous because analytes are introduced in the liquid phase at atmospheric pressure, making it an ideal ionisation technique for coupling LC and MS.

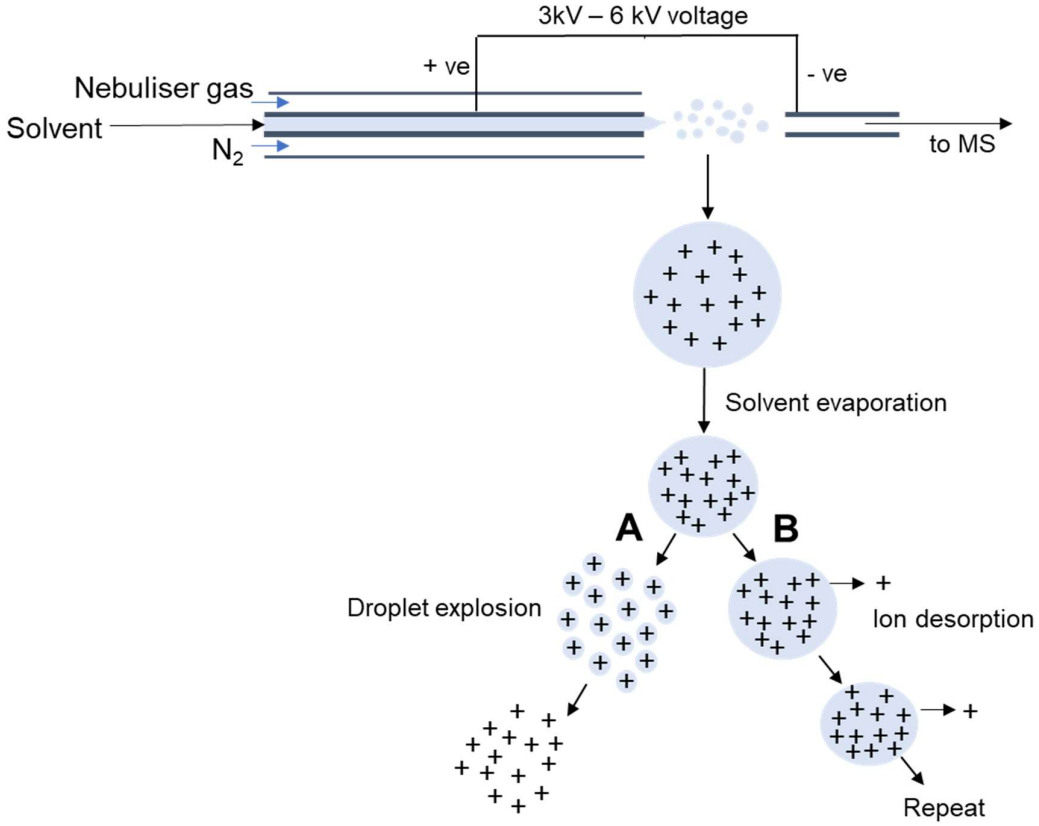


Figure 3. Schematic of ESI with proposed models for gas-phase ion production (A) charge residue model (CRM) and (B) ion evaporation model (IEM).

Briefly, analyte-containing solvent flows through a capillary to which an electric potential is applied. Analytes, charged in solution move either towards or away from the capillary walls, depending on their charge. Repulsion of like charges in solution causes the solvent to form a droplet at the end of the capillary. Charge accumulates until

repulsion over-takes surface tension and droplets containing the charged analytes are released. Simultaneously, a nebulising gas (usually N_2) is passed coaxially along the capillary, which when the droplets are released from the capillary helps form a spray. Once in the spray, the mechanism by which charged ions are desolvated and enter the gas-phase is debated.[86] Two models, the charged residue model (CRM)[87] and the ion evaporation model (IEM)[88] have been suggested. The CRM proposes that as solvent evaporates, droplet size is reduced and charge density increases until this charge density reaches a critical threshold, the Rayleigh limit. At this point the charge density is unstable and as a result the droplet bursts into smaller droplets. This process is repeated until fully desolvated ions exist in the gas phase (Figure 3A). Alternatively, the IEM proposes that as solvent evaporates charge density increases, similarly to the CRM. The difference is than when charge density becomes unstable gas-phase ions desorb directly from the solvent droplet (Figure 3B).

1.3.2 Quadrupole mass analysers

The principles of the quadrupole mass analyser were first described by Paul and Steinwedel in the 1950s. Since then the quadrupole has become one of the leading mass analysers for environmental pollutants due to its low cost and maintenance, compactness, high sensitivity, and the qualitative/quantitative information that can be obtained.[89] The quadrupole acts as a mass filter, in which a dynamic electric field is applied, permitting only ions of a specific *m/z* to pass through the quadrupole. A quadrupole consists of two pairs of parallel rods (Figure 4A) to which potentials are applied by supplying radio frequency (RF) and direct current (dc) voltages. The polarities of the potentials applied are opposite for the two pairs of rods (Figure 4A). Positive ions entering the quadrupole are repelled by the positively charged rods, keeping the ions in the centre of the quadrupole. The polarity of the voltage applied is predominately positive, but switches briefly to negative as indicated by the amplitude of the voltage in Figure 4B. Low *m/z* ions are drawn towards the rods during this negative polarity period, while heavier ions remain stable between the rods as the period of negative polarity is too short for them to react. As low *m/z* ions are drawn towards the now negative set of rods, those which are stable travel towards the rod but do not reach it before the polarity returns to positive, consequently repelling the ions back towards the centre of the quadrupole (Figure 4B). Ions below the stable *m/z* hit the rods and are discharged before

the rods return to positive polarity. Therefore, the predominately positively charged rods act as low pass filters, as they filter out low *m/z* ions (Figure 4B).

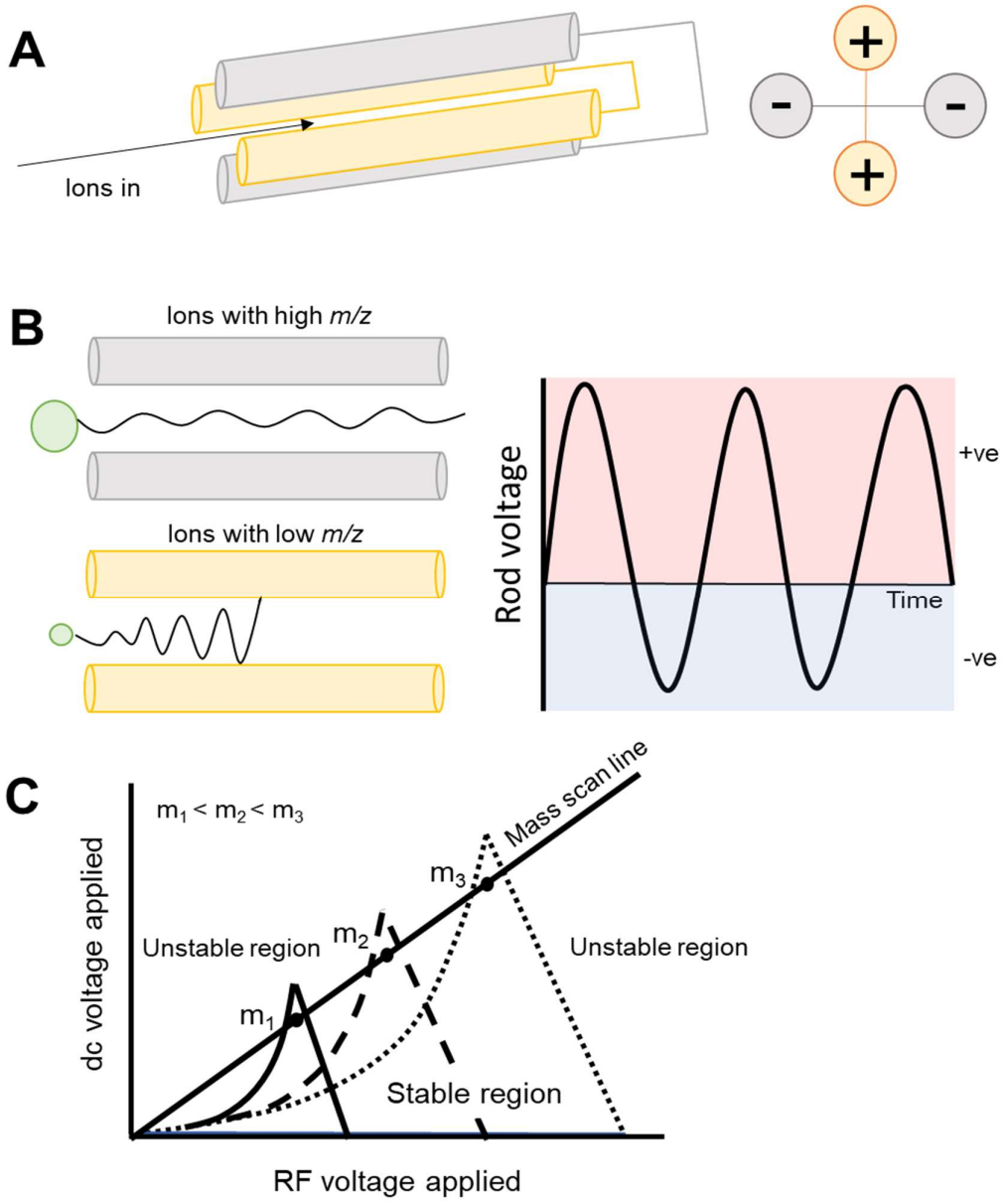


Figure 4. (A) A simple schematic of the quadrupole showing opposing rod pairs and the opposing predominate polarity applied to each pair. B) A schematic of a how the quadrupole acts as a low pass filter. C) Ions of different *m/z* can be brought into the stable region at different points along the mass scan line by altering the RF and dc voltage, but keeping the ratio between these changes constant. In a collision cell no dc voltage is applied, moving all ions into the stability region. Adapted from Steel and Henchman.[90]

The predominately negatively charged pair of rods act as high pass filters, removing high *m/z* ions. Positively charged ions are drawn towards the negatively charged rods, but when the rods briefly change polarity, low *m/z* ions are repelled back

to the centre of the quadrupole and so remain between the rods (opposite of Figure 4B). High *m/z* ions have too much momentum to move back to the centre of the quadrupole during the brief period of positive polarity and so ions above the stable *m/z* hit the rods and are discharged. Ions are only transmitted through the quadrupole when they are stable to both the low and high pass filters. This is achieved by scanning through RF and dc voltages to sequentially bring to stability and thus transmit ions of increasing *m/z* to record a spectrum (Figure 4C). This stability condition is represented by the mass scan line in Figure 4C. Ions of different *m/z* fall at different positions along this mass scan line; when the line passes through an ion's stability region, this indicates the voltage combination under which the ion is transmitted through the quadrupole. Ions at different points along the mass scan line can be brought into the stable region by altering the RF and dc voltage, but keeping the ratio between these changes constant (Figure 4C).[90] The mass scan line in Figure 4C depicts the quadrupole operating in scan mode, but it can also be operated in fixed mode. In fixed mode, instead of scanning along the line, the voltage ratio will remain constant (to transmit a single m/z) or jump between several voltages to transmit particular ions. In addition to being operated as a mass filter, a quadrupole can also be operated as a collision cell. In the collision cell, all ions are stable and pass through the quadrupole. This is achieved by only applying an RF voltage (i.e. no dc) (Figure 4C). The collision cell is also pressurised with inert gas with which ions collide, causing them to fragment in the process of collision induced dissociation.

1.3.2.1 Triple quadrupole mass analysers

In a QqQ, quadrupoles are arranged in series and for targeted quantitative analysis, operated in multiple reaction monitoring (MRM) mode. Ions enter the first quadrupole (Q1) which is operated as a mass filter, only permitting precursor ions with stable (preselected) *m/z* values through (Figure 5). The selected precursor ions enter the second quadrupole (q2), which is operated as a collision cell. Precursor ions are fragmented and the resulting fragment ions are transmitted to the third quadrupole (Q3), which, similarly to Q1 is operated as a mass filter permitting only fragment ions of certain preselected *m/z* values stable trajectories and thus to be transmitted to the detector (Figure 5). In environmental samples, many small organic molecules with similar *m/z* to pharmaceuticals could be present and subsequently pass through Q1 along with the

target analyte. For this reason, at least two unique transitions (precursor-fragment pairs) are monitored, which together with the chromatographic retention time, help ensure correct identification of the target analyte. The MRM approach limits background from the complex sample matrix, which helps lower limits of detection.[80]

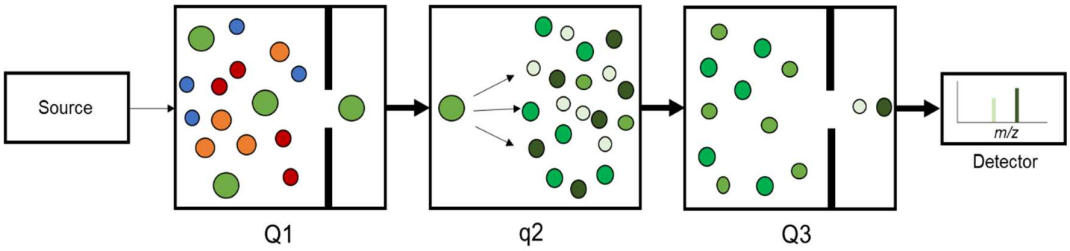


Figure 5. Schematic of a triple quadrupole mass spectrometer operating in multiple reaction monitoring mode where Q1 and Q3 are operated as mass filters of the precursor ions (Q1) and fragment ions (Q3) and precursor ions are fragmented in the collision cell (q2).

1.3.3 Liquid chromatography

To ensure analytes are ionised efficiently from 'dirty' matrices (e.g. surface water, WWTP influent or effluent) a pre-separation technique, such as liquid chromatography (LC) can be used. In the ion source, analytes are competing for charge. With greater matrix complexity, target analytes may not be ionised efficiently enough to be detected. LC can be used to strategically elute components within the matrix based on physico-chemical properties, effectively separating sample components and reducing charge competition at the source.

In liquid chromatography, sample is introduced in a solvent (mobile phase) which is then pumped through a column packed with particles (solid phase). Separation of the analytes depends on their differing affinities for the stationary phase, into which they partition. The interaction with the stationary phase will prolong elution, permitting separation of sample components. More efficient separations require smaller column packing particle sizes, which incur greater system pressure to pump mobile phase through. Therefore, high-performance liquid chromatography (HPLC) systems were introduced able to pump solvent at the higher pressure (400 bar), enabling faster separation in shorter and narrower columns. For environmental pharmaceutical analysis, HPLC is commonly used.[77,91–94] To separate pharmaceutical analytes, reversed phase chromatography (i.e. polar mobile phase) is used. Briefly, the analyte mixture (sample) is

carried by a polar mobile phase (e.g. an aqueous-organic mixture) through a column packed with hydrophobic stationary phase particles (usually silica with C18 alkyl chains). Polar analytes are eluted from the column first (i.e. partition back out into the polar mobile phase), with more hydrophobic analytes being retained for longer by the stationary phase.

Elution efficiency can be improved by use of a mobile phase gradient, in which the composition of the mobile phase is varied over time, starting with low organic and rising to high organic composition. The effect is to speed up the elution of more hydrophobic analytes and thus to improve overall chromatographic performance. The chromatographic program (e.g. % organic modifier, temperature, flow rate) is optimised to elute analytes separately and predictably (retention time). This is highly advantageous as it reduces the number of components entering the MS at any one time, improving both ionisation efficiencies as well as allowing duty cycles to be optimised.

In the early 2000s, a new category of separation technology was introduced by Waters©, Ultra High-Performance Liquid Chromatography (UHPLC). UHPLC and HPLC are similar, however the UHPLC systems are designed to withstand backpressure in excess of 400 bar, enabling higher flow velocities, smaller stationary phase packing material particle sizes and reduced column length.[79] This is advantageous as it can reduce the time needed to separate analytes, improve chromatographic resolution and narrow peak widths, desirable for quantification.[95,96] UHPLC is increasingly used for the separation of pharmaceuticals in environmental samples.[95,97,98]

1.3.4 Sample preparation

Prior to chromatographic separation and MS determination, sample preparation employing one or more pre-concentration/clean-up step is common practice.[99] The most commonly used method for pharmaceutical analysis in aqueous matrices is solid phase extraction (SPE).[79] The SPE cartridge used is packed with a reversed-phase sorbent which retains analytes based on non-polar or hydrophobic interactions. SPE cartridges are wetted and equilibrated prior to loading with diluted sample. Sample is applied at a consistent flow rate to ensure molecules are optimally retained on the cartridge. A wash step can then be used to remove co-retained interferences using diluted organic solvent, however this step will only remove less hydrophobic interferences and could result in

target analyte losses. Molecules retained on the column are then eluted using organic solvent (e.g. methanol), then dried down and reconstituted in LC mobile phase to a volume suitable for analysis. Pharmaceuticals are ionisable, therefore pH buffering is usually necessary during the loading and washing phases to ensure molecules are in their neutral (more hydrophobic) form.

It is often difficult to selectively retain a large group of physico-chemically diverse pharmaceuticals. Therefore the hydrophobic retention mechanism, which is poorly selective, is commonly used. This can result in interfering molecules pre-concentrating along with target molecules, which could increase matrix effects (discussed in Chapter 4). SPE can also result in analyte losses, leading to poor analyte recoveries. Regardless, these SPE-LC-MS/MS methods have been highly successful at achieving low limits of detection (low to sub-ng/L) for a range of pharmaceuticals in difficult matrices (surface water, WWTP influent and effluent).[42,95,97,100–104]

Recently, pharmaceutical quantification methods for use in aqueous matrices have been developed that do not employ sample pre-concentration/clean-up.[77] Larger than normal sample injection volumes (e.g. 100 µL versus 10 µL) are used instead of pre-concentration to increase chances of detection.[44] The trade-off is that these direct LC injection methods can incorporate large numbers of target analytes (e.g. 40 – 110), experience limited analyte losses during sample preparation (i.e. filtering), reduce analysis costs and greatly enhance sample throughput,[77,105] but detection of certain analytes may be reduced without sample pre-concentration. The application of these approaches is promising, especially for large monitoring campaigns.[44,105,106]

1.4 Pharmaceutical effects in the environment

A vast range of chemical contaminants is unintentionally released to the environment through use or manufacture. These chemicals, for example plasticisers, pharmaceuticals, personal care products, detergents, insecticides, and brominated flame retardants, have been detected in the environment, but little is known of their emission, fate and potential to cause adverse effects, thus they are referred to collectively as emerging contaminants. Of this group, pharmaceuticals have gained specific ecotoxicity concerns as they are designed to illicit a biological response at low concentrations, similar

to concentrations being reported in the environment. A wide range of effects due to unintended pharmaceutical exposure have been observed in the environment. One of the most well-known effects was the decimation of an Indian vulture population (*Gyps vulgaris*) from exposure to diclofenac (a non-steroidal anti-inflammatory) through the food chain.[107] Farmers treated cattle with the drug; when the cattle died, they were preyed upon by the vultures. A known side effect of diclofenac, renal failure, was found to be the reason for the vulture deaths.[108] While this revelation was particularly striking, a number of other effects in the environment have also been observed. For example, the feminisation of male fish downstream of WWTPs, which was hypothesized to be, at least partly, due to the presence of the synthetic hormone, ethinylestradiol, used for female birth control.[109] Results from laboratory studies provide further evidence of this male feminisation, which could impact wild fish populations and consequently the ecosystem.[110,111] This has spurred much more research into the potential effects that pharmaceuticals could illicit in exposed organisms, with environmental risk assessment now required prior to market authorisation in the European Union and US.[112] As a result, much of the data that have been collected has been based around traditional standard ecotoxicological, mainly acute (sometimes chronic), endpoints pertaining to survival, reproduction and growth of standard test species of fish, daphnia or algae. Brauch et al.[113] compiled an excellent review of acute and chronic ecotoxicity data available, identifying 150 pharmaceuticals with acute ecotoxicity data and 65 with chronic data. Acute effect concentrations generally fell in the mg/L range and chronic effects at the µg/L range and the majority of data available pertains to freshwater invertebrates and fish.[113] The mode of action (MoA) in acute exposures is expected to be non-specific narcosis,[114] which is likely to occur at concentrations much greater than concentrations where effects arise from the intended MoA of the pharmaceutical through chronic exposure.[115] Therefore, acute testing strategies could be underestimating risks of these substances. Moreover, standardised chronic data, while more suitable for risk assessment than acute data, still focuses on survival/reproduction/growth endpoints and therefore could also be missing key effects related to the MoA of the drug.[113]

Pharmaceuticals target specific metabolic, enzymatic or cell-signalling pathways. Many of the receptors involved are evolutionarily conserved to various degrees in aquatic species including fish, amphibians and reptiles, and invertebrates.[116,117] Current theories

suggest that effects could occur if the receptor is present and organism internal concentrations near the human therapeutic (or side effect) concentration are achieved in the organism.[118] The receptor pathways and physiological systems are usually not wholly conserved,[116] therefore dissimilar effects from the intended therapeutic effect can be observed in exposed organisms.[119] There is an increasing evidence base supporting this. For example, snails exposed to environmentally relevant concentrations (ng/L) of the antidepressants venlafaxine and fluoxetine, displayed physiological effects (i.e. foot detachment from the tank surface).[120] This was proposed to be due to the conservation of the 5-HT cell signalling pathway, which in humans results in increases of serotonin, but in invertebrates is an important physiological controller.[71,121] The freshwater plant *Lemna gibba* was exposed to the antibiotic sulfamethoxazole, because the MoA receptor pathway (i.e. disrupts folate synthesis) was also identified in *Lemna gibba.*[122] The authors found the 50% effect concentration (EC50) for the standard chronic ecotoxicity endpoints (e.g. weight, number of fronds) was 20 and 40 times higher than the EC50 from the sulfamethoxazole MoA based endpoint. This MoA endpoint relates to an increase in p-aminobenzoic acid content, a precursor for folate synthesis.[113] A growing number of these subtle molecular, physiological, behavioural and histopathological effects are being observed in a growing range of aquatic species; however, the impact these non-standard effects have on individual fitness, populations and ecosystems is not well understood unlike traditional ecotoxicity testing outcomes.[37]

In addition to single chemical concerns, there is also a growing body of research on the ecotoxic potential of pharmaceutical mixtures. In the environment, organisms are exposed to a pharmaceutical cocktail, which could have important implications in terms of ecotoxicity.[3] Several studies have demonstrated that the effect of pharmaceuticals on aquatic organisms are additive,[114,123,124] which would imply that due to being present as a mixture, organisms would be at greater risk than when based on single compounds. Others have demonstrated synergistic effects can occur (i.e. an unexpected response based on the responses to the mixture components singly).[125] For example, daphnia exposed to anticancer drugs exhibited an effect at the lowest concentration tested as a mixture, but not when tested singly.[126] This has also been observed for the antibiotics sulfamethoxazole and trimethoprim which, when exposed in combination, resulted in synergistic toxicity in algae.[127] The drugs' MoAs are similar (inhibiting synthesis of a

bacterial enzyme), but they have different molecular targets, which could explain the synergy.[128] Conversely, other research has demonstrated that pharmaceuticals when exposed as a mixture, have antagonistic effects. For example, mussels exposed to the β-blocker propranolol as well as the anti-depressant fluoxetine exhibited effects when exposed to each singly, but no effects were observed when exposed concurrently.[121] The authors hypothesized that since both pharmaceuticals targeted the same receptor (one increased occupation of 5-HT receptors while the other blocked the 5-HT receptor), co-exposure resulted in counteractive (antagonistic) effects.[121] The potential for additive, synergistic or antagonistic effects of pharmaceuticals has been demonstrated and adds further complexity to understanding and quantifying the extent of effects pharmaceuticals could be having in the environment.

Another environmental concern is the potential selection of antibiotic resistant strains of bacteria resulting from exposure to antibiotics.[129] This potential resistance could have major impacts on human or livestock health as antibiotics could lose their efficacy.[130] A recent evaluation of river basins in China found that resistance rates in hospitals and rivers was correlated with antibiotic usage.[131] In addition to resistance, antibiotics can have devastating impacts on microbial communities in the environment, such as microbes responsible for organic matter or chemical degradation.[132]

Freshwater ecosystems have been the major focus of pharmaceutical ecotoxicity assessment, possibly due to the multiple bioaccumulation routes possible and the breadth of exposure work conducted in this matrix. Pharmaceuticals can enter an aquatic organism *via* the diet or directly from the water column through the gills or dermally (bioconcentration).[133] Pharmaceutical bioconcentration is the main aquatic uptake mechanism, with bioconcentration factors (BCF) ranging from 2.2 (carbamazepine, algae) up to 185900 (fluoxetine, crustacean) as compiled in a review by Huerta et al.[134] These bioconcentration factors are important to consider as internal concentrations could be much higher than the surrounding water; they also indicate that food chain interactions could lead to exposure in predators.[135] Limited work on potential effects in predators in aquatic food chains has been undertaken.[136] Furthermore, research on terrestrial species effects and food chains has also been limited, despite the potential for exposure as shown in Figure 1.[137,138]

1.5 Prioritisation of pharmaceuticals in the environment

There are more than 1900 active pharmaceutical ingredients currently in use and despite the growing knowledge base surrounding pharmaceuticals in the environment, knowledge of environmental exposure, fate and effects is limited to a relatively small proportion of these compounds. It is highly likely that far more pharmaceuticals not yet studied or present below current analytical detection limits are in the environment. The presence of pharmaceuticals in the environment alone does not necessarily mean effects are occurring. Limited persistence or low potency could indicate that the pharmaceutical is unlikely to accumulate in the environment at a concentration great enough to pose a risk. In the European Union, environmental risk assessments are only required for pharmaceuticals released to market after 2006, Figure 6.[112] This risk assessment process is heavily biased towards the aquatic environment and based on our growing knowledge of pharmaceutical fate, exposure through sediment or soils could be significant.[139]

It would take a tremendous amount of effort, animals and cost to assess the ecotoxicity and exposure of all pharmaceuticals in each environmental compartment in a timely manner.[37] Furthermore, it is likely unnecessary to assess all pharmaceuticals and each ecotoxicity endpoint for species representative of each environmental compartment due to the particular MoA of the drug, emission rate or fate on entering the environment. For example, Küster and Adler[140] evaluated the risks of 120 human medicinal products with standardised ecotoxicity data available from the German Federal Environment Agency and found that only 10% were noteworthy in terms of environmental risks.[140] While this assessment focuses on standardised ecotoxicological effects and as a result could be missing risks, it still demonstrates that not all pharmaceuticals in use are expected to pose risks to the environment.

Prioritisation methodologies provide a useful tool for identifying which of the 1900 pharmaceuticals in use have the greatest potential to cause unintended effects in non-target organisms and which therefore should be experimentally tested in terms of their fate and effects.[6] Several prioritisation approaches have been proposed for pharmaceuticals (Chapter 2). For example, hazard-based approaches have involved the prediction of persistence, bioaccumulation, and toxicity of a pharmaceutical and these

have then been used to develop an overall hazard score. Compounds with the highest scores are considered to have the highest priority.[141] Risk-based approaches have involved the estimation or measurement of pharmaceutical concentrations in environmental media and the comparison of these concentrations with an effect endpoint. Examples included predicted no-effect concentrations derived from acute or chronic ecotoxicity data[142–144] or predictions,[145] plasma therapeutic concentrations,[118] acceptable daily intakes for humans[146] or a combination of these.[147] Risk-based methods have been identified as preferable due to the consideration of effects and environmental occurrence, ruling out the possibility of prioritising compounds that have little chance of accumulating in the environment at ecologically relevant concentrations.[6,146]

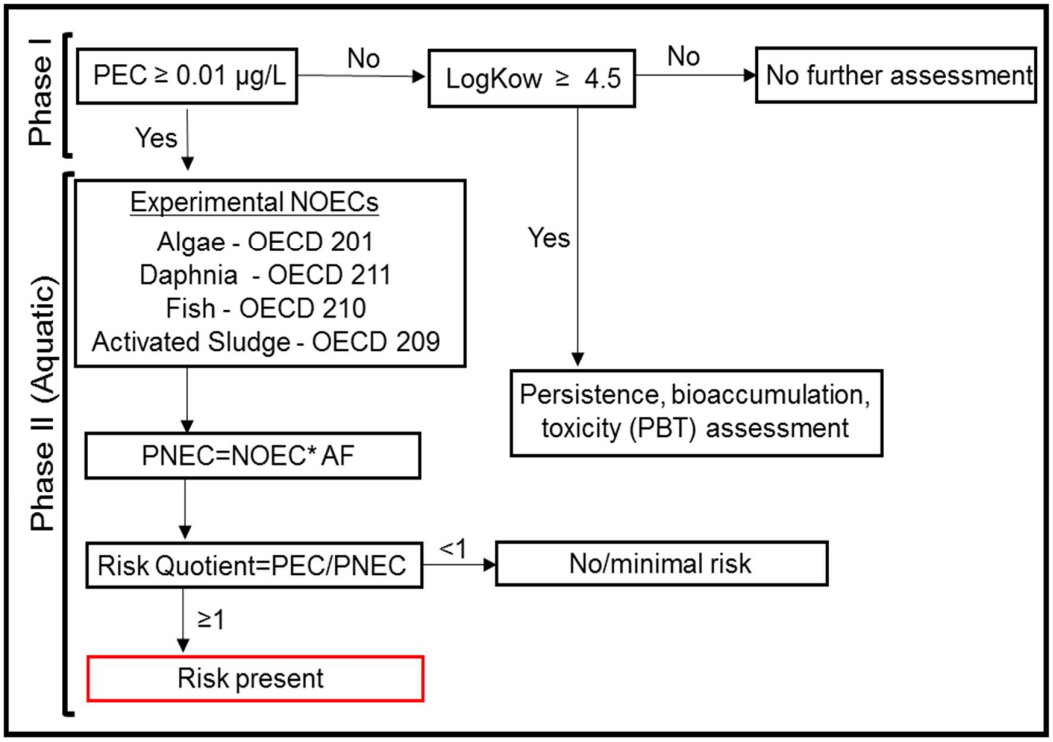


Figure 6. Simplified EMA risk assessment framework.[112] Phase I: Predicted environmental concentrations (PEC) are calculated and when greater than 0.01 µg/L, Phase II is initiated. In Phase II, standard Organisation for Economic Co-operation (OECD) ecotoxicity tests are conducted to obtain no observable effect concentrations (NOECs), which is multiplied by an assessment factor (AF) to incorporate species inter- and intra-variability to calculate a predicted no effect concentration (PNEC). The PEC is then compared to the PNEC to determine whether a risk is present.

Prioritisation methodologies are a tool that can be used to identify which pharmaceuticals pose the greatest risk to the environment, in order to focus research effort on the pharmaceuticals that matter.[6] A good prioritisation incorporates as many

pharmaceuticals as possible and assesses them equally without bias towards data rich or poor compounds.[148] The conundrum central to the development of a successful prioritisation approach, is how can potential risks be identified with limited or no fate and ecotoxicity data? This problem has spurred the development of many different prioritisation methodologies, which are reviewed in detail in Chapter 2. At the core of these approaches, are models that estimate pharmaceutical exposure and/or effects or estimate risks, hazards or likelihood of presence in the environment. The methods can then rank the pharmaceuticals and designate priority compounds based on exposure, hazard or risk.

All risk- and exposure-based approaches require an assessment of the concentration of pharmaceuticals in the environment. Real environmental data are desirable; however, monitoring data are generally lacking for a wide range of pharmaceuticals. Moreover, when monitoring data are available, the relevance of the data is often questionable due to sampling designs that do not consider seasonal biases, hydrologic conditions or spatiotemporal fluctuations.[60] As a result, comparing absolute measured concentrations of pharmaceuticals for prioritisation has been questioned.[149] Furthermore, sufficiently sensitive analytical methods, suitable for complex environmental matrices, or isotopically labelled standards necessary for accurate quantitation are not yet available for the majority of pharmaceuticals in use, making determination of pharmaceuticals in environmental matrices challenging.[143,145] Consequently, many risk-based prioritisation methods have employed exposure prediction models or algorithms to derive predicted environmental concentrations (PECs) in order to prioritise pharmaceuticals that have no monitoring data and/or to provide conservative estimates of environmental concentrations.[150]

1.5.1 Aquatic exposure modelling of pharmaceuticals

Pharmaceutical exposure models can be used to predict the concentrations of pharmaceuticals for either risk assessment, prioritisation exercise or to design a monitoring campaign. PECs are typically derived based on data on pharmaceutical usage, degree of metabolism in humans, removal in wastewater treatment plants (WWTP) and environmental dilution. The method most commonly used is based on the approach recommended in the EMA guidelines for assessment of the risk of human

pharmaceuticals in the environment, referred to as the simple PEC.[6,112,143,145,151–156] Default parameters (e.g. for dilution of wastewater production) proposed by the EMA guidance are regularly used in these prioritisation exercises, regardless of their suitability.[6,144,153,154] The use of site-specific data when performing these calculations for prioritisation is a rarity.[155]

Higher-tier spatial exposure modelling approaches have also been developed and applied to pharmaceuticals.[157–159] These models operate at the catchment scale by digitizing the river network and spatially incorporating pharmaceutical source inputs (i.e. WWTPs). In-stream decay can also be incorporated and the result is spatially explicit PECs within a river network.[157] This spatial advantage could be very important for the accurate prediction of pharmaceuticals as many rivers have multiple pharmaceutical inputs and complex hydrological dynamics which will affect pharmaceutical exposure.

The impact of using PECs for prioritisation has not been explored, although several authors have explored how well simple PECs compare to measured environmental concentrations (MECs).[7,150,160–166] These comparisons have provided varied results, with some studies showing that simple PECs adequately represent MECs,[161–165] while others suggest the differences are too great to be useful, or that simple PECs generally under represent MECs.[150,160,166] Validation of higher-tier exposure models has been limited to only a few studies, but results have indicated predictions are reasonably accurate (within a factor of 2).[158,167,168] Finally, these comparative studies for both simple and higher tier exposure models, concentrate on pharmaceuticals that have been identified as being of concern, or of high usage and generally focus on fewer than ten compounds,[165] limiting the relevance of their prioritisation and risk assessment conclusions across the broader spectrum of physico-chemically diverse pharmaceuticals known to be present in the environment globally.

Exposure models are crucial for pharmaceutical prioritisation and risk assessment; however validation of these models is limited. Furthermore, the implications of possible under- or over- predictions of exposure for risk assessment and prioritisation are not known. The suitability of these models is especially important to quantify as pharmaceutical effects in non-target organisms are increasingly reported and a vast number of pharmaceuticals have yet to even be prioritised or risk assessed.

1.6 Thesis aims and objectives

The aims of the work presented in this thesis were to: 1) characterise the key factors affecting exposure of aquatic systems to pharmaceuticals across space and time; and 2) develop approaches for better integrating spatial and temporal considerations of exposure into pharmaceutical prioritisation approaches and the regulatory environmental risk assessment process. These aims were met using the following objectives:

1. Evaluate previous pharmaceutical prioritisation approaches and identify their strengths and weaknesses. Based on this assessment develop an optimum prioritisation framework and evaluate the validity and availability of data and models underpinning the framework (Chapter 2).
2. Conduct a scoping study using an existing analytical method to evaluate pharmaceutical exposure in the York river system. Using this data, evaluate the accuracy of the simple exposure model and the impact this simple model has on prioritisation outcomes (Chapter 3).
3. Develop and validate a rapid LC-MS/MS method for the quantification of 33 pharmaceuticals present in the York river system as identified by the scoping study (Chapter 4).
4. Apply the analytical method to a year-long monitoring study of 11 sites in the York river system to determine the spatial and temporal variability of pharmaceutical concentrations in this system and identify the key exposure drivers behind this variability (Chapter 5).
5. Evaluate the simple exposure model and a higher-tier spatial exposure model with the spatiotemporally robust monitoring dataset from the York river system. Conduct a York river system risk assessment using the higher-tier spatial exposure model (Chapter 6).
6. Develop recommendations to improve current aquatic exposure modelling approaches, based on the key spatiotemporal exposure drivers (Chapter 5) to ultimately improve the effectiveness of prioritisation approaches and the regulatory risk assessment process. (Chapter 7).

Chapter 2

Prioritisation of pharmaceuticals in the environment

2.0 Introduction

An environmental risk assessment (ERA) is required as part of the marketing authorisation process for new active pharmaceutical ingredients in many regions of the world. For example, in the European Union, the European Medicines Agency (EMA) ERA for human medicinal products registered after 2006.[112] Detailed criticism of this process is outside the scope of this thesis, however many limitations have been identified which include the type of testing conducted (both ecotoxicity and fate), the required testing thresholds and the default assumptions pertaining to environmental exposure.[139,169] EMA based ERAs have only been conducted for a small proportion of the approximately 1900 active pharmaceutical ingredients (APIs) currently granted market authorisation in the UK.[4] To gauge the availability of these ERAs for the top 350 pharmaceuticals prescribed by mass in England (compiled from the National Health Service prescription cost analysis 2012[170]), European public assessment reports (EPAR) compiled by the EMA were then identified.[171] Of the 350 pharmaceuticals, only 71 had ERA data available as an EPAR (Appendix 1). Highly used pharmaceuticals such as paracetamol, carbamazepine, or amoxicillin, all commonly cited in the literature as high priority compounds possess no EMA environmental risk assessment. Other regional ERA initiatives exist, for example, Sweden's Wikipharma.[172] These assessments may not be directly comparable, but if pooled into a new central database would be a more efficient tool for identifying what ERA data is currently available.

The difference between the number of pharmaceuticals currently authorised for market use and those with environmental data is large (Figure 7). Potentially, those pharmaceuticals with little or no environmental data could pose risks. To conduct full ERAs on all pre-2006 authorised pharmaceuticals would be a substantial and likely unnecessary task, as the majority of currently assessed pharmaceuticals demonstrate a good margin of safety with studies focused in Europe predicting that approximately 5-10% of pharmaceuticals in use will pose any appreciable risk to the environment,[6,140] therefore it would be valuable to identify those pharmaceuticals most likely to pose the greatest risk then assess these through experimental testing and monitoring. Desk-based prioritisation approaches which screen pharmaceuticals based on either hazard or risk could help as they can be used to focus monitoring campaigns, effects testing, or to

decide which pharmaceuticals most urgently require a formal ERA.[37] This will help ensure that risky pharmaceuticals are identified while minimising any unnecessary organism testing.

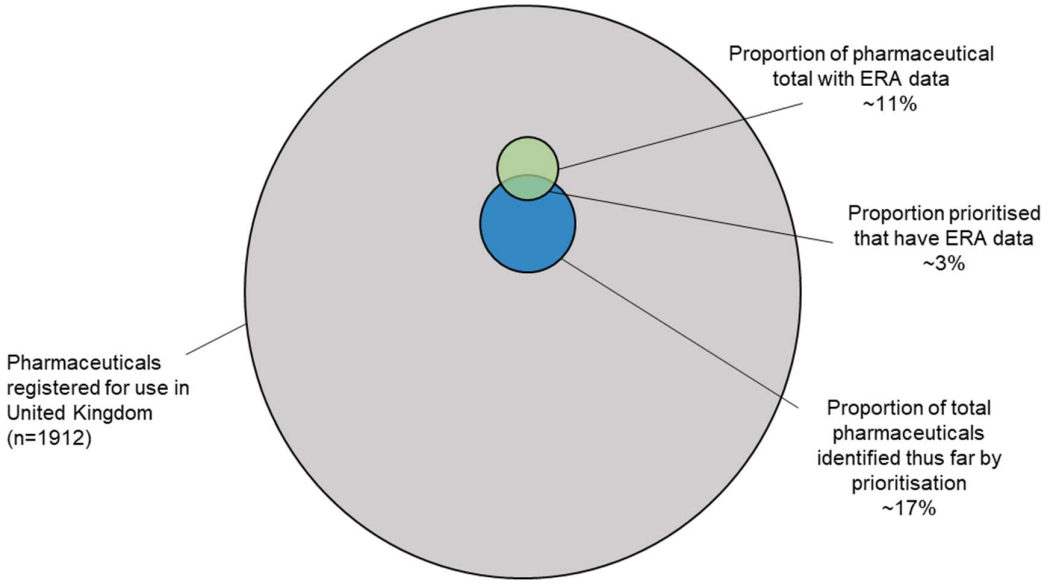


Figure 7. A qualitative representation of the estimated active pharmaceutical ingredients (APIs) registered for market use in the UK[8] (grey, n=1912), proportion of pharmaceuticals identified thus far by prioritisation exercises (blue, n=332), roughly the portion of total UK registered APIs that have EMA ERA data (green) and the overlap between APIs prioritised thus far and also assessed within the EMA ERA framework (blue and green).

A variety of prioritisation approaches has been suggested, which employ different methodologies.[145,147,149,173–176] This critical review examines a range of past pharmaceutical desk-based prioritisation exercises, to identify best practice along with what is missing and the likely data availability for different operating systems. A systematic literature search was conducted using combinations of the keywords: 'pharmaceutical' with either 'prioritisation', 'ranking' or 'priority' within the Web of Science™ and Scopus® databases as well as the Google Scholar search engine. Additional targeted searching was included where appropriate from identified literature references. Prioritisation exercises and risk assessments that either identified a pharmaceutical of concern or several priority compounds were included. A total of 73 papers were identified, several of which included multiple priority lists (total 76) either representing different environmental compartments or prioritisation approaches. Prioritisation approach references are reported in Appendix 2. To limit the scope, only prioritisation approaches that considered human-use pharmaceuticals were included.

Pharmaceutical mixtures, while gaining significant attention in terms of pharmaceutical risk assessment, are beyond the scope of the framework currently. Ultimately, the aim is to present a holistic prioritisation framework that can be applied to various understudied exposure scenarios and regions of the world, to serve as a guide to researchers and regulators whilst highlighting what work is needed to address knowledge gaps.

2.1 Previous prioritisation approaches

2.1.1 Geographical spread

In total, 76 prioritisation exercises were identified covering 24 countries (Figure 8). Multiple prioritisation exercises have been performed in the USA, France, Switzerland and Sweden. In each of these regions the prioritisation exercises utilised a variety of approaches i.e. risk, hazard or exposure and were both generic and country-specific (e.g. usage data). The most common approach for countries with a single prioritisation is risk-based (i.e. combined exposure and effects). Environmental pharmaceutical exposure will vary based on the prescribing practices, water treatment and hydrological conditions, which can affect the potential risk posed by particular compounds, i.e. a priority pharmaceutical in one country could be different from a neighbouring country, despite using similar prioritisation methodologies (Figure 8). While the majority of pharmaceutical prioritisations have been focused on Europe and the USA, and to a lesser extent Asia, the rest of the world is scarcely covered (Figure 8). These understudied areas could be harbouring hot spots of pharmaceutical exposure and risks, for example due to environmental inputs from pharmaceutical manufacturing and formulation sites with inadequate effluent treatment,[8] large urban populations (e.g. India, Brazil, Nigeria) or the fact that many of these regions have limited or no sewage treatment connectivity.

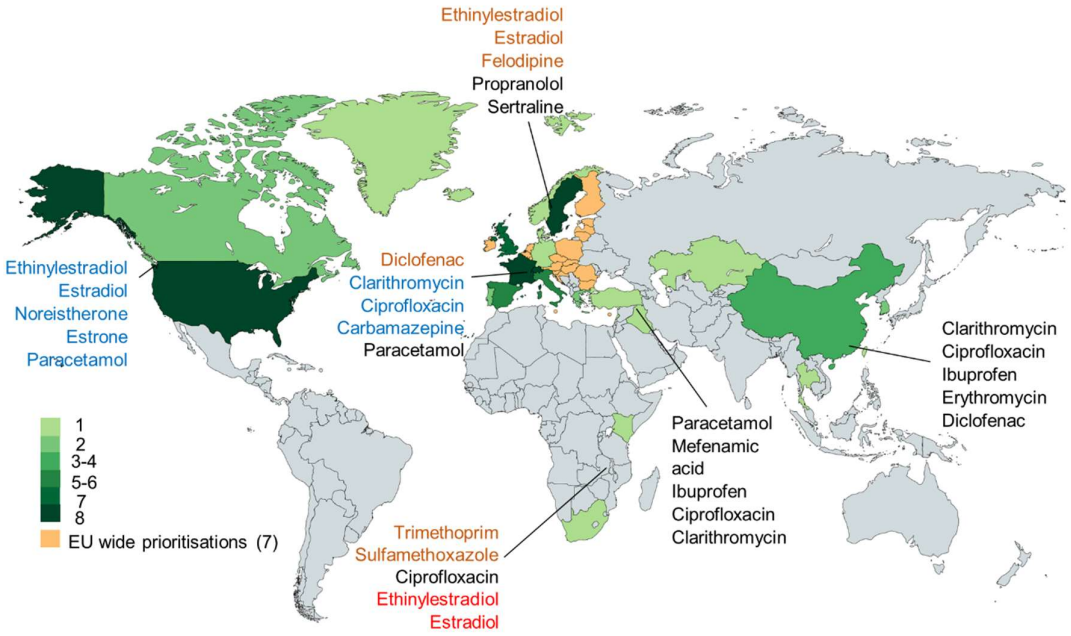


Figure 8. Areas of the world where priority pharmaceuticals have been identified by either risk-, hazard- or exposure-based approaches. Colouring corresponds to the number of prioritisations undertaken within that region (i.e. 1 to 8). The five pharmaceuticals most commonly identified as priority compounds in each region are presented. The font colour indicates the type of prioritisation that identified these compound: black: entirely or vast majority risk-based, red: entirely hazard based, green: entirely exposure based, blue: at least 50% risk-based while remainder are exposure-based and orange: at least 50% risk-based while remainder are hazard-based.

2.1.2 General approaches

The different prioritisation approaches used can be characterised into three general categories: exposure-based; hazard-based and risk-based. These approaches are discussed in more detail below.

2.1.2.1 Exposure-based methods

Exposure-based methods prioritise pharmaceuticals solely based on predictions or measurements of concentrations of compounds in the environment. In general, this type of approach is used to develop monitoring campaigns by selecting pharmaceuticals most likely to be present, thereby focusing costly monitoring efforts.[164,177,178] For example, the greater the pharmaceutical usage the greater the load that is expected to reach the environment and the higher the priority.[164] To achieve this, predicted environmental concentrations (PECs) are usually calculated using approaches such as that defined by the EMA.[164] Briefly, a pharmaceutical consumption estimate based on sales or prescription data is converted into population equivalents and multiplied by a patient

excretion percentage which is derived from peer-reviewed pharmacokinetic studies of the pharmaceutical. This pharmaceutical load is refined again for wastewater treatment (WWTP) removal, which typically is either predicted using quantitative structure activity relationships (QSARs), for example the STPWIN program (which predicts removal of a chemical in a typical activated sludge WWTP), part of the United States Environmental Protection Agency's (USEPA) EPISuite software package which aims estimates of environmental fate using physico-chemical properties[179] or based on measured values previously reported in the literature. Removal can also be assumed to be zero to reflect the worst case exposure scenario.[180] The predicted pharmaceutical load is diluted based on the average amount of wastewater entering a WWTP and again by the receiving waterbody. These dilution values are generally defined using defaults suggested in the EMA guidance,[112] a value of 200 L/per person·day is assumed to be generated, although Henze and Comeau[181] suggest 50 to 400 L/per person·day reflects the range in actual water usage practices throughout the world. The default environmental dilution factor is 10, which depending on the region or season could under- or over-estimate dilution.[182]

Exposure-based prioritisations can also be based on measured environmental concentrations (MECs). These approaches are limited to pharmaceuticals already detected in the environment, have analytical methods available or which are already of concern.[164,178,183] Exposure-based prioritisations do offer a means of overcoming limited ecotoxicological knowledge of pharmaceuticals by putting a greater focus on what is persistent and present in the environment.[178,184]

2.1.2.2 Hazard-based methods

A small number of pharmaceutical prioritisations identify priority compounds based on those that present the greatest hazard to the environment. Hazard-based approaches are unbiased by environmental occurrence and therefore can highlight low or concentrated local use pharmaceuticals that have the potential to be very harmful (e.g. synthetic hormones or anti-cancer drugs). These could be missed in some risk-based methods.[185] Hazard-based methods can also be useful for informing pharmaceutical substitution policies as part of a risk mitigation measure.[8] Generally, hazard-based methods identify and score pharmaceuticals based on their persistence, bioaccumulation, and toxicity (PBT)[141,186,187] or simply their persistence and

bioaccumulation (P&B).[188] These approaches are based entirely or are heavily reliant on QSARs to predict PBT characteristics because experimental PBT data is limited. These data are usually obtained from systems such as the USEPA's PBT Profiler or EPISuite software programs: BIOWIN, BCFBAF and ECOSAR.[179,189]

Hazard-based prioritisation can also involve 'read across' from readily available pharmacokinetic data.[190] This leveraging of parameters, derived during the drug development process, enables a consistent comparison across all pharmaceuticals instead of biasing the prioritisation towards data rich or poor compounds. For example: adsorption, distribution, metabolism and excretion (ADME) has been related to how a pharmaceutical will behave in an organism and therefore the likelihood of causing an adverse effect in the environment.[190,191] A simpler approach assumes that the plasma concentration of a pharmaceutical that causes a therapeutic response in a human, could potentially cause an effect in a fish at similar plasma concentrations.[118] The lower the environmental concentration required to reach this concentration in a fish, the higher the priority.[6,149] These simpler 'read across' hazard-based methods not based on ADME parameters rely on predicting internal organism concentrations based on the bioconcentration factor (BCF) of a compound. BCFs are not experimentally available for most pharmaceuticals, therefore a number of QSARs exist to predict the BCF based on the octanol-water partition coefficient (Kow).[192] While these approaches attempt to overcome the heavy dependence on predicted PBT data to select priority compounds, their validity as indicators of hazard has yet to be extensively assessed.

2.1.2.3 Risk-based methods

The majority of prioritisation methods and exercises reported in the literature are risk-based, where a measure of risk resulting from the ratio of exposure to effect is ranked by decreasing severity.[193] By putting effects or hazards in the context of environmental occurrence, resources are focused not just on detectable or hazardous pharmaceuticals, but those present at a concentration likely to result in an appreciable risk. Previous critical assessments of prioritisation methods have concluded that risk-based approaches are most appropriate for prioritising pharmaceuticals.[6,194]

The most common approach to risk-based prioritisation is to calculate a risk-quotient (RQ) based on the comparison of the PEC of a substance to its predicted no-

effect concentration (PNEC), PEC/PNEC. The PNEC can be extrapolated from the most sensitive ecotoxicological endpoint by adjustment with a safety factor, depending on the source and nature of the ecotoxicity data (i.e. measured or predicted, acute or chronic) to help ensure risks are not missed.[147] These safety factors are based on assessment factors which are applied to derive a PNEC for an ERA based on the amount of ecotoxicity data available outlined by regulatory bodies such as the EMA[112] and the US Food and Drug Administration.[195] Similarly to the PBT hazard-based methods, the ecotoxicity data used in many prioritisation studies is often derived from QSAR models such as ECOSAR,[145] again putting a heavy reliance on predictive rather than experimental methods.

Other methods have focused on assessment of the risks posed by secondary poisoning *via* exposure from food or water to predators or humans. In the case of human unintended exposure, the acceptable daily intake (ADI).[146,196,197] Other approaches 'read-across' from pharmacokinetic data to make ecotoxicological predictions.[118,198] Some risk-based studies have considered multiple endpoints including mammals and humans, but rely heavily on predicted data[147] or complex weighting schemes to deliver rankings.[145,147,173]

Whilst there are a number of published approaches which present methods to establish a PNEC, with little or no prior ecotoxicity data, there is much less diversity among the options for estimating exposure.[199] Risk-based prioritisations rely on measured environmental concentrations (MECs),[200,201] PECs,[153] or a mixture of the two.[6] Relying on monitoring data limits the number of compounds than can be considered to those that are already in the environment or a small fraction of the pharmaceuticals in use. Prioritisations dependent on this data may not be comprehensive enough to provide meaningful results to risk assessors.[194] Likely as a result of this and reduced cost, PECs are more commonly used in risk-based prioritisations, similarly to the method described for exposure based approaches. These PECs are derived for entire countries or regions,[147] which may not identify important pharmaceutical hot spots due to localised hydrologic conditions and populations. Geographical information systems (GIS) approaches can generate spatially refined PECs by combining population estimates, WWTP technology, discharge locations and dilution from the receiving water body, e.g. P*h*ATE (US) and

GREAT-ER (EU), to make spatially explicit exposure predictions for large-scale river basins.[146,154] Oldenkamp et al.[157] refined this concept further by creating a smaller-scale screening tool for Europe capable of deriving potential pharmaceutical environmental hotspots. The model generates emissions aggregated into 100 km x 100 km grids and also includes environmental fate considerations based on the SimpleBox model such as hydrolysis, biodegradation and photolysis and as well as partitioning to sediment and releases to soils.[202] Inclusion of all these factors to derive localised concentrations could be necessary to manage risk at the local/regional level,[182] as prioritisation results have been shown to be influenced by localised conditions as well as by the scale at which the exercise is undertaken (e.g. European Union, country, or locally).[203]

2.2 Prioritisation results and current limitations

2.2.1 What pharmaceutical classes are most commonly prioritised?

In total 332 pharmaceuticals have been identified as a priority in the 76 prioritisation exercises. There were 197 compounds identified only once, while 76 pharmaceuticals were selected as priority compounds by 3 or more exercises. In Table 1, the 76 pharmaceuticals prioritised 3 or more times are categorised by prioritisation approach, then by therapeutic class. A marked difference can be seen in the dominant therapeutic classes selected based on the type of prioritisation approach employed (Table 1). It has been previously noted that the prioritisation category an exercise belongs to affects the priority outcome.[6] The results presented here support this, for example hazardous low-dose pharmaceuticals or those with generally higher limits of detections, or both, such as hormones are completely overlooked by exposure based methods. This problem can also be encountered in risk-based prioritisations which rely on MECs, where limits of detection can be near or higher than the PNEC, especially in the case of ethinylestradiol.[204]

Table 1. Pharmaceuticals classified 3 or more times (n=76) sorted initially by prioritisation approach that identified each of the 76 compounds, then by therapeutic class within each approach.

Category	Therapeutic Class	Pharmaceutical
Risk	Antibiotics (16)	Amoxicillin, ampicillin, azithromycin, cephalexin, ciprofloxacin, clarithromycin, clindamycin, clotrimazole, erythromycin, levofloxacin, lincomycin, metronidazole, ofloxacin, oxytetracyline, sulfamethoxazole, trimethoprim
	Hormones (including synthetic) (10)	Equilenin, estradiol, estriol, estrone, ethinylestradiol, levonorgestrel, medoxyprogesterone, mestranol, noreistherone, testosterone
	Analgesic (8)	Acetylsalicylic acid, dextropropoxyphene, diclofenac, ibuprofen, mefenamic acid, naproxen, paracetamol, tramadol, ketoprofen
	Antidepressant (6)	Amitriptyline, citalopram, fluoxetine, norfluoxetine, paroxetine, sertraline
	Lipid-lowering agent (5)	Atorvastatin, bezafibrate, clofibrate, gemfibrozil, simvastatin
	Cardiovascular agent (4)	Felodipine, fenofibrate, losartan, valsartan
	Anti-cancer (4)	Cyclophosphosphamide, ifosfamine, tamoxifen
	β-blocker (3)	Atenolol, metoprolol, propranolol
	Antidiabetic (2)	Metformin, glyburide
	Contrast agent (2)	Iopamidol, iopromide
	Diuretic (2)	Furosemide, hydrochlorothiazide
	Anaesthetic (1)	Lidocaine
	Antiarrhythmic (1)	Amiodarone
	Antibacterial (1)	Triclosan
	Antifungal (1)	Ketoconazole
	Antihistamine (1)	Loratadine
	Anti-convulsant (1)	Carbamazepine
	Antineoplastic (1)	Mitotane
	Antiretroviral (1)	Ritonavir
	Benzodiazepine (1)	Oxazepam
	H2 Blocker (1)	Ranitidine

Table 1. (continued) Pharmaceuticals classified 3 or more times (n=76) sorted initially by prioritisation approach that identified each of the 76 compounds, then by therapeutic class within each approach.

Category	Therapeutic Class	Pharmaceutical
Risk	Proton pump inhibitor (1)	Omeprazole
Exposure	Antibiotic (10)	Amoxicillin, azithromycin, ciprofloxacin, clarithromycin, erythromycin, levofloxacin, lincomycin, ofloxacin, sulfamethoxazole, trimethoprim
	Analgesic (5)	Diclofenac, ibuprofen, naproxen, paracetamol, ketoprofen
	Lipid-lowering agent (4)	Atorvastatin, bezafibrate, gemfibrozil, simvastatin
	Anti-cancer (3)	Cyclophosphosphamide, ifosfamine, tamoxifen
	Cardiovascular agent (3)	Irbesartan, losartan, valsartan
	Diuretic (2)	Furosemide, hydrochlorothiazide
	Antidiabetic (2)	Metformin, glyburide
	Antidepressant (1)	Paroxetine
	β-blocker (1)	Atenolol
	Anti-convulsant (1)	Carbamazepine
	H2 Blocker (1)	Ranitidine
Hazard	Hormones (including synthetic) (9)	Equilenin, estradiol, estriol, estrone, ethinylestradiol, etonogestrel, levonorgestrel, medoxyprogesterone, testosterone
	Antidepressant (6)	Amitriptyline, citalopram fluoxetine, norfluoxetine, Paroxetine, Sertraline
	Lipid-lowering agent (5)	Atorvastatin, bezafibrate, clofibrate, gemfibrozil, Simvastatin
	Antibiotic (5)	Amoxicillin, ciprofloxacin, clotrimazole, erythromycin, sulfamethoxazole
	Cardiovascular agent (3)	Felodipine, fenofibrate, irbesartan
	Analgesic (2)	Diclofenac, ibuprofen
	Antihistamine (2)	Clemastine, ioratadine
	Contrast agent (2)	Iopamidol, iopromide
	Anticancer (1)	Tamoxifen
	Antineoplastic (1)	Mitotane

Table 1. (continued) Pharmaceuticals classified 3 or more times (n=76) sorted initially by prioritisation approach that identified each of the 76 compounds, then by therapeutic class within each approach.

Category	Pharmaceutical	Therapeutic Class
Hazard	β-blocker (1)	Propranolol

The top three therapeutic classes represented in risk-based approaches were antibiotics (16) followed by hormones (10) and analgesics (9). When exposure-based prioritisation systems were evaluated, antibiotics (10) comprised the largest therapeutic class followed by analgesics (5) and lipid-lowering agents (4). Hormones were not selected in any of the exposure-based exercises despite their prevalence in hazard- and risk-based prioritisation methods. This is expected because hormones are administered in very small doses, which, despite prevalent usage results in low environmental inputs in terms of mass and therefore are less likely to be detected in the environment than their higher mass use counterparts (e.g. antibiotics and analgesics). On the other hand, antibiotics and analgesics, are prevalent in exposure-based priority lists, similar to risk-based priorities, indicating that their associated risks may be related to high exposures.

Hazard-based methods identified hormones (9) followed by antidepressants (6), cardio-vascular agents (5) and antibiotics (5) as pharmaceutical classes of highest priority. Analgesics were much less of a priority according to these approaches despite their prevalence in risk-based and exposure-based prioritisation outcomes, again indicating that the perceived risks of analgesics are likely a result of their high exposure than potency. Antidepressants were the fourth most highly selected therapeutic class in risk-based studies and second in hazard-based studies, but again overlooked almost entirely by exposure-based approaches.

This analysis indicates that prioritisation relying solely on hazard- or exposure-based approaches could be misleading as key therapeutic classes of known environmental risk were respectively under-represented in comparison to risk-based methods, whether it be hormones or analgesics. The knowledge is currently not available to determine the accuracy of the risk-based approaches, they too are flawed in the fact that they are reliant on combinations of PECs and MECs for exposure and predicted or

empirical data for effects. Research effort should focus on increasing the certainty in exposure and effects approaches to be employed in a reliable prioritisation approach, for example validation of exposure models through targeted environmental monitoring or validity of ecotoxicity QSARs and read across theories for range of pharmaceuticals with differing physico-chemical characteristics and modes of action. Despite this, the balance between exposure and hazard is most likely the most effective approach for prioritisation.[6,194]

2.2.2 What drives priority compound selection?

The most common priority pharmaceuticals were diclofenac and ethinylestradiol (EE2), designated as priority compounds in 36% of reviewed priority lists or 26 and 25 times respectively (Figure 9). This is predictable considering the substantial focus on these two compounds in the literature, documented environmental effects, and their inclusion on the Water Framework Directive (WFD) watch list.[205] Risk-based methods dominate priority selection (Figure 9) due to the greater number of studies using this approach (78%), while hazard- and exposure-based make up 12% and 9% of prioritisations respectively. Of the entire list of identified pharmaceutical priorities (n=332) or roughly 20% of pharmaceuticals in UK use, only 17 pharmaceuticals were selected by all three method types. An equal number, 46, were selected by both hazard and risk-based methods as well as exposure and risk based methods. Risk-based methods identified 96% of the pharmaceuticals in Figure 9, while hazard- and exposure-based methods identified 49% and 45% respectively. Clemastine and etonogestrel were identified exclusively by hazard-based methods. Whilst identifying which pharmaceuticals are selected most often indicates that these could be the highest risk pharmaceuticals, the driving factor behind these selections is uncertain. It seems to be equally driven by hazard and exposure, which is a similar conclusion as to when results were grouped by therapeutic class.

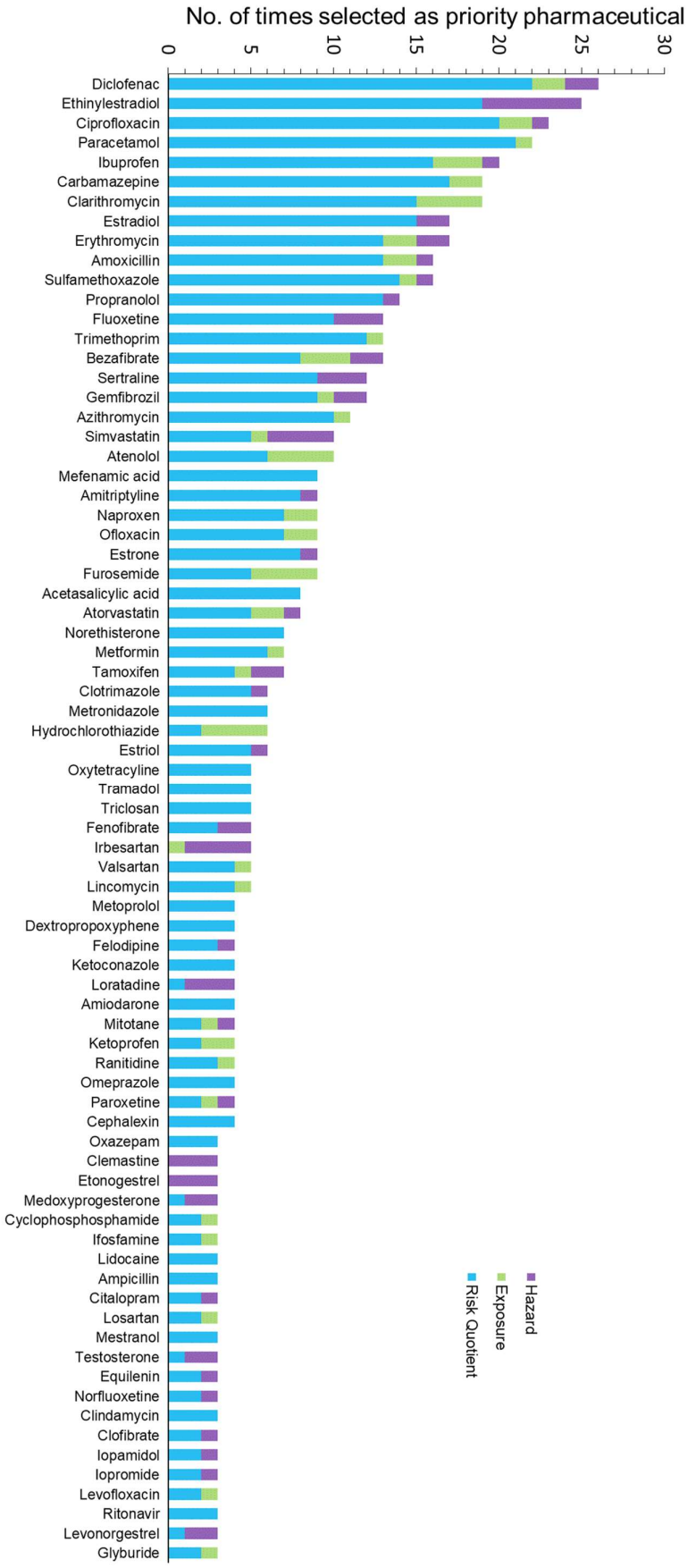


Figure 9. Pharmaceuticals identified as priorities three or more times (n=76) from on the 76 approaches reviewed in the literature.

2.2.3 Limitations with current methodologies

Prioritization methodologies have differing goals, whether it is deciding on which pharmaceuticals to conduct standard or non-standard effects testing, environmental monitoring, selecting legacy pharmaceuticals for a targeted ERA, or underpinning risk management options. Many different variations of the three main prioritization approaches have been undertaken, which suggests that consensus on a suitable method has yet to be reached. Based on the collated previous prioritization results, exposure- and hazard-based approaches likely overlook pharmaceuticals that may pose a potential risk. We therefore conclude that the use of risk-based approaches for prioritization is preferable. While several risk-based methods are available, these can employ very different approaches. Some used experimental monitoring data while others used exposure predictions. Some use toxicity 'read-across' approaches while others use QSARs developed for general chemicals. In Table 2 we therefore highlight the strengths, limitations, threats and opportunities of the different approaches that have been employed previously for risk-based prioritization of pharmaceuticals.

In terms of exposure (Table 2), it is evident that relying on monitoring data limits the number of compounds than can be considered to those already present in the environment or a small fraction of the pharmaceuticals in use. Prioritization based on MECs can be skewed by methodological limitations including: limited number of detections, analytical detection limits, compounds considered, or the risks being overstated by using maximal MECs.[206,207] Therefore prioritizations dependent on MECs may not be comprehensive enough to provide meaningful results to risk assessors.[194] Conversely, a simple PEC (based on the EMA (2006) approach) permits the inclusion of a larger range of pharmaceuticals in a prioritization, however PECs can be complicated by a lack of, or variability in, the parameters required to calculate them. Access to regionally defined usage data is important, but is sometimes difficult to obtain or does not capture all relevant usage pathways. For example, over-the-counter pharmaceutical usage could be missed by prescription-based usage, while sales data could overlook generic formulations. The public availability of prescription, hospital, and over-the-counter pharmaceutical sales data is uncommon and generally only available at the national or regional scale, which may not be representative of localized conditions. In most countries/regions, pharmaceutical consumption datasets are only available through

expensive market research. To overcome this, calculations of per capita pharmaceutical usage can be estimated similarly to an approach used recently in a prioritization in Kazakhstan where usage estimates were based on the number of products available for each active ingredient used in the country.[208] This accuracy of this method is unknown, as it has yet to be validated against monitoring data.

Another difficulty encountered is the diversity in patient metabolism estimations, compounded with the variability in WWTP removal efficiency along with the potential of conjugated metabolites (e.g. glucuronide or sulfato-conjugates) to reform the active parent compound due to cleavage of the conjugate during water treatment processes.[209] Environmental dilution exhibits substantial spatial and temporal variability, which is another significant source of uncertainty. For example, the impact of local dilution variability on simple PECs was investigated by Verlicchi et al.[7] and estimated to cause an uncertainty of up to 695%. Environmental fate is generally overlooked; this includes dissipation processes (e.g. biodegradation) and partitioning to sludge and sediment, both of which will affect exposure estimates and potentially prioritization rankings.[210,211] Furthermore, simple PECs are limited to single source systems (e.g. a single WWTP on a river with no upstream contribution), which is an uncommon scenario. Higher-tier spatial models provide an opportunity to move past these limited simple PECs by incorporating multiple pharmaceutical sources and upstream contributions along with in-stream fate to generate spatially relevant PECs. Inclusion of all these factors to derive localized concentrations could be necessary to manage risk at the local/regional level,[182] as prioritization results have been shown to be influenced by localized conditions as well as by the scale at which the exercise is undertaken (e.g. European Union, country, or locally).[203]

In terms of the PNEC (Table 2), experimental ecotoxicity data for pharmaceuticals is limited and to compensate for this, models are extensively utilized which may be inappropriate for all or for specific groups of pharmaceuticals and/or have yet to be validated for pharmaceuticals specifically. For example, ECOSAR was commonly cited as the source of modelled ecotoxicity data despite many pharmaceuticals falling outside its applicability domain.[145,147,176] Moreover, the relevance to pharmaceuticals is questionable as ECOSAR was originally validated using a small set of industrial chemicals with simple

molecular structures dissimilar to those of pharmaceuticals and mainly acting *via* a non-specific narcosis mode of action.[16,212] Furthermore, non-specific narcosis and apical acute toxicity endpoints (i.e. endpoints related to growth, reproduction and mortality) used for ERAs, are likely to occur at concentrations much higher than those arising through chronic exposure as they do not reflect low level continuous exposure. Chronic experimental data derived for ERAs, while more suitable for risk assessment than acute data, still focuses on apical endpoints and therefore could also be missing key effects related to the intended mode of action (MoA) of the pharmaceutical.[113,115] Therefore a prioritization approach which captures MoA-based concerns alongside the apical endpoints, should be incorporated into a prioritization framework aimed at informing risk assessment monitoring and testing strategies to ensure these potential risks are not overlooked.

The results of the reviewed prioritizations are useful; however there are several general scope limitations that potentially diminish confidence in the results. Certain prioritization approaches remove pharmaceuticals that lack relevant experimental data,[144,151,152] so a criticism of prioritization is that it will continually prioritize pharmaceuticals that have already been studied; the phenomenon is termed the 'Matthew Effect'.[148] Suitable models, which are validated and can be applied across the physico-chemically diverse range of pharmaceuticals are required to overcome this limitation. All prioritizations reviewed considered a single pharmaceutical source to the environment, WWTP discharge. Many pharmaceuticals are manufactured in countries such as India or China, where studies have shown manufacturing effluent can reach concentrations of 237 mg/L in production heavy regions, leading to localized pharmaceutical hot spots.[8–10] Moreover, while less manufacturing is done in Europe and North America, increased pharmaceutical loads in surface water due to manufacturing have been documented at concentrations 30-500 times higher than those in unaffected areas,[14] highligting the fact that this is a global consideration; not considering these sources could thus dramatically underestimate risks and therefore identification of priority compounds.[8]

The main focus of the reviewed prioritizations was on a single environmental compartment, surface water. The reason for limiting the scope to surface water could be

a current lack of validated exposure models suitable for predicting concentrations in other relevant environmental compartments such as sediment, biosolids, soils and porewater. Only three prioritizations included or focused on the sediment compartment.[213–215] Another exposure pathway overlooked in the vast majority of the reviewed approaches is the application of biosolids[147] and reclaimed irrigation waters[216] to agricultural fields. Agricultural soil exposure is derived from the sludge concentrations of pharmaceuticals in WWTPs, which is the result of sorption characterized by the sludge/water partition coefficient (K_d).[53] Most sorption models are driven by hydrophobicity (i.e. logKow > 4), however many pharmaceuticals are ionizable at environmentally relevant pH values and it has been shown that sorption is also affected by ionic state.[217] Therefore models that estimate sorption and do not consider the ionic state of a compound may be unreliable; an example is the commonly used STPWIN model,[179] as estimates of pharmaceuticals in both WWTP sludge and the aquatic environment could be over/underestimated.[16,145] Models that do include ionic state considerations (e.g. SimpleTreat 4.0) should be preferred.

The lack of ecotoxicity data or validated ecotoxicity models available, is another factor which may have contributed to the scope of reviewed prioritizations encapsulating only the water column. There is evidence that pharmaceuticals have been detected in invertebrate organisms in the benthos and soil;[138,218–220] therefore, risks to these compartments should be considered. Additionally, the potential risks to predators and humans have, with a few exceptions,[147,221] been overlooked, despite recent findings to suggest that these risks may be present.[222–226] Therefore, several opportunities to improve prioritization exist, such as including understudied environmental pathways and compartments, diet and food chain assessments for predators, pharmaceutical sources beyond the WWTP, and the inclusion of MoA-based concerns alongside apical ecotoxicological effects data. In the following section, we therefore bring together the strengths of existing methods and attempt to overcome current limitations in scope to develop an optimum prioritization framework which could be used in the future for pharmaceutical prioritization.

Table 2. Strengths, limitations, opportunities and weaknesses of major parameters in current risk-based prioritisation approaches.

	Parameter	Strengths	Limitations	Opportunities	Threats
Exposure	Simple PEC	•Cost effective •Tailored to be local or regional •Applied to all APIs for which consumption data available •Simple algorithms and EMA defaults available. •Provide basis for local/regional monitoring campaigns	•No over-the-counter usage •No hospital usage •Prescription data only available for select regions •Single environmental pathway (WWTP) •Not representative of local wastewater usage/environmental dilution. •Variability in patient metabolism	•Alternative methods to derive usage •Incorporate other major sources (e.g. manufacturing effluent) •Approach that can assess the 87% of APIs in use that without ERA data •Development/validation of exposure models for understudied compartments such as soil, sediment and porewater.	•First tier may unknowingly eliminate compounds (e.g. assessment trigger values) •Unsuitable exposure models (e.g. ionisable)
	Higher-tier spatial PEC	•Multiple pharmaceutical sources •Incorporate mixing with pharmaceuticals transported from upstream •Identify local concentration hot spots •Incorporate hydrological characteristics/long term flow trends	•Only developed for specific regions/watersheds •Access limited •Similar pharmaceutical consumption, WWTP removal and metabolisms issues to simple PEC	•Development of open-access platforms to make predictions •Open-access tools to develop spatial models for currently unstudied areas •Incorporate sludge and soil sorption models which can account for ionisable compounds •Expand past surface water to include sediment and vulnerable soils (e.g. agriculture) •Probabilistic risk assessment	
	MEC	•Confidence in results •All environmental pathways considered (when representative sampling used) •Localised	•Limited number of APIs/compounds •Costly •Maximal MECs •Limits of detection •Unrepresentative sampling •Limited to pharmaceuticals already detected/already of concern •Data quality	•Lower cost monitoring approaches •Improved limits of detection •Use to confirm risky predictions	• Poorly representative sampling

Table 2. (continued) Strengths, limitations, opportunities and weaknesses of major parameters in current risk-based prioritisation approaches.

	Parameter	Strengths	Limitations	Opportunities	Threats
PNEC	Experimental (chronic/acute)	• Regulatory relevance • Confidence in results	• Limited availability • Limited relevance of acute data • Chronic ECOSAR not yet validated for APIs and likely not robust • Considers only apical endpoints (mortality, reproduction, growth)	• Create comprehensive database of industry held data to prevent 'Matthew Effect'	• Missing specific MoA concerns • Experimentally filling data gaps defeats purpose of desk-based prioritization.
	ECOSAR	• Rapid, can be applied to all APIs.	• Heavy reliance on predicted data, 'Matthew Effect' • Large arbitrary safety factors	• Improve chronic QSAR	• 'Matthew effect' • Missing specific MoA concerns
	FPM	• Readily available pharmacokinetic parameters • QSAR to predict BCF based on octanol-water partition coefficient (Kow) • Can be applied to vast majority of APIs • Covers mode of action (MoA) concerns	• BCFs not experimentally available for most APIs • Applicability of BCF QSAR • Only relevant for fish • Experimental validation limited, but growing	• Expand to invertebrates (water column, benthos and soil) • Reduce animal testing by prioritising legacy APIs and focusing efforts towards those most likely to have an adverse impact • Use internal concentrations to develop predator exposure models	• Unsuitable uptake models (e.g. ionizables) • Miss specific MoA concerns pertaining to an API's side effect/off label use
	ADI	• Can calculate for all APIs which have mammalian toxicity studies	• Based on arbitrary uncertainty factors to ensure conservative risk assessment • Focuses on water exposure (surface and drinking)	• Include a diet component (fish, meat, water, crops)	• Diet (fish, meat, water, crops)

2.3 Proposed prioritisation framework

The proposed prioritisation methodology uses a tiered, risk-based approach. The method is holistic in that it considers: all the relevant environmental compartments; assesses specific risks to plants, invertebrates, vertebrates and mammals (human and non-human) as well as incorporating food chain interactions; and endpoints related to the MoA or side effects in addition to standard acute and chronic ecotoxicological endpoints of a pharmaceutical. It is based on models capable of leveraging existing data to overcome bias towards data-rich or poor pharmaceuticals when generating risk ranks. The framework also accounts for differences in pathways of exposure for different regions as well as differences in the drivers of exposure (e.g. differences in water chemistry) which can affect pharmaceutical uptake and equilibrium partitioning. The framework will be underpinned by thorough model validation and defined applicability domains to give greater confidence in results whilst by highlighting current weaknesses and knowledge gaps. In the future, the overall approach needs to be validated against both laboratory and field data to demonstrate it as a reliable tool in pharmaceutical prioritisation.

2.3.1 Navigating the Framework

The starting point and progression through the framework is dependent on the question being asked which generally falls into one of four main categories: i) Identifying highest risk pharmaceuticals from the approximately 1900 in use to determine which are in greatest need of effects testing or formal ERAs, ii) developing a catchment-scale or national monitoring campaign to determine the status of predicted risks in reality, iii) identifying pharmaceuticals for effects research that based on predicted effects data, pose a risk at regional or local scales, or iv) identifying risk mitigation measures which aim to minimise the mass of pharmaceutical reaching the environment, for example risk-benefit analysis, WWTP upgrades, reductions of incorrect disposal, increased pharmaceutical bioavailability, or incorporation into legislation such as the WFD. The acquisition of relevant pharmaceutical consumption data is critical to progressing through the exposure component of the framework regardless of the research question (Figure 10). Publicly available prescription, hospital usage, over-the-counter pharmaceutical sales data or all three is uncommon and generally only available at the

country or regional scale which may not be representative of localised conditions. In most countries/regions pharmaceutical consumption datasets are only available through prohibitively expensive market research. To overcome this, calculations of per capita pharmaceutical usage can be estimated similarly to an approach used recently in a prioritisation in Kazakhstan where usage estimates were based on the number of products available for each active ingredient used in the country.[208] This method is limited, as it has yet to be validated against monitoring data. Approaches using the fraction of market penetration (Fpen) and defined daily dose should be avoided as they lead to inaccurate PECs.[169] Despite the shortcomings of PECs, they should still be favoured when attempting a risk-based prioritisation over a MEC to ensure a wider range of pharmaceuticals are considered and potent pharmaceuticals with high limits or detection are not overlooked.

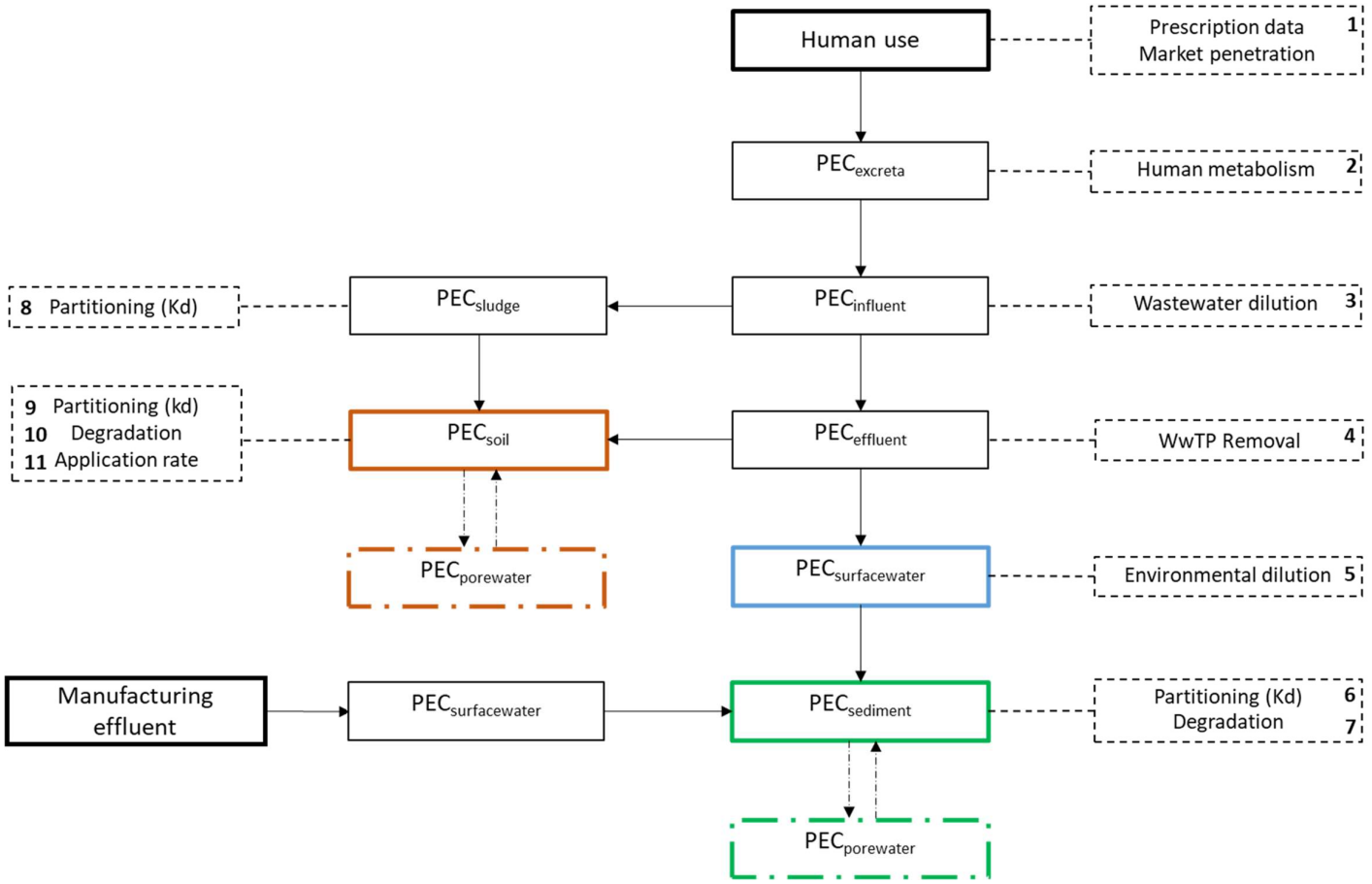
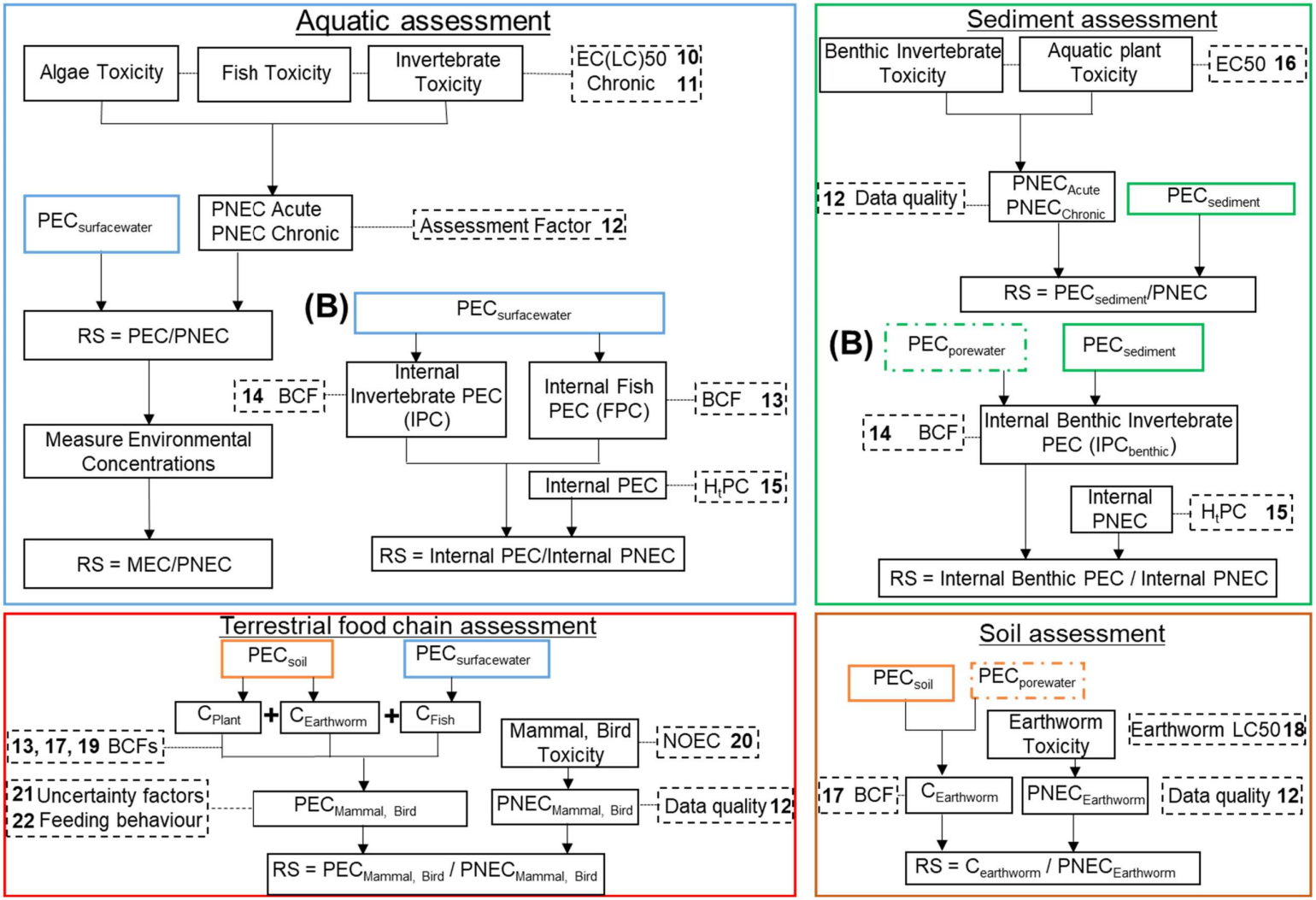


Figure 10. Breakdown of the relationship between exposure scenarios and the generalised parameters required to calculate the emissions.

Effluents from pharmaceutical manufacturing facilities will need to be treated differently as many compounds are produced through batch production causing transient pharmaceutical hot spots in contrast to low level continuous therapeutic use. Manufacturing PECs require industry knowledge of manufacturing schedules, batch production and a mass balance of pharmaceutical recovery and losses[221] paired with localised effluent and environmental dilution. In the case of hospitals treating and releasing their own effluent, these sources also need to be accounted for. Prioritisations have also been developed to include PECs for major active metabolites.[144,147,227] Evidence has shown that the majority of metabolites are less potent than the parent compound,[228] however exceptions do exist.[76,199] In addition, glucoronide or sulfated metabolites may convert back to the parent drug during water treatment,[61] therefore the fraction of these metabolites excreted should be added to the parent excretion estimate. For a conservative approach, when the excretion or potency of an active metabolite is unknown, a total residue approach (e.g. no metabolism), similar to that used for environmental risk assessment, can be used to account for potentially risky metabolites with limited data which can then be assessed in greater detail at later stage.

A prioritisation should begin by considering as many pharmaceuticals as possible, allowing investigation of the large proportion of compounds in the 'unknown' region (Figure 7). It is suggested to begin with the aquatic compartment (Figure 11), then assess the sediment compartment as $PEC_{surfacewater}$ is required to calculate $PEC_{sediment}$ (Figure 10). Both the A (apical ecotoxicity endpoints) and B (MoA-based concerns) methods should be used for both aquatic and sediment compartments. For the terrestrial assessment (Figure 11), the mass of pharmaceutical sorbed to sludge in the WWTP (PEC_{sludge}) along with $PEC_{effluent}$ to represent irrigation with reclaimed wastewater both contribute to the PEC_{soil} estimate (Figure 10). The food chain assessments, (terrestrial or aquatic) should be triggered for all pharmaceuticals assessed in the relevant environmental compartment (aquatic or soil). This is due to the lack of experimental biomagnification and bioaccumulation factors and therefore understanding of how pharmaceuticals may accumulate through the food chain. The human assessment can also be done for each pharmaceutical that undergoes a food chain assessment (dietary exposure) (Figure 11). The $PEC_{surfacewater}$ is used in this assessment to reflect the worst case scenario.


Aquatic assessment
Algae Toxicity
Fish Toxicity
Invertebrate Toxicity
EC(LC)50 10
Chronic 11
PEC surfacewater
PNEC Acute
PNEC Chronic
Assessment Factor 12
RS = PEC/PNEC
Measure Environmental Concentrations
RS = MEC/PNEC
(B)
PEC surfacewater
14 BCF
Internal Invertebrate PEC (IPC)
Internal Fish PEC (FPC)
BCF 13
Internal PEC
HtPC 15
RS = Internal PEC/Internal PNEC
Sediment assessment
Benthic Invertebrate Toxicity
Aquatic plant Toxicity
EC50 16
12 Data quality
PNEC Acute
PNEC Chronic
PEC sediment
RS = PEC sediment/PNEC
(B)
PEC porewater
PEC sediment
14 BCF
Internal Benthic Invertebrate PEC (IPC benthic)
Internal PNEC
HtPC 15
RS = Internal Benthic PEC / Internal PNEC
Terrestrial food chain assessment
PEC soil
PEC surfacewater
C Plant + C Earthworm + C Fish
13, 17, 19 BCFs
Mammal, Bird Toxicity
NOEC 20
21 Uncertainty factors
22 Feeding behaviour
PEC Mammal, Bird
PNEC Mammal, Bird
Data quality 12
RS = PEC Mammal, Bird / PNEC Mammal, Bird
Soil assessment
PEC soil
PEC porewater
Earthworm Toxicity
Earthworm LC50 18
17 BCF
C Earthworm
PNEC Earthworm
Data quality 12
RS = C earthworm / PNEC Earthworm

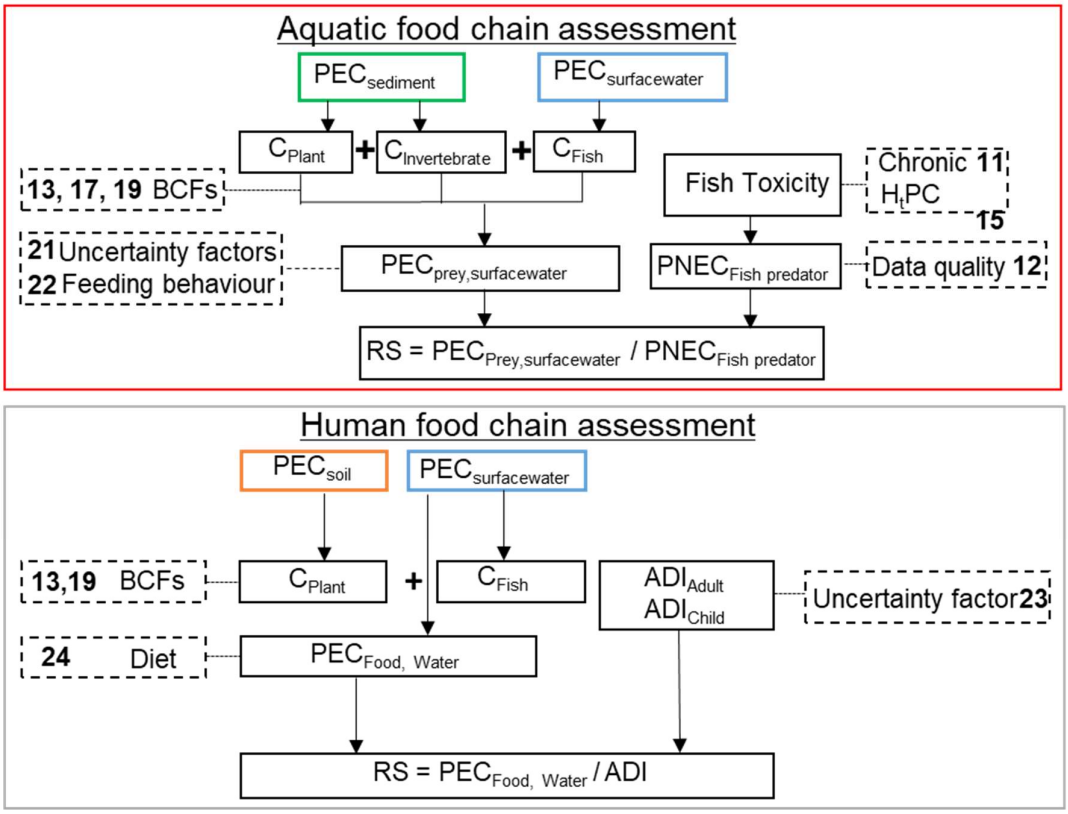


Figure 11. High level schematics demonstrating the prioritisation of risk posed to three trophic levels in aquatic, sediment and soil systems. Secondary approaches to prioritising risks to aquatic/sediment systems based on pharmaceutical uptake and internal concentrations compared to human therapeutic concentrations are also shown (B). Predatory terrestrial and aquatic wildlife food chain risk prioritisation which considers exposure to multiple prey/environmental sources. Risks to humans considering both water intake as well as other dietary sources such as plants. Numbers refer to additional information provided in Appendix 3 and 4.

These sequential assessments lead to risk score (PEC/PNEC) lists for each considered environmental compartment as well as two risk score lists in cases where an A and B scenario are presented, Figure 11. A risk score of 1 or greater indicates that the PEC is equivalent to or higher than the PNEC, thus a risk may be present. As part of a conservative approach, any compound with a risk score (RS) using any PNEC (A or B, Figure 11) greater than 0.1 should be ranked as a priority, with the largest risk score ranking as highest priority. The parameters needed to apply each of the models presented in each of the assessments scenarios is listed in the dotted box with a number (Figures 10 and 11). The availability of these parameters and the reliability of the models/experimental data that are used to derive them is crucial to the success of the

prioritisation and a frequently cited limitation.[143,147] The current knowledge surrounding these parameters and models is detailed in Appendix 3 & 4.

2.3.2 Why a new prioritisation framework is needed

It has been demonstrated that 76 human pharmaceutical prioritisations have been undertaken throughout the world (Figure 8), however the multitude of suggested approaches indicates that credibility for any particular approach is low, especially when prioritisations are repeated in the same region. Different sets of priority compounds are expected based on the region and scale of assessment (Figure 8), due to differences in populations, prescribing practices and hydrology. Therefore deriving some level of standardisation, such as our framework, could be important for harmonisation of research and regulatory goals across the world as prioritisation results obtained using the same methodology will be comparable. Moreover our approach goes beyond the aquatic compartment to prioritise risks in sediment, soil and exposure *via* the food chain to provide a compressive assessment of all relevant environmental compartments. These considerations are especially important when put in a global context. For example, biosolids containing pharmaceuticals have been demonstrated as a significant pathway by which they can enter and accumulate, in the terrestrial environment.[22] In addition, pharmaceuticals can persist in soils and build up to detectable concentrations after repeated applications of reclaimed wastewater.[25,229] The use of reclaimed wastewater both treated and untreated and biosolids in agriculture is a widely adopted practice in countries suffering from water shortages such as Mexico, Israel, Australia and Southern Europe.[216,230–232] Furthermore, crops are grown on agricultural soils and cattle producing meat and milk are grazing on grasslands that have been amended with sludge based biosolids and/or reclaimed wastewater, which poses a potential risk of indirect human exposure *via* these products,[25,233,234] which has been demonstrated.[222–225]

The expansion beyond exposure to the aquatic compartments is important, however this needs to be paired with intelligent approaches to effect assessment capable of catching pharmaceutical potency, even when exposure is low and parameters are limited to consumption and leveraging physicochemical properties (e.g. logkow and pKa) across the diverse range of pharmaceuticals in use. This is achieved through a combination of apical and non-apical endpoints in aquatic, benthic and terrestrial species

belonging to multiple trophic levels. The prioritisation of risks from food chain exposures in predators as well as humans has only been documented in a single prioritisation.[147] The framework builds upon this and reflects realistic dietary habits consisting of multiple prey sources and/or vegetation.

While many aspects of the framework are similar to previous prioritisation approaches, a major departure concerns the consideration of apical ecotoxicological endpoints. Due to the larger assessment factor, acute PNECs based on experimental data have been demonstrated as consistently lower than chronic PNECs (except when a chronic mode of action (MoA) of concern is present) and therefore protective,[235] the application of this approach will only continue to replicate prioritisation of acute endpoints, while the more environmentally relevant chronic concerns could go unaddressed. Acute data in previous prioritisations is almost entirely predicted with unsuitable approaches (e.g. ECOSAR) and has little relevance to real world exposures.[236,237] Instead, development of new chronic QSARs may be more useful for identifying pharmaceuticals without a concerning MoA, while focused effects testing based on real exposure concerns can incorporate non-apical MoA endpoints to ensure these compounds are not overlooked.

The non-apical endpoints involve predicting internal concentrations and relating them to therapeutic effect concentrations in humans, for example, using the fish plasma model (FPM).[118] Endocrine disruption which could be related to human side effects/off label uses were recently observed in fish at concentrations lower than the therapeutic level.[238] Also, the decline of vultures in Pakistan (*Gyps bengalensis*) was linked to the organism exhibiting a known side effect of diclofenac (renal failure).[107] This suggests the therapeutic concentration alone may not be protective enough to encompass the concentrations at which side effects can occur, therefore application of a safety factor originally suggested to encompass cross-species sensitivity could also be appropriate to account for potential side effects.[118,239]

Teleost fish possess approximately 80% of drug targets through ortholog conservation, while certain invertebrate species conserved 50-60% of drug targets.[117] Exposure of *Daphnia magna* to pharmaceuticals with highly conserved drug targets resulted in predictable molecular effects, while exposure to pharmaceuticals with non-

conserved drug targets did not illicit an effect, implying that read-across approaches could also be important for invertebrates.[119] Therefore, expanding past fish to also predict internal invertebrate concentrations and leveraging human therapeutic concentrations could be useful for certain pharmaceuticals. The FPM still requires further experimental work but is a promising tool,[240,241] while the development of an invertebrate plasma model which considers invertebrate specific uptake (BCFs) could be an important step to ensuring potential risks are not missed.

In summary, our proposed approach builds upon many of the ideas presented in previous prioritisation exercises and brings them together in a coherent and comprehensive framework. It is recognised that simplicity is advantageous, but difficulties arise when using unsuitable QSARs for one size fits all fate and effects estimation. Extensive use of these QSARs leads to similar compounds being identified as priority pharmaceuticals again and again (Figure 9), which is not beneficial when attempting to identify knowledge gaps. Our framework tackles these biases by promoting more intelligent assessment approaches and clearly identifying where research is needed to implement this optimum framework. The following section describes the data availability and state of the models required to implement our framework.

2.4 Data availability and quality

Each of the experimental parameters required to parameterise the exposure and effect models mentioned in Figures 10 and 11 have been evaluated for availability in Tables 3 and 4. For brevity, we present a high-level overview of each parameter, while specifics such as OECD tests, default values and QSARs can be found in Appendix 3 & 4. The intention of these tables is to detail the relative availability of the data required for our prioritisation and highlight where research is needed most to achieve parameterisation of the optimum framework. In addition to the optimum framework, further development and validation of the tools and models in Tables 3 and 4 could also be useful for incorporating environmental considerations earlier in the pharmaceutical development process itself.

The experimental sources in Table 3 relate to environmental measurements or data from peer-reviewed literature. We recommend tailoring the parameters listed as defaults. For example, wastewater generation practices can vary throughout the world by 50-400 L/per person·day,[181] while the environmental dilution of 10 may not be protective enough in some regions or overly conservative in others.[7,182,242]

Table 3. Generalised overview of the availability of parameters required to estimate environmental pharmaceutical exposure in multiple compartment.

Model	Parameter		Experimental source	Model	Default (Table S1)
Human use	(1) API consumption	mg/yr	Table S1	Table S1	
$PEC_{excreta}$	(2) Human metabolism	$F_{excreta}$			
$PEC_{influent}$	(3) Wastewater dilution	L/person·day			
$PEC_{effluent}$	(4) WWTP Removal	% removal			
$PEC_{surfacewater}$	(5) Environmental Dilution				
$PEC_{sediment}$	(6) Equilibrium partitioning	Sediment (Kd)			
$PEC_{sediment}$	(7) Degradation	DT50 (sediment)			
PEC_{soil}	(9) Equilibrium partitioning	Soil (Kd)			
PEC_{soil}	(10) Degradation	DT50 (soil)			
PEC_{soil}	(11) Application rate	kg/ha·year			

Available or applicable to all pharmaceuticals.
Available for majority of pharmaceuticals, but applicability domains exist.
Available or applicable to few pharmaceuticals.
Model to predict parameter does not exist.

It is immediately clear from the status of parameters in Table 4, that models are lacking that can adequately predict the behaviour of ionisable compounds. A red designation in Table 3 & 4 indicates a research gap that needs to be filled in order to effectively implement the prioritisation. Compiling currently held industry pharmaceutical fate and effect data in conjunction with data from the academic and regulatory sector into a publicly available database would be advantageous. The database can be used as a data source or to reduce the 'Mathew Effect' where previously studied compounds are subject to similar tests repeatedly. The database would contain high quality data to use and to validate models thus reducing the reliance on unsuitable

QSARs. Prioritisation is an exercise in efficiency, a database such as this will vastly improve the efficiency of the process.

Table 4. Availability of parameters needed to predict adverse pharmaceutical effects in the models contained within the prioritisation framework.

Model	Description	Experimental source	Model	Default
Algae/fish/daphnia toxicity	(10) $EC(LC)_{50}$			
	(11) Chronic			
PNEC	(12) Assessment factor			
Internal PEC (fish, invertebrate)	(13) BCF_{fish}			
	(14) $BCF_{invertebrate}$			
Internal PNEC	(15) Therapeutic plasma concentration (H_tPC)			
Benthic invertebrate toxicity	(16) EC_{50}			
$C_{Earthworm}$	(17) $BCF_{earthworm}$			
Earthworm toxicity	(18) LC_{50}			
C_{Plant}	(19) BCF_{plant}			
Toxicity mammal, bird	(20) NOEC mammal			
	(20) NOEC bird			
$PEC_{Diet(wildlife)}$	(21) Uncertainty factor			
	(22) Feeding behaviour			
Acceptable daily intake (ADI)	(23) Uncertainty factor			
$PEC_{Diet(human)}$	(24) Diet			

Full dataset for pharmaceuticals released to market post 2006.
Available or applicable to majority pharmaceuticals.
Available but not applicable to ionisables or other applicability domain exist.
Available or applicable to few pharmaceuticals.
Model to predict parameter does not exist.

2.4.1 Status of optimum scheme

We evaluated the overall reliability of the models required to progress through the proposed prioritisation framework (Figures 10 and 11) based on the status of parameters from the previous section and experimental validation from the literature. Detailed results can be found in Appendix 3 & 4, while a summary of the results is presented in Table 5. The largest knowledge gaps and therefore greatest research needs are easily identifiable by the red colours and pertain largely to terrestrial species and invertebrates (both aquatic and terrestrial). This summary can be used as a guide to direct further development of predictive models that are a) suitable and validated for pharmaceuticals and b) have applicability domains encompassing the majority of pharmaceuticals. As these knowledge gaps are filled, the optimum prioritisation scheme could emerge and be suitable for assessing risks to relevant environmental compartments globally so that a greater focus can be put on risk mitigation where is it most needed.

Table 5. Summary of the current reliability of models included in the optimum prioritisation framework based on the suitability of relevant parameter estimation and reported validation in the literature.

Endpoint	Exposure		Effects	
	Model	Model reliability	Model	Model reliability
Aquatic plant	$PEC_{surfacewater}$		Acute	
Aquatic plant			Chronic	
Aquatic invertebrate	$PEC_{surfacewater}$		Acute	
			Chronic	
Aquatic invertebrate	Invertebrate plasma concentration (IPC)		Read across	
Aquatic wildlife (fish)	PEC_{sw}		Acute	
			Chronic	
Aquatic wildlife (fish)	Fish plasma concentration (FPC)		Read across	
Terrestrial plant	PEC_{soil}		Acute	
			Chronic	
Terrestrial invertebrate	PEC_{soil}		Acute	
Terrestrial invertebrate	$PEC_{sediment}$		Chronic	
Terrestrial invertebrate	Invertebrate plasma concentration (IPC)		Read across	
Terrestrial wildlife (birds)	PEC_{diet}		Acute	
			Chronic	
Terrestrial wildlife (birds)	PEC_{bird}		Chronic	
Human	PEC_{diet}		ADI	
Human	$PEC_{surfacewater}$			

Model exists and is validated for pharmaceuticals.
Model exists and used for pharmaceuticals, but lacks validation.
Model exists, but not designed for pharmaceuticals.
No model has been developed yet.

2.5 Conclusion

The majority of pharmaceuticals do not have a formal environment risk assessment. There is therefore a real need for prioritisation methodologies to identify those molecules that have not been tested which are of a potential concern in the environment as well as potentially identifying where risk mitigation measures for which molecule risk mitigation measure may be most appropriate. Many prioritisation approaches have been proposed in the literature. The majority of these use a risk-based approach whereas some approaches simply combine limited hazard- and effect-based methods. The methods have tended to focus on the aquatic exposure scenario and on a few regions of the world. In this chapter, the most promising from several approaches have been brought together

and presented as part of a holistic approach for prioritising the risks posed by pharmaceuticals to multiple environmental compartments. There is still much work to be done before this approach can be applied, including both model development and validation. Initial investigations should focus on the validity of existing and commonly used exposure and effect models. This could include determining the impact using these models have on prioritisation outcomes. An assessment of the influence different effect models have on pharmaceutical prioritisation has been undertaken previously.[6] Therefore further investigations should focus on the impact of commonly used simple exposure models to prioritise pharmaceuticals in the environment.

In the next Chapters, simple exposure models were therefore evaluated against monitoring data from the York river system to explore whether prioritisation based on measured and predicted data resulted in similar outcomes. The accuracy of these simple exposure predictions in comparison to monitoring data was also evaluated.

CHAPTER 3

Evaluation of simple exposure predictions used for the prioritisation of pharmaceuticals environment

3.0 Introduction

Several prioritisation approaches have been proposed for pharmaceuticals (Chapter 2). The optimum prioritisation approach was determined to be a risk-based. All risk-based approaches require an assessment of the concentration of pharmaceuticals in the environment. Simple algorithms are commonly used to predict environmental concentrations (PECs) of pharmaceuticals for both prioritisation and risk assessment, however the suitability of this simple PEC for this purpose is not known. These PECs, based on the EMA approach[112] are generally used with default parameters for wastewater usage and environmental dilution, regardless of the appropriateness to the situation being assessed.[6,144,153] The use of site-specific data when performing these calculations simple PECs for prioritisation is a rarity.[155]

Several studies have undertaken a comparison of PECs to measured environmental concentrations (MECs) to determine how well these predictions preform.[7,150,160–166] The conclusions from these studies has been divided, with several suggesting that PECs are sufficiently accurate, while others state that PECs are too great an over- and underestimate to be useful.[150,160,166] Usually the assessment of PEC relevancy is reliant on determining a PEC/MEC ratio. The acceptability of the PEC depends on how close this ratio is to 1,[166] however the acceptable range varies between studies.[165] This poses a problem when trying to assess the relevance of results across studies because the derivation of these ranges is subjective and dependent on the motive of the study (e.g. prioritisation or risk assessment). Furthermore, it has not been investigated whether prioritisation results obtained using PECs or MECs differ, as the difference between the two may not be large enough to affect the selection of priority compounds.

In the present study, simple PEC models for use in prioritisation were evaluated by comparing modelled and monitoring data for a comprehensive set of 95 pharmaceuticals derived from a wide range of therapeutic classes with different modes of action, an extensive range of chemical and physical properties, high and low usage, as well as select pharmaceuticals not thought to be prescribed in the UK. The city of York (population of 227 000) was chosen as the study system due to the availability of local prescription data, a well-defined and accessible hydrological system (i.e. two rivers that pass through the

city), and numerous access points to the rivers *via* bridges, which enables a detailed characterisation of pharmaceutical concentrations throughout the city. The prioritisation approach used to compare PECs and MECs was based on the Fish Plasma Model (FPM).[118] Studies of this nature that assess a large range of compounds (95), are an important check on ensuring that priority compounds identified, using common modelling approaches, are comparable to those using environmental data representative of key seasonal, locational, water treatment and hydrological differences.

3.1 Methods

3.1.1 Study site and sampling

Samples were collected and analysed river water samples from eight sites along the Rivers Ouse and Foss in the city of York in the UK where flow conditions were below the long term mean flow and near the Q50 (i.e. where flow is equal or exceeded 50% of the time) in February 2015 (Figure 12).[243] Site locations were chosen based on ease of access and their position in relation to WWTP outfalls discharging into these river systems (Appendix 5). Two WWTPs serve the city of York that impact the sampling network. There is a third WWTP; however, it is downstream of the city and sampling points (not included in Figure 12). The first of these two WWTPs (WWTP A) serves a population of 27 900, employs conventional activated sludge (CAS) as secondary treatment, nitrifying filters as a tertiary treatment option and a hydraulic residence time of approximately eight hours, and the second (WWTP B) serves a population of 18 600 and uses trickling filter technology as secondary treatment paired with biological aerated filtration for tertiary treatment and a hydraulic residence time of approximately 20 hours.

At each site, three 1 L samples were collected at points distributed equidistant across the width of the river channel and homogenised into a single 1 L composite sample. Three 10 mL aliquots were taken from the composite sample and filtered through 0.7 µm glass microfiber (GF/F) disposable filters (Whatman Inc.). To ensure that filtration and field handling of samples did not result in cross-contamination, high-performance liquid chromatography (HPLC)-grade water was also filtered and prepared in the field identically to river samples (i.e. a field blank) three times during the sampling. Samples were frozen directly in the field using dry ice and transported in dry ice to the U.S.

Geological Survey (USGS) National Water Quality Laboratory in Denver Colorado, USA. The frozen samples arrived four days later and were immediately thawed and analysed.

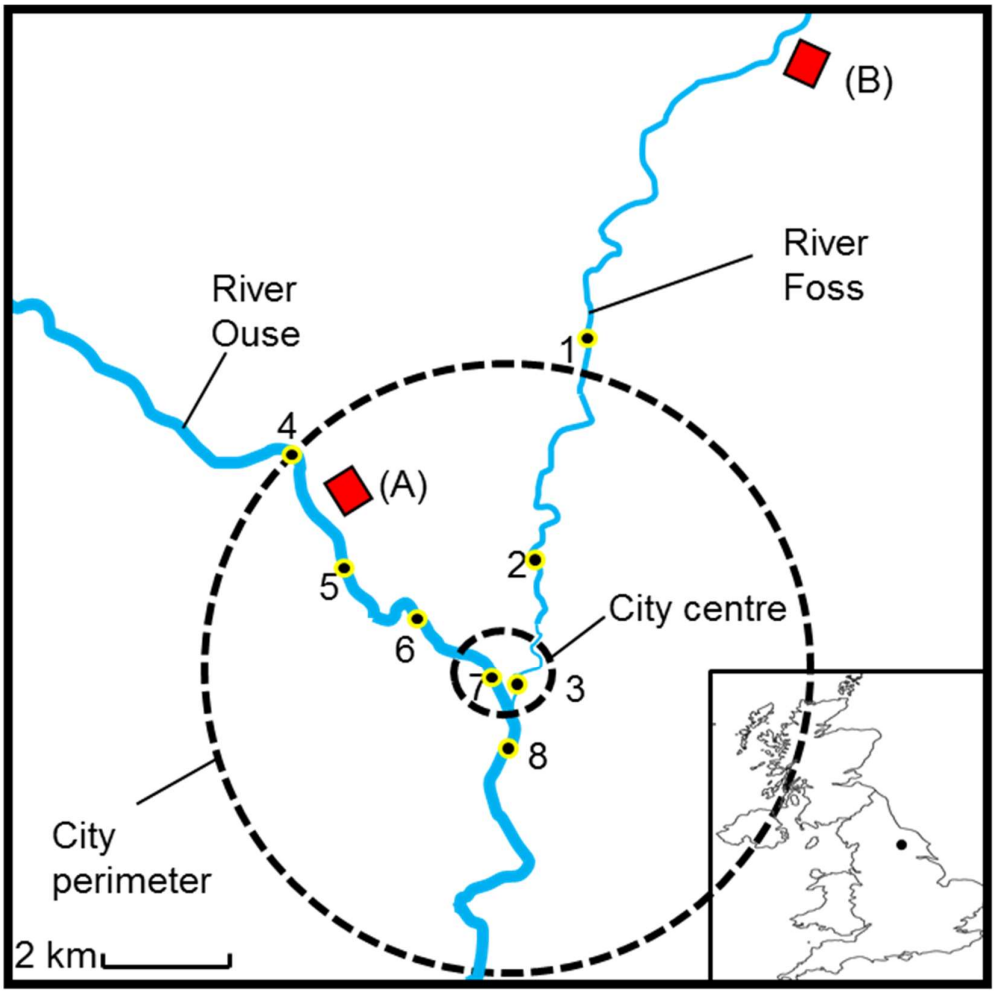


Figure 12. Locations of the 8 sampling sites around the city of York, UK. A and B represent the WWTPs that service the city. Grab samples were collected in February 2015.

3.1.2 Analytical methods

Samples were analysed using a direct injection (100 µL) high-performance liquid chromatography/tandem mass spectrometry with an electrospray ionization source (LC-ESI-MS/MS) method for the determination of 110 pharmaceuticals, pharmaceutical degradates, and wastewater indicator compounds.[77] The Furlong et al. [77] method was developed and fully validated on the instruments at the National Water Quality Laboratory in Denver where the analysis was undertaken. Therefore, repetition of those validation study results are not duplicated here, but can be found in the original method. The presentation of key validation parameters such as accuracy, precision and linearity can be found in Appendix 8.[77] Of the 110 compounds, 95 pharmaceuticals were targeted

in the present study with method detections limits (LOD) as defined by the USEPA[244] down to 0.45 ng/L, previously determined by Furlong et al.[77] (Table 6). The determination of the LOD by Furlong et al.[77] is the same method as that reported in Section 4.8.3.

Instrumentation included an Agilent 6410 triple quadrupole MS/MS system coupled with an Agilent 1200 Series HPLC. Mobile phases were HPLC-grade water modified with 1M formic acid and 1M ammonium formate (A) and 100% HPLC grade methanol (B). Chromatography gradient and conditions are detailed in Appendix 6. Quantification and identification was achieved by external calibration with known standards for each of the pharmaceuticals and completed using Agilent Mass Hunter software in accordance with the (USGS) methodology described in Furlong et al.[77] Due to the occurrence of matrix effects resulting from the variable ionisation efficiency of the target ions in the ESI source, the results reported can only be treated semi-quantitatively as there was no strategy employed to compensate for matrix effects, such as internal calibration. The MS/MS was operated in multiple reaction monitoring (MRM) mode, where two MRM transitions and correct retention times were required for ion qualification, while quantification was based on the major transition (Appendix 7). Additionally, ion ratios between the major and secondary transitions were required to fall within a compound-specific range determined from the corresponding analytical standard.[77] Concentrations reported in the present study are the median of three aliquots taken from each site.

3.1.2.1 Statistical analysis and quality control

The limit of quantification (LOQ) was established as 2 to 5 times the LOD where the probability of incorrectly reporting the presence of an analyte is less than 1% when concentrations are equal to or greater than the LOQ.[245] Concentrations greater than the LOQ were fully quantitative while concentrations detected between the LOQ and LOD were considered semi-quantitative estimates. To enable the consideration of as many pharmaceuticals as possible, both quantitative and semi-quantitative data were used in subsequent data analyses.

Quality control samples were analysed to (1) assess matrix recovery efficiency and identify the presence of matrix interferences that could induce ion suppression or enhancement,[246] and (2) identify any blank contamination from sampling and analysis.

For matrix recovery assessment, all environmental samples were amended with the pharmaceuticals of interest (matrix spike) to a concentration of 400 ng/L. The ambient concentration was also determined prior to the matrix spike and subtracted from the determined matrix spike concentration and divided by the theoretical spike concentration and multiplied by 100 to get a recovery percentage. The matrix recovery assessment was undertaken for all environmental samples because internal standards were not used to compensate for matrix effects and it will give a general assessment of matrix effects in all samples. The aforementioned field blank samples were analysed to identify any potential contributions of pharmaceuticals during sample collection, laboratory processing and analysis. In addition to the field blank and matrix spike samples, analogous laboratory spike and blank samples, using high purity HPLC-grade water, also were analysed with each batch of environmental samples.

3.1.3 PEC modelling

The calculation of PECs for the 95 pharmaceuticals was based on Equation 3.1.[112]

$$PEC = \frac{\text{consumption} * F_{excreta} * (1\text{-WWTP removal})}{\text{inhabitants} * WW_{inhab} * \text{dilution}} \qquad \text{Equation 3.1}$$

Where the numerator represents the river input rate (ng per day): consumption = amount used per day (ng/day); $F_{excreta}$ is the fraction of pharmaceutical excreted unchanged by patients; and WWTP removal is the fraction of a pharmaceutical removed by water treatment. The denominator is the river flushing rate where: inhabitants = population served by the WWTP; WW_{inhab} = amount of wastewater generated (L/day·person), which has a default value of 200; dilution was based on site-specific conditions in each river.

Pharmaceutical usage was generated from localised prescription data released monthly by the National Health Service for January 2015.[247] Relevant medical practices were selected by postal code (Appendix 9). The $F_{excreta}$ term was obtained from either the peer-reviewed literature or online databases such as Drugbank, MedSafe and RXmed, as well as publicly available pharmaceutical data sheets released by government organisations such as MedSafe New Zealand or the Food and Drug Agency (Appendix 10). When a pharmaceutical was metabolised to conjugated metabolites (e.g. glucuronide or sulfato-conjugates), the portion released as a conjugate was added to the

unchanged parent excretion estimate. These metabolites can undergo reactions during water treatment such as cleavage and thus be converted back into their parent compounds, increasing the parent pharmaceutical load in wastewater effluent.[248] Estimates of unchanged pharmaceutical excretion varied across sources; this led to a range of possible unchanged excretion estimates, which were used to calculate a PEC range. For ophthalmic and topical preparations, metabolism was assumed to be zero and therefore the $F_{excreta}$ was set to 1.[153]

Wastewater treatment removal was considered in two ways due to the limited availability of removal estimates for all pharmaceuticals in the present study.[249] Firstly, removal values from the literature were collected and, similarly to $F_{excreta}$ estimates, varied substantially (Appendix 10). A range of possible WWTP removal estimates was compiled to account for variability in hydraulic residence times and WWTP treatment plant designs, which will affect removal efficiency, in order to calculate a possible PEC range. Secondly, data gaps were filled using the USEPA's EPISuite software STPWIN program,[179] similarly to a recent prioritisation exercise in Asia.[154]

3.1.4 Evaluation of PECs

Separate PEC ranges were calculated for pharmaceuticals for both the River Foss and River Ouse. The PEC range incorporated a river-specific dilution factor reflecting hydrological conditions on the day of sampling. The lowest $F_{excreta}$ and highest WWTP removal values found in the literature were paired to give a minimum PEC, while the maximum was derived using the highest $F_{excreta}$ and lowest WWTP removal found in the literature. A PEC (worst case) was also calculated which only considered site-specific dilution (ie. $F_{excreta}$ = 1, WWTP removal = 0).

3.1.5 Prioritisation approach

The fish plasma model (FPM) approach,[118,250] which has been used in previous prioritisation exercises,[6,147] was selected as the method used for prioritisation. Bioconcentration factors (BCFs) for neutral and ionisable compounds were estimated according to the approach of Fu et al.[192] (Appendix 11) and used to determine fish plasma concentrations (FPCs) based on either PECs or MECs. FPCs were then compared to human plasma therapeutic concentrations (indicated by the peak plasma concentration after therapeutic dosing (C_{max})) using Equation 3.2 to determine the risk quotient (RQ).

The Kow and C_{max} for all compounds were collected from the MaPPFAST database complied by Berninger et al.[191]

$$RQ=\frac{PEC*BCF}{C_{max}} \qquad \text{Equation 3.2}$$

RQs are ranked from highest to lowest risk, where a larger RQ indicates a greater potential risk. Using this approach, two ranking lists were obtained, one based on FPCs obtained from PECs, the other using FPCs obtained from MECs.

3.2 Results and Discussion

3.2.1 Pharmaceutical occurrence

No pharmaceuticals were detected in the field blanks collected indicating that sample collection, handling, and analysis did not result in measurable contamination of the water samples (i.e. protocols did not generate false positives for the present study). Calculated recoveries from quality control matrix spike samples generally fell within 60-120% and were considered acceptable.[251] Recoveries failing to meet these criteria are identified and subsequently interpreted with caution. Reported values were not corrected for percentage of analyte recovered in environmental matrix spikes.[252] The median matrix recovery was 88% while the 25 and 75 percentiles were 81 and 160% respectively; this distribution suggests that some matrix enhancement is occurring.

Of the 95 pharmaceuticals surveyed, 25 compounds were detected and quantified (Figure 13) in the eight water samples collected from the York network. A further 19 pharmaceuticals were detected, however only qualitative or semi-quantitative assessment was appropriate due to either quantification limits (11) or unacceptable matrix interferences (7) (Table 6). Of the 25 pharmaceuticals quantified, 10 have not been previously identified in the UK aquatic environment to the authors' knowledge: acyclovir, diphenhydramine, glyburide, hydrocodone, lidocaine, methocarbamol, oseltamivir, sitagliptin, triamterene and loratadine. The remaining 15 pharmaceuticals detected were consistent with the ranges reported previously in the literature (Table 6). Ten pharmaceuticals included in the analysis are not prescribed in the UK and were not detected in any samples. Median and maximum detected concentrations, along with detection frequency and matrix recovery for all target analytes are reported in Table 6.

The concentrations and number of detections between the Rivers Ouse and Foss varied (Fig. 13) with concentrations of six pharmaceuticals in the River Foss being significantly higher than in the Ouse (Student's T-test, $p < 0.05$). A greater number of and more consistent detections occurred in the River Foss, (Fig. 14) which has both a lower dilution factor and the corresponding WWTP (WWTP B) provides less sophisticated water treatment (trickling filter) compared to the treatment used by WWTP A discharging to the River Ouse (conventional activated sludge).

Table 6. Occurrence data for the eight water samples collected during February 2015 from the sampling network with matrix recovery and method detection limits for each of the pharmaceuticals, pharmaceutical degradates and wastewater indicators targeted.

Pharmaceutical	Source or use	LOD (ng/L)	LOQ (ng/L)	Detection Frequency % (n=8)	Max (ng/L)	Median (ng/L)	Matrix recovery % (median)	Detected in the UK (ng/L)
10-Hydroxy-amitriptyline	Degradate of amitriptyline	1.7	8.3	0	ND	ND	110	
Abacavir	Antiviral	4.1	8.2	0	ND	ND	73	
Acyclovir[a]	Antiviral	4.4	22	13	7.9	7.9	60	
Albuterol[a]	β2-adrenergic receptor	1.2	6.1	0	ND	ND	180	38 – 470[39,253]
Alprazolam	Benzodiazepine	4.3	21	0	ND	ND	75	
Amitriptyline	Antidepressant	19	37	25	<LOD	<LOD	250	1.0 – 72[45,46]
Amphetamine	Psychostimulant	4.1	8.1	0	ND	ND	76	1.1 -4[46]
Antipyrine[b]	Analgesic	58	116	20	<LOD	<LOD	87	
Atenolol	β-blocker	2.7	13	13	25	25	97	<1 – 530[39]
Benztropine[b,c]	Anticholinergic	7.9	15	0	ND	ND	300	
Bupropion	Antidepressant	3.6	17	0	ND	ND	86	
Carbamazepine	Anticonvulsant	0.84	4.2	38	27	22	80	<0.5 – 52[39,43]
Carisoprodol	Muscle relaxant	2.5	12	0	ND	ND	81	
Chlorpheniramine[a,c]	Antihistamine	0.94	4.7	13	2.4	2.4	220	
Cimetidine[c]	H2-receptor antagonist	5.6	28	38	<LOD	<LOD	100	<0.5 – 202[39]
Citalopram[c]	Antidepressant	1.3	6.6	50	37	14	170	53[254]
Clonidine	Antihypertensive	30	61	0	ND	ND	87	
Dehydronifedipine	Nifedepine metabolite	4.9	25	0	ND	ND	78	
Desmethyl-diltiazem[c]	Degradate of diltiazem	2.5	12	25	48	44	210	

Table 6. (continued) Occurrence data for the eight water samples collected during February 2015 from the sampling network with matrix recovery and method detection limits for each of the pharmaceuticals, pharmaceutical degradates and wastewater indicators targeted.

Pharmaceutical	Source or use	LOD (ng/L)	LOQ (ng/L)	Detection Frequency % (n=8)	Max (ng/L)	Median (ng/L)	Matrix recovery % (median)	Detected in the UK (ng/L)
Desvenlafaxine	Antidepressant, venlafaxine metabolite	3.8	7.5	88	85	16	87	7.3 – 290[44,254]
Dextromethorphan[a,c]	Cough suppressant	1.6	8.2	25	6.7	6.0	140	
Diazepam	Benzodiazepine	0.45	2.2	63	1.3	1.0	81	0.6– 1.1[45,46]
Diltiazem[c]	Calcium channel blocker	5.1	10	63	44	9.1	180	<1 – 49[39]
Diphenhydramine[a]	Antihistamine	2.9	5.8	25	6.0	5.6	100	
Erythromycin[c]	Macrolide antibiotic	27	53	25	180	170	250	<0.5 – 1000[255,256]
Ezetimibe[c]	Cholesterol-reducing agent	13	64	25	<LOD	<LOD	160	
Fadrozole[b]	Aromatase inhibitor	1.5	7.3	0	ND	ND	92	
Fenofibrate	H2-receptor antagonist	1.3	6.3	0	ND	ND	100	
Fexofenadine	Antihistamine	4.0	20	100	130	18	90	64[44]
Fluconazole[a]	Antifungal	36	71	0	ND	ND	76	
Fluoxetine[c]	Antidepressant	5.4	27	0	ND	ND	360	6.2 – 34[46,257]
Fluticasone[c]	Synthetic corticosteroid	0.92	4.6	63	<LOD	<LOD	86	
Glipizide	Antidiabetic	17	35	0	ND	ND	82	
Glyburide	Antidiabetic	0.79	4.0	88	3.1	<LOD	81	
Hydrocodone	Opioid, codeine metabolite	2.1	11	25	39	34	110	
Hydrocortisone	Natural glucocorticoid	29	147	0	ND	ND	77	

Table 6. (continued) Occurrence data for the eight water samples collected during February 2015 from the sampling network with matrix recovery and method detection limits for each of the pharmaceuticals, pharmaceutical degradates and wastewater indicators targeted.

Pharmaceutical	Source or use	LOD (ng/L)	LOQ (ng/L)	Detection Frequency % (n=8)	Max (ng/L)	Median (ng/L)	Matrix recovery % (median)	Detected in the UK (ng/L)
Hydroxyzine	Glucocorticoid hormone	1.5	7.4	0	ND	ND	110	
Iminostilbene	Carbamazepine degradate	73	145	0	ND	ND	98	
Ketoconazole[c]	Antifungal	56	113	0	ND	ND	430	
Lamivudine[c]	Antiretroviral	3.2	16	0	ND	ND	160	
Lidocaine[a]	Topical anesthetic	3.1	15	75	9.6	8.9	84	
Loperamide [c]	Antidiarrheal	5.7	11	0	ND	ND	420	
Loratadine[a]	Antihistamine	1.4	6.9	88	8.5	1.5	120	
Lorazepam	Benzodiazepine (anxiolytic)	58	116	0	ND	ND	84	
Meprobamate	Anxiolytic	17	86	0	ND	ND	74	
Metaxalone[b]	Muscle relaxant	7.8	16	0	ND	ND	80	
Metformin	Antidiabetic	6.6	13	100	1300	630	120	2300[44]
Methadone[c]	Synthetic opioid	3.8	7.6	0	ND	ND	200	10 – 18[45]
Methocarbamol	Muscle relaxant	4.4	8.7	25	10	8.7	81	
Methotrexate	Chemotherapy agent	11	52	0	ND	ND	76	<6.3[258]
Metoprolol[c]	β-blocker	14	28	0	ND	ND	86	<0.5 – 12[39]
Morphine	Analgesic (opioid)	2.8	14	30	21	19	84	0.6 – 36[45,46]
Nadolol	β-blocker	16	81	0	ND	ND	85	
Nevirapine[c]	Antiretroviral	3.0	15	25	<LOD	<LOD	81	

Table 6. (continued) Occurrence data for the eight water samples collected during February 2015 from the sampling network with matrix recovery and method detection limits for each of the pharmaceuticals, pharmaceutical degradates and wastewater indicators targeted.

Pharmaceutical	Source or use	LOD (ng/L)	LOQ (ng/L)	Detection Frequency % (n=8)	Max (ng/L)	Median (ng/L)	Matrix recovery % (median)	Detected in the UK (ng/L)
Nizatidine[c]	Acid inhibitor (ulcers)	9.5	19	0	ND	ND	240	
Noreistherone	Oral contraceptive component	2.2	11	13	<LOD	<LOD	85	<10 – 17[259]
Nordiazepam	Benzodiazepine, diazepam metabolite	21	41	0	ND	ND	82	0.1 – 6.8[46]
Norverapamil[c]	Verapamil metabolite	1.7	8.6	0	ND	ND	400	
Omeprazole[c] + esomeprazole	Proton pump inhibitor	2.8	5.6	0	ND	ND	260	
Oseltamivir	Antiviral	2.9	15	38	3.6	<LOD	85	
Oxazepam	Benzodiazepine (anxiolytic)	28	140	0	ND	ND	81	0.9 – 21[46]
Oxycodone	Opioid analgesic	5.0	25	0	ND	ND	90	0.4 – 7.1[45,46]
Paracetamol[a]	Analgesic	3.6	7.1	63	1000	260	88	52 – 2400[39,253]
Paroxetine[c]	Antidepressant	4.1	21	0	ND	ND	300	
Penciclovir[c]	Antiviral	8.1	40	0	ND	ND	160	
Pentoxyfylline[c]	Cardiovascular drug	4.7	9.3	10	<LOD	<LOD	86	
Phenazopyridine[b]	Urinary tract analgesic	2.7	13	0	ND	ND	84	
Phendimetrazine[b]	Appetite suppressant	16	31	0	ND	ND	86	
Phenytoin	Antiepileptic	94	188	0	ND	ND	78	
Piperonyl butoxide[b]	Pesticide, lice treatment	1.5	3.1	13	2.8	2.8	87	
Prednisolone	Synthetic corticosteroid, prednisone metabolite	75	150	0	ND	ND	91	

Table 6. (continued) Occurrence data for the eight water samples collected during February 2015 from the sampling network with matrix recovery and method detection limits for each of the pharmaceuticals, pharmaceutical degradates and wastewater indicators targeted.

Pharmaceutical	Source or use	LOD (ng/L)	LOQ (ng/L)	Detection Frequency % (n=8)	Max (ng/L)	Median (ng/L)	Matrix recovery % (median)	Detected in the UK (ng/L)
Prednisone	Synthetic corticosteroid	84	168	0	ND	ND	120	
Promethazine[a,c]	Antihistamine	10	50	50	<LOD	<LOD	190	
Propoxyphene	Opioid analgesic	3.4	17	0	ND	ND	140	9 -680[255,260]
Propranolol	β-blocker	13	26	50	27	18	110	3.9- 220[99,255,]
Pseudoephedrine[a] + ephedrine	Decongestant	5.5	11	13	8.5	8.0	81	12 – 17[45]
Quinine[a,c]	Antimalarial, flavouring agent	16	80	50	41	23	140	
Raloxifene	Selective estrogen receptor modulator	4.9	9.7	0	ND	ND	420	
Ranitidine[a]	Acid inhibitor (ulcers)	38	192	100	180	72	100	<3 – 73[39, 41,43]
Sertraline[c]	Antidepressant	3.3	16	0	ND	ND	300	
Sitagliptin	Antihyperglycemic	20	97	25	36	20	81	
Sulfadimethoxine[b]	Sulfonamide antibiotic	33	66	0	ND	ND	83	
Sulfamethizole[b]	Sulfonamide antibiotic	21	102	0	ND	ND	82	
Sulfamethoxazole	Sulfonamide antibiotic	13	26	38	<LOD	<LOD	80	1.8 – 8[39,44]
Tamoxifen[c]	Cancer treatment	11	52	0	ND	ND	3300	<10 – 210[255,260]
Temazepam	Benzodiazepine	9.2	18	25	<LOD	<LOD	81	1.4 – 78
Theophylline	Diuretic	8.3	42	0	ND	ND	75	
Thiabendazole[b]	Fungicide	0.82	4.1	0	ND	ND	83	
Tiotropium[c]	Bronchodilator	8.6	43	0	ND	ND	220	

Table 6. (continued) Occurrence data for the eight water samples collected during February 2015 from the sampling network with matrix recovery and method detection limits for each of the pharmaceuticals, pharmaceutical degradates and wastewater indicators targeted.

Pharmaceutical	Source or use	LOD (ng/L)	LOQ (ng/L)	Detection Frequency % (n=8)	Max (ng/L)	Median (ng/L)	Matrix recovery % (median)	Detected in the UK (ng/L)
Tramadol	Opioid analgesic	3.0	15	50	77	49	90	3.0 – 7700[39,46]
Triamterene	Diuretic	2.6	5.3	25	4.2	<LOD	80	
Trimethoprim	Antibiotic	3.8	19	75	31	22	86	<1.5 – 180[39,50]
Venlafaxine	Antidepressant	0.90	4.5	38	15	12	95	1.1 – 85
Verapamil[c]	Calcium channel blocker	3.1	16	0	ND	ND	550	
Warfarin	Anticoagulant	3.0	6.0	25	<LOD	<LOD	84	

% = percentage; ng/L = nanograms per litre; LOD = Method detection limit; ND = Not detected

[a] Available over-the-counter in the UK

[b] Not prescribed in York, UK in January 2015

[c] API reported as estimate due to being only qualitatively confirmed (<LOD) or environmental matrix recovery quality assurance criteria (60-120%) according to Furlong et al.[77] reported values are not corrected for percentage of analyte recovered in environmental matrix spikes according to Wershaw et al.[252]

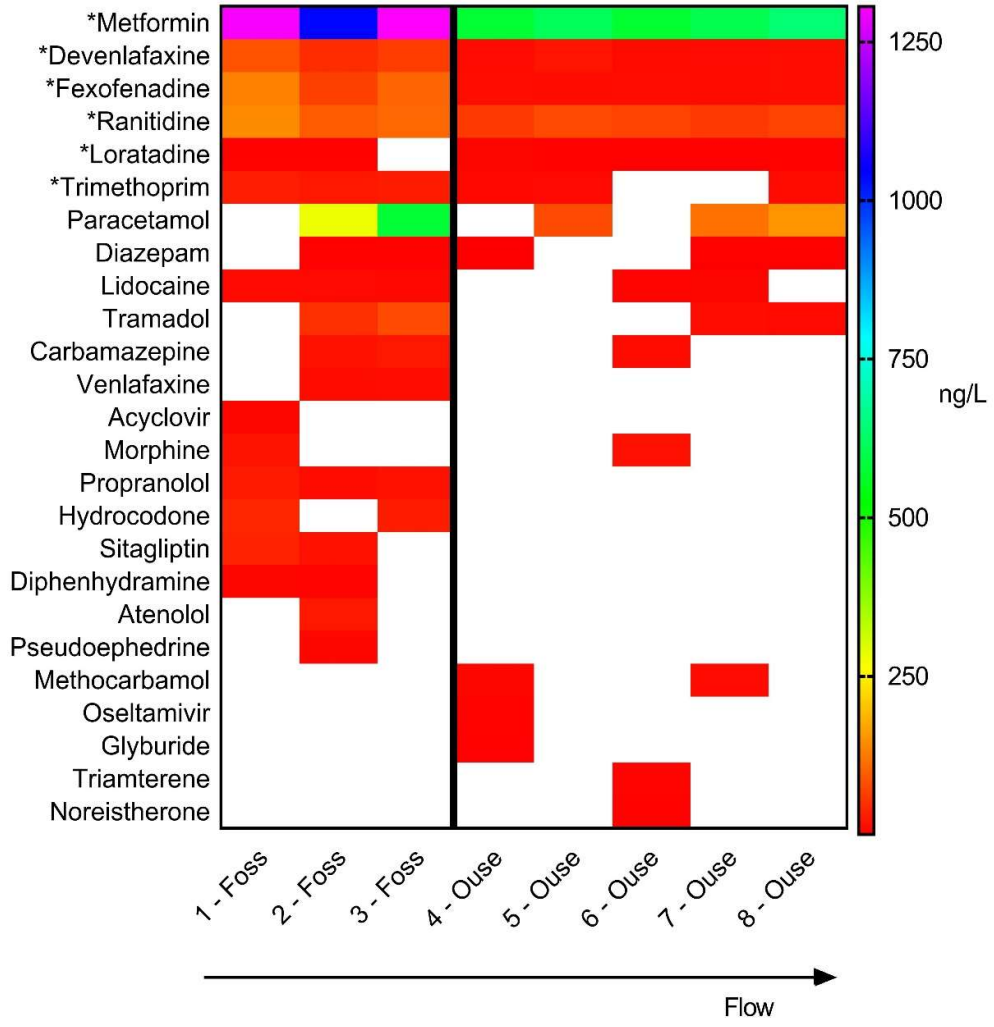


Figure 13. A heat map of the mean pharmaceutical concentration at each of the 8 sampling sites along the Rivers Ouse and Foss. Numbers refer to the specific sampling sites listed in Figure 1. Significant differences in concentrations between the River Ouse and Foss were found for the 6 pharmaceuticals that were detected frequently enough to compute a student's t-test, * indicates a $p \leq 0.05$.

3.2.2 Evaluation of modelled concentrations with monitoring data

The EMA PEC model describes an annual average concentration for the region the consumption data cover; in general, usage data from the whole of a country is averaged to give a single PEC.[147] Evaluating this approach with localised, temporally limited samples would introduce a source of potential error as it has been shown that seasonal usage is important for some pharmaceuticals and that demographics in a specific area may differ substantially from the national average.[162,163] To reduce these potential biases,

local usage data, corresponding to time of sampling, was used. In addition, site-specific dilution factors were incorporated to avoid the use of EMA[112] default dilution factors (i.e. 10). The WW_{inhab} term could not be refined to actual discharge because both WWTPs are highly variable and discharge measurements were not available for the sampling dates. This permits a focus on other factors that could be affecting the suitability of PECs such as WWTP removal and metabolism.

3.2.2.1 Overall PEC performance

Many pharmaceuticals targeted were not detected in the monitoring campaign, however based on their PECs, this was not unexpected. To assess the overall performance of the PECs, a semi-quantitative approach was taken. Each of the 77 pharmaceuticals for which a PEC could be calculated were sorted into one of four possible categories (Figure 14). Pharmaceuticals that were expected to be detected in the monitoring campaign (i.e. PEC greater than the corresponding analytical LOD) were sorted into either detected or not detected categories. Similarly, pharmaceuticals not expected to be detected (i.e. PEC less than the respective analytical LOD) were sorted into detected and not detected categories. Overall in the semi-quantitative analysis, the PECs in the two rivers performed well with 79% and 86% of predictions correctly confirmed in the River Foss and Ouse, respectively, by the monitoring data.

The large difference in dilution between the two rivers, factors of 17.8 and 540 for the Foss and Ouse respectively, led to larger PECs in the River Foss and therefore a higher number of expected detections. A larger proportion of expected detections were not identified in our monitoring campaign in the Foss in comparison to the Ouse; it could be that pharmaceuticals were missed by our sampling effort, however our results indicate that pharmaceutical concentrations are stable throughout the River Foss over an 8-hour period (Figure 13), which diminishes the likelihood of missing a detection. Conversely, the metabolism or WWTP removal selected from the literature may have produced PECs larger than real-world concentrations. The number of unexpected but detected pharmaceuticals is greater in the River Ouse, despite corrections for upstream contributions detected at site 4, (Figure 14). The River Ouse could be subject to a greater number of sources not reflected in our usage estimate in contrast to the more rural River Foss. Sources of pharmaceuticals beyond the scope of localised prescription data exist

within the city include, for example, a substantial tourism industry and two post-secondary institutions. Recent studies have demonstrated the impact of post-secondary institutions[30] and music festivals[261] on MECs, and it is likely that MECs in the Ouse are influenced by demographic factors not inclusive of localised prescription-based usage estimates.

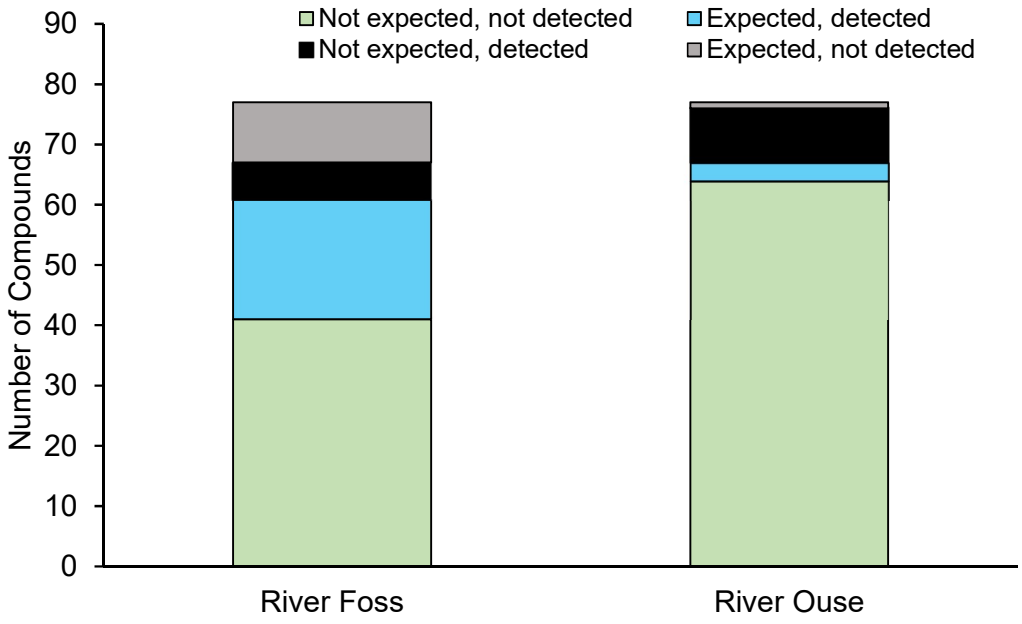


Figure 14. A semi-quantitative analysis of PEC performance in the rivers based on the monitoring campaign results. A compound is expected to be detected when the PEC is greater than the respective analytical method detection limit.

3.2.3 Impact of metabolism and WWTP removal uncertainty on PECs

3.2.3.1 Underestimated PECs

A breakdown of how each pharmaceutical PEC performed in comparison to the MEC is shown for the River Foss (Figure 15) and the River Ouse (Figure 16). While the overall semi-quantitative performance of PECs in the River Ouse was slightly better than the Foss, these results were not repeated when quantitative data were compared. In the Foss and the Ouse, 38% and 78% respectively, of the MEC ranges were entirely greater than the corresponding PEC range. This drops to 12% and 44% respectively when the PEC (worst case) is considered. The PEC (worst case) does not include metabolism or WWTP removal, only dilution, and when this PEC still falls below the MEC it indicates a problem with the consumption estimate. The analytical matrix spike recoveries indicated

that matrix enhancement is occurring, which could affect the comparisons with PECs. To investigate, each compound with a MEC range greater than the PEC range was theoretically corrected based on the compound specific matrix recovery. All of the theoretically corrected MEC ranges were still greater than the corresponding PEC ranges in the River Ouse and Foss with one exception, erythromycin, where the MEC range corresponded with the top of the PEC range in the River Foss. Therefore we do not expect our results to be significantly altered by the distribution in matrix recoveries.

In the River Foss, three pharmaceuticals (dextromethorphan, diphenhydramine and pseudoephedrine) had greater MECs than PEC (worst case) estimates and are all available over-the-counter (OTC). This consumption pathway was not considered in our consumption estimate as we were unable to access data on sales of OTC medicines. As a result, PECs for these pharmaceuticals should be systematically underestimated.[7,161,164] This was not reflected for all OTC pharmaceuticals, similarly to a recent study in Canada.[165] This highlights the need for a new approach to incorporate OTC consumption into WWTP pharmaceutical loadings.[147,164] The results from the River Ouse (Figure 16) are more complicated, a mixture of both OTC and prescription-only pharmaceuticals had MECs which were greater than the PEC (worst case) estimates. This supports our semi-quantitative findings where a problem exists with the consumption estimate and is likely a result of the specific demographics impacting pharmaceutical loads for the River Ouse.

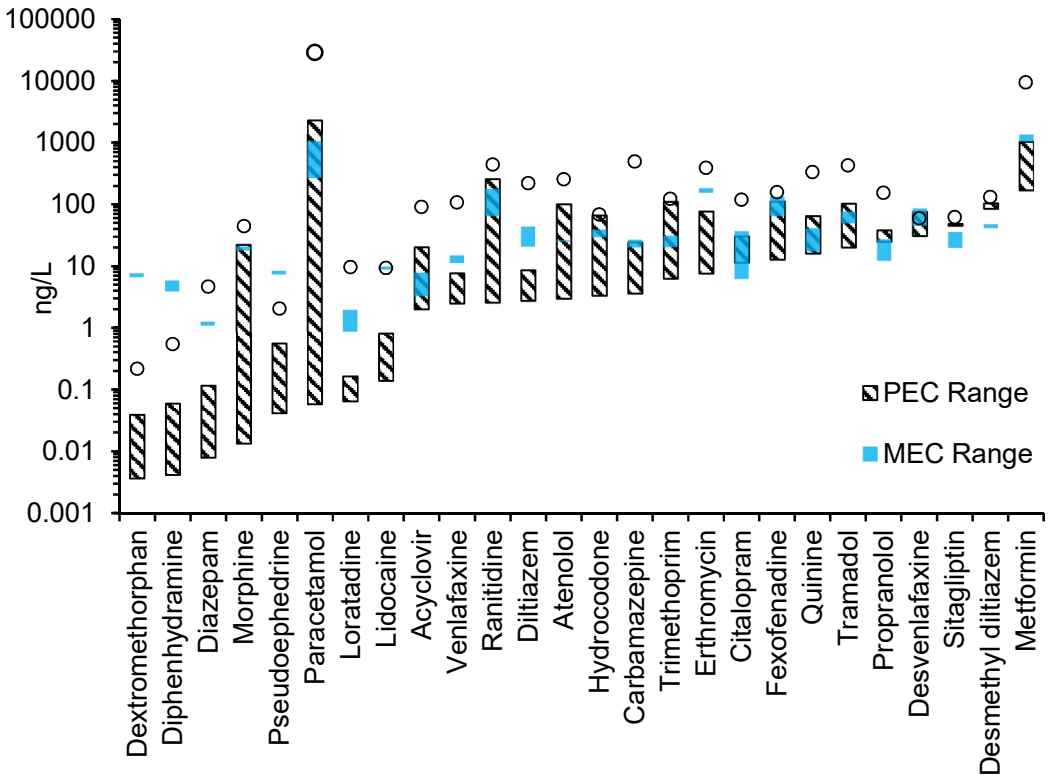


Figure 15. PEC range and MEC range for compounds quantified in the River Foss. The worst case PEC is also plotted (open circles) where $F_{excreta}$ = 1 and WWTP removal = 0. The MEC range is based on the results from sampling sites 1-3 (Figure 12).

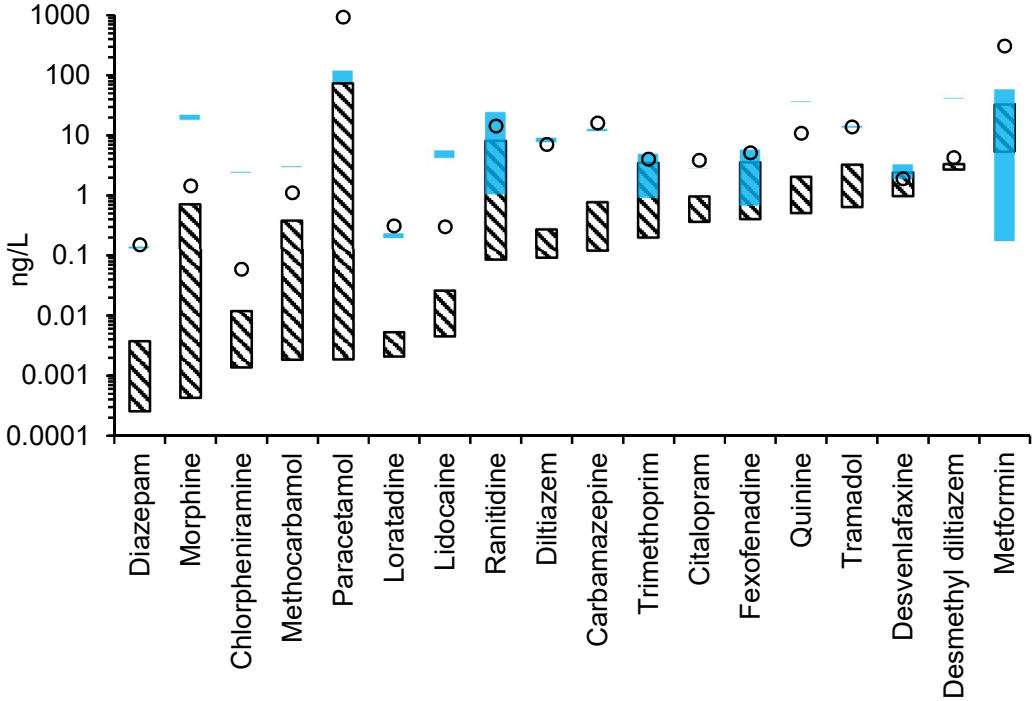


Figure 16. PEC range and MEC range for compounds quantified in the River Ouse. The worst case PEC is also plotted (open circles) where $F_{excreta}$ = 1 and WWTP removal = 0. The MEC range is based on the results from sites 5-7 (Figure 12) and corrected for the upstream contributions.

3.2.3.2 PEC ranges

The PEC range is large for many of the pharmaceuticals. For instance, the paracetamol PEC range covers over 4 orders of magnitude (Figure 14). This large uncertainty is a result of the extensive variability in experimental WWTP removal and $F_{excreta}$ estimates obtained from the literature. In both rivers, the majority of PEC ranges vary by at least 2 orders of magnitude, which could be important from both a risk assessment and prioritisation perspective. The large PEC range does mean that, in general, the MEC range did correspond with predictions in the River Foss (Figure 15). The MEC range is typically near the top of the PEC range, where the smallest WWTP removal was paired with the highest unchanged excretion found in the literature. This finding has two implications: firstly, choosing the worst-case fate parameters to estimate PECs is likely the best approach to avoid underestimations of PECs, which is in agreement with PEC approaches in the literature,[262] secondly, anything short of an exhaustive literature review could lead to underestimated PECs in the majority of cases shown in Figures 15 and 16. This is because the PEC ranges determined herein are the result of an exhaustive literature review; in a larger scale prioritisation exercise the time resources required to thoroughly check each compound would be impractical and the process itself highly subjective. This could lead authors to different conclusions about the resulting risks and priority compounds as it is a single value computed for the PEC, not a range, which is a substantial flaw not often considered when the fate data used in a PEC are collected in this manner.

Our results indicate that consideration of metabolism and WWTP removal is essential when calculating PECs because PEC (worst case) is a large overestimate of actual concentrations in the majority of cases (Figure 15), also shown by others.[6,144,156] In the River Foss, prescription pharmaceuticals are described well using the PEC approach. This is in sharp contrast to the River Ouse, where multiple consumption sources are likely affecting concentrations of the pharmaceuticals in the environment, making it impossible to evaluate the effect of the fate parameters with the current dataset. Further monitoring that incorporates sampling WWTP influents and effluents to compute actual removals will be critical to assessing PECs relative to MECs.

There are several major limitations with the sampling strategy and quantification approach utilized in this study. Firstly, the grab sampling strategy, while simpler and

cheaper, is a major limitation of the current study and concentrations reported are representative of a snapshot in time and thus may not truly reflect pharmaceutical exposure in these rivers. To help compensate for this limitation, the rivers were sampled at a high spatial resolution to build up concentration profile of the city as samples were collected over an eight-hour period and to increase the chances of detecting pharmaceuticals which may be transient in the system. Time-proportional composite sampling, where samples are collected a specified time periods, usually over a 24-hour period, by an automated device are preferable as they provide an indicate of the average daily pharmaceutical exposure in the system. These composite samplers with either pool the collected samples into a single sample or keep each sample collected separate. Time-proportional composite sampling strategies reduce the uncertainty in measured concentrations as they are representative of the average conditions in the rivers.[60] Therefore, PECs not matching with MECs could be due to limitations associated with the grab sampling method rather than the modelling (Figure 16).

Secondly, the sampling and analysis only focused on the dissolved phase of the aqueous compartment. The physico-chemical properties of many of the pharmaceuticals included in the method indicate that sorption to the solid phase, indicated by a high logKow, is possible. The solid phase constitutes either the suspended particulate matter (SPM) in the water column or sediment. Pharmaceuticals have been detected in both sediment[206] and SPM[263] which could be important for pharmaceutical modelling and source apportionment. Due to methodological and logistical limitations analysis of sediment and SPM was not included in this study. For example, a very large sample is required to obtain enough SPM to analyse which was not practical to ship to the US from the UK. There can also be difficulties with maintaining sample integrity prior to analysis as without filtration microbes, associated with the SPM could degrade the pharmaceuticals present.[264] Furthermore the analytical method is designed for river water, no extraction step is required. To analyse SPM and sediment samples by LC-MS, an extraction is first required which can be time consuming, costly and difficult to optimize. Despite this, the inclusion of sediment and SPM could help explain why many pharmaceuticals concentration predictions were underestimated (Figure 16). Partitioning between the solid and aqueous phase could be occurring and thus would be missed by the analysis approach used here.

Thirdly, the quantification method relied on external calibration, which due to the frequent and variable occurrence of matrix effects could potentially impact the accuracy of the reported concentrations. Therefore, the data reported in this scoping studying should be viewed with caution and considered semi-quantitative. The lack of internal standards to improve quantification accuracy could also be a reason for MECs not agreeing with PECs for several compounds (Figure 16). Taken together, these limitations highlight the shortcomings of this scoping study and need to be taken into account in future work where robust monitoring data is required. Further work which includes a seasonal monitoring campaign is suggested to quantify the seasonal variability in MECs to serve as a check of the findings from the present initial scoping study.

3.2.4 Implications for prioritisation

Risk ranking order is important as it dictates which pharmaceuticals are of highest risk and thus, most likely to receive further costly investigations into effects and occurrence.[147] Therefore we evaluated the similarities and differences between risk rankings obtained based on MECs and rankings based on PECs for the River Foss (Figure 17A) and River Ouse (Figure 17B). In the River Foss, while there was some variability in the ranking position of individual compounds, generally, the rankings based on MECs and PECs followed a similar trend. Compounds identified as highest risk based on MECs also were identified as highest risk based on PECs and those ranked as lower risk based on MECs also ranked as lower risk using PECs (Figure 17A). The exceptions were dextromethorphan and diphenhydramine where the rank position was much higher based on MECs than based on PECs. This degree of similarity was not observed in the River Ouse (Figure 17B). Eight of the MEC ranks are higher risk than their PEC rank counterparts, which visually, is a more variable but gentler rise (Figure 17B). This indicates that the degree in which PECs were underestimated in the River Ouse affects prioritisation ranking order trend.

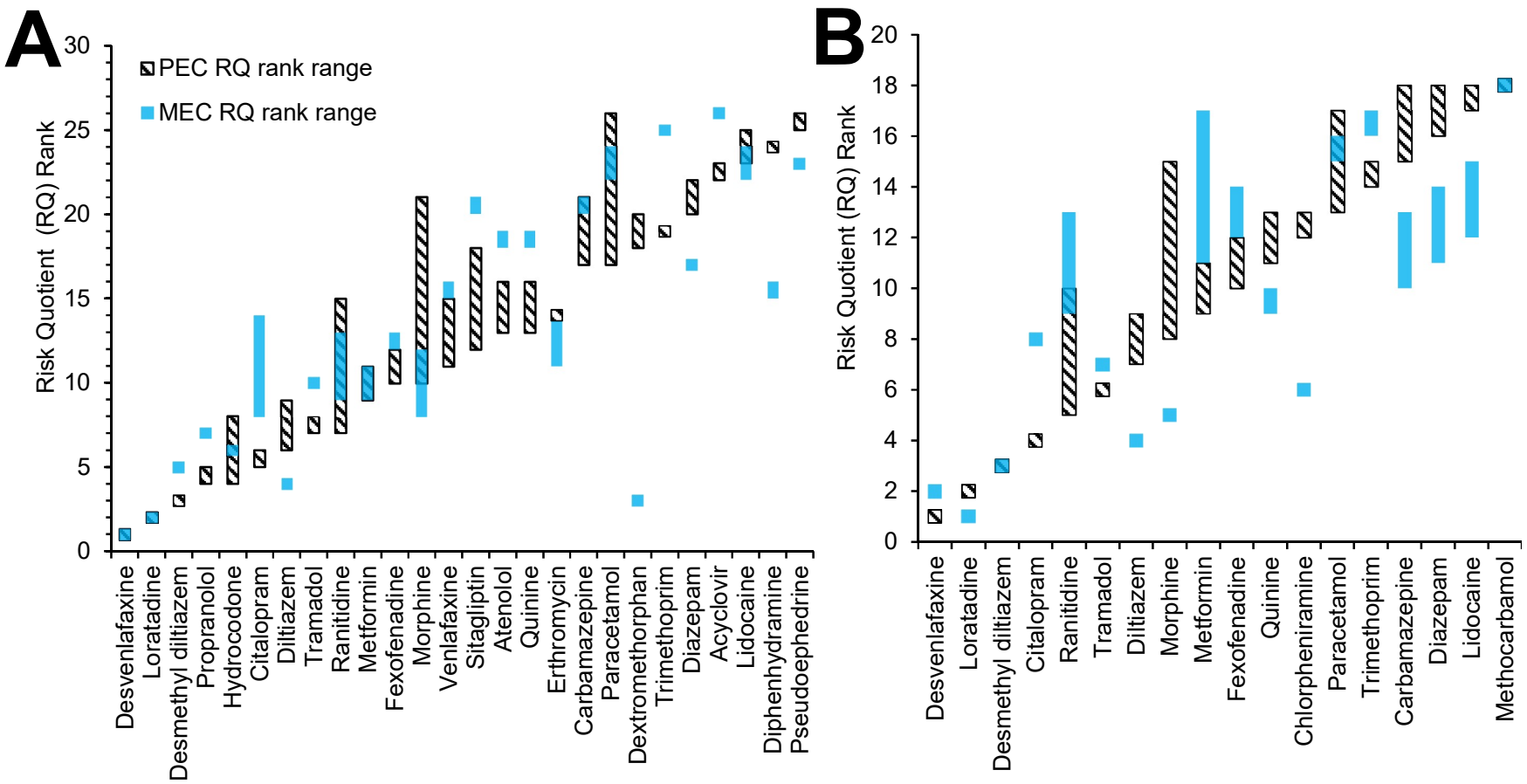


Figure 17. (A) The range of possible ranks resulting from risk quotients calculated using MECs or PECs in the River Foss. (B) The range of possible ranks resulting from risk quotients calculated using MECs or PECs in the River Ouse. Ranks are presented by decreasing risk, where rank 1 corresponds to highest risk.

3.3 Conclusion

Monitoring data for a comprehensive set of 95 pharmaceuticals in two rivers that run through the city of York, UK was presented. This data was collected during a snapshot sampling where flow conditions were below the long-term mean and near the Q50 in February 2015, 25 pharmaceuticals were quantified (i.e. detected), 10 of which had not been previously measured in the UK aquatic environment. Site-specific PEC ranges varied up to four orders of magnitude due to the variability in metabolism and WWTP removal values found in the literature. The largest unchanged excretion paired with the lowest WWTP removal approach provided the greatest comparability to measured concentrations. When PECs and MECs were used to prioritise the detected pharmaceuticals based on risk, generally the two approaches provided similar ranking outcomes for well-defined systems such as the River Foss, but were less comparable the more complicated system, the River Ouse. The findings for the Foss, in particular, provide some confidence in the use of PECs in prioritisation exercises for pharmaceuticals. Further work should include building up a robust monitoring dataset representative of spatial and temporal variations in pharmaceutical concentrations in the present river system. From this detailed dataset, annual average MECs can be derived which would provide a more robust validation of the PECs reported here.

Therefore in the next Chapters, an LC-MS/MS method for the quantitation of compounds observed in the York river system in this Chapter was developed and validated. This method was then applied to a year-long monitoring campaign to build up a robust monitoring dataset more appropriate for validating the simple PEC.

Chapter 4

Part A: Experimental sampling and analytical methodologies

4.0 Introduction

Identification and monitoring of pharmaceuticals in environmental matrices has become a substantial area of research since the 1990s.[33] This expansion has been largely due to significant analytical advances in mass spectrometry (MS) which enabled low to sub-ng/L detection levels in complex samples.[105] In general this is achieved by the pairing of high performance liquid chromatography (HPLC) with low resolution mass analysers (e.g. quadrupoles) operating in multiple reaction monitoring mode (MRM) which provides excellent selectivity and sensitivity in complex matrices.[265]

A major drawback of pharmaceutical analysis in aqueous environmental samples is a time consuming and costly extraction step than can result in analyte losses.[68,98,266] Recently, aqueous methods have been developed that achieve comparable limits of detection (LODs) with no sample pre-concentration or clean-up step.[267] This is accomplished by utilising larger than normal injection volumes (~100 µL) to increase the likelihood of detection, paired with a MS/MS operating in MRM.[44] Removal of the extraction step greatly reduces sample preparation time, which can increase the number of samples than can be processed; this is highly beneficial to large monitoring campaigns.

Several analytical methods have been described for the detection of pharmaceuticals using a so-called 'direct injection' LC-MS/MS approach.[44,77,105,106,267,268] In the environmental science literature, 'direct injection' is referring to the lack of pre-concentration and sample clean-up prior to LC injection, rather than direct injection into the MS itself. Therefore, in the interest of maintaining appropriate nomenclature across analytical disciplines, the 'direct injection' methods are referred to subsequently as 'rapid screening' methods. The analytical methods described in this study build upon the methods developed by Furlong[77] for similar environmental matrices (surface water, wastewater influent and effluent) used for the detection of pharmaceuticals in Chapter 3. The aim here was to optimise the method described by Furlong[77] by tailoring it to the 33 study compounds, reaching an average LOD < 10 ng/L and reducing run time to 30 minutes. Method validation is addressed in Chapter 4 Part B. In addition to the analytical method, this chapter also describes the sample collection protocols.

4.1 Collection of surface water samples

4.1.1 Field filtration and storage

All water samples collected were subject to the same sampling protocol. Sample filtration was completed in the field wearing appropriate protective equipment (e.g. nitrile gloves). Clean glass 1 L sample bottles (Fisher Scientific Ltd.), pre-washed and triply rinsed with HPLC grade methanol, were triply rinsed with sampling matrix before filling with sample. A disposable 24 mL Norm-Ject® luer-lock syringe Sigma-Aldrich (Dorset, UK) was triply rinsed with sample and 20 mL of sample drawn. To prime the disposable 25 mm diameter a 0.7 µm glass-fibre filter (GF/F) (Whatman Inc.) 10 mL of sample was passed through and discarded (Figure 19). A 10 mL aliquot was then filtered through the primed filter into a Thermo Scientific™ Chromacol 20 mL amber glass screw top vial (Figure 18). Samples were immediately frozen on pure dry ice and transported back to the laboratory as soon as possible, where they were stored at -18 °C until analysis.

4.1.2 Grab samples

Grab samples were collected using a weighted sampler designed and built for the study (Figure 18). The sampler greatly reduced the likelihood of cross-contamination as a clean triply methanol rinsed bottle could be fitted at each site. Three field replicates were always collect from each site visited (i.e. bottle contents emptied and a fresh litre of river water retrieved for the 2nd and 3rd replicates).

Figure 18. Left and centre: weighted bottle sampler built for collecting surface water samples. Right: Example of sample syringe filtering.

4.1.3 Composite samples

Three composite samplers built by the USEPA using an in-house design[269] were available for experimental work over the summer of 2016 (Figure 19). Briefly, the samplers collected 26 mL of water once every 15 minutes and deposited it in a collapsible 20 L polyethylene container submerged under water. The samplers are simple and cost-effective, however no cooling or filtering of samples occurred as they were collected. The collection bag was sampled using the protocol described in section 4.1.1 at the desired time intervals (e.g. 24 hours). There is the potential for degradation of analytes over the 24 hour sample collection period and this is a clear limitation of these particular composite samplers. The validation of these samplers and thus the potential degree of degradation was not investigated as part of this study, therefore results from composite samplers should be treated with caution. The time intervals and sites at which the composite samplers were deployed are detailed in Chapter 5.

Figure 19. Left: deployment of the composite sampler in the River Foss, note the sampling bag is submerged. Right: one of the three composite samplers on loan from the US EPA, design detailed in Kahl et al.[269]

4.2 Collection of wastewater treatment plant samples

There are three WWTPs of varying size and treatment capabilities that service the city (Chapter 5), therefore influent and effluent from each WWTP was sampled during summer 2016. Due to access restrictions, 24-h composite samples for influent and effluent could only be collected once. All samples were collected by Yorkshire Water personnel following the protocol detailed in section 4.1.1. Therefore composite samples reflecting the hydraulic residence time of the WWTPs could not be collected, as it was

only possible to collect 24 hour composite samples. The composite samplers used in the WWTPs were owned by Yorkshire Water and thus a holding study to check sample degradation could not be undertaken. The composite samplers used by Yorkshire Water do have a cooling mechanism, therefore sample degradation is less likely than for the composite samplers deployed in the rivers.

4.3 Instrumentation, standards and reagents

4.3.1 Analytical instrumentation

A Dionex Ultimate 3000 HPLC equipped with a 100 µL sample injection loop and autosampler maintained at 4°C, was coupled with a Thermo Scientific™ TSQ Endura triple-stage quadrupole MS interfaced with an EASY-Max NG™ heated electrospray source (located in the York Centre of Excellence in Mass Spectrometry). Xcalibur™ Qual Browser version 4.0 was used for qualitative peak processing while TraceFinder™ 4.1 General Quantification software was used for sample quantification and calibration. Chromatography was performed with a guard column followed by a Zorbax Eclipse® Plus-C18 HPLC column with a 1.8 µm particle size, 3.0 mm internal diameter and 100 mm length. This instrumentation was used for all described sample analyses with the exception of the experiments reported in Chapter 3.

4.3.2 Solvents and reagent solutions

HPLC grade water and methanol (VWR Chemicals) were used in all sample or standard preparation. Mobile phases for chromatographic separation were prepared as follows: aqueous solvent consisted of water amended with 12 mL of 1 M formic acid and 10 mL of 1 M ammonium formate in a 1 L volumetric flask. Organic solvent was 100% HPLC grade methanol.

4.3.3 Standards

Due to the cost and limited availability of many study pharmaceuticals, standards (≥ 98 % purity) were purchased from a range of different vendors. Metformin HCl, gabapentin, citalopram HBr, venlafaxine HCl, *o*-desmethylvenlafaxine, oxazepam, diltiazem HCl, sertraline HCl, temazepam, diazepam, amitriptyline HCl, paracetamol, codeine, hydrocodone, cimetidine, trimethoprim, triamterene, lidocaine, tramadol HCl, propranolol HCl, loratadine and diphenhydramine were purchased from Sigma-Aldrich

(Dorset, UK). Sitagliptin phosphate, raloxifene HCl, oseltamivir phosphate, noreistherone were obtained from Cambridge Bioscience (Cambridge, UK). Fexofenadine HCl, ranitidine HCl and erythromycin were purchased from Santa Cruz Biotechnology (Heidelberg, Germany) while noreistherone was obtained from Tokyo Chemical Industry UK Ltd. (Oxford, UK). Standards were either purchased at or prepared to give a 1 mg/mL in methanol stock concentration. All stock standard solutions were stored at -18°C.

4.3.4 Internal standards

Internal calibration was used to quantify the pharmaceuticals in the method described. For reasons of expense and availability, not all pharmaceuticals had a corresponding isotopically labelled internal standard (ILIS) (Table 7). In these cases, atrazine-d_5 was the internal standard (ILIS) and has been previously determined suitable for this role.[77]

The following isotopically labelled standards were purchased from Sigma Aldrich (Dorset, UK): amitriptyline-d_3, codeine-d_6, citalopram-d_6, diphenhydramine-d_3, Temazepam-d_5, diazepam-d_5, gabapentin-d_{10}, hydrocodone-d_3, paracetamol-d_4, propranolol-d_7, sertraline-d_3, venlafaxine-d_6, atenolol-d_5, oxazepam-d_5, *o*-demesmethylvenlafaxine-d_6. Diltiazem-d_3, trimethoprim-d_9, raloxifene-d_4, verapamil-d_7, lidocaine-d_6, triamterene-d_6, sulfamethoxazole-d_4, sitagliptin-d_4, oseltamivir-d_5 and carbamazepine-d_{10}. Finally, metformin-d_6 was purchased from Cambridge Bioscience (Cambridge, UK). Internal standards were either purchased or prepared to a stock concentration of 0.1 mg/mL in methanol and stored at -18°C. A spiking solution, called the internal standard solution (ISS) of 16 µg/L was prepared in methanol. The ISS, when 5 µL was spiked into 995 µL of sample, gave a final concentration of 80 ng/L of each pharmaceutical.

4.4 Sample preparation for analysis

4.4.1 Environmental samples

Samples were fully thawed, vortexed and a 995 µL aliquot pipetted into a 1.5 mL LC vial (Fisher Science, UK). A 5 µL spike of ISS was added and the vial vortexed for at least 5 seconds to ensure adequate mixing. Samples were immediately analysed after preparation. After analysis, samples were returned to -18°C for storage.

Table 7. The optimised mass spectrometer operating conditions. The bold transition is the quantification transition. When no corresponding internal standard (ILIS) is reported, atrazine-d_5 was used as the IS.

Pharmaceutical	Quantifier and qualifier transitions	Collision energy	Retention time
Amitriptyline	**278.2 → 233.1**	18.0	12.45
	278.2 → 191.1	25.6	
Amitriptyline-d_3	**281.2 → 91.2**	23.9	12.46
Atenolol	**267.2 → 145.0**	26.1	4.30
	267.2 → 190.0	19.2	
Atenolol-d_7	**274.3 → 145.1**	24.9	4.25
Atrazine-d_5	**221.2 → 179.1**	18.3	12.41
Carbamazepine	**237.1 → 194.1**	25.0	11.61
	237.1 → 192.1	31.0	
Carbamazepine-d_{10}	**247.2 → 204.0**	20.2	11.49
Cimetidine	**253.2 → 159.0**	14.4	4.39
	253.2 → 95.2	24.2	
Citalopram	**325.2 → 109.1**	26.5	10.02
	325.2 → 262.0	19.6	
Citalopram-d_6	**331.2 → 109.1**	26.1	10.03
Codeine	**300.1 → 215.1**	28.1	4.80
	300.1 → 225.1	26.5	
Codeine-d_6	**306.3 → 165**	39.4	4.82
Desvenlafaxine	**264.1 → 58.3**	17.9	7.27
	264.1 → 107.1	30.3	
Devenlafaxine-d_6	**270.2 → 64.3**	17.4	7.22
Diazepam	**285.0 → 193.0**	31.7	14.02
	285.0 → 154.0	26.5	
Diazepam-d_5	**290.1 → 198.0**	33.8	13.95
Diltiazem	**415.1 → 177.9**	24.1	11.1
	415.1 → 150.0	42.1	
Diltiazem-d_3	**418.2 → 178.0**	23.5	11.09

Table 7. The optimised mass spectrometer operating conditions. The bold transition is the quantification transition. When no corresponding internal standard (ILIS) is reported, atrazine-d_5 was used as the IS.

Pharmaceutical	Quantifier and qualifier transitions	Collision energy	Retention time
Diphenhydramine	**256.0 → 167.1**	10.3	10.20
	256.0 → 152.1	35.6	
Diphenhydramine-d_3	**259.0→ 167.0**	11.2	10.25
Erythromycin	**734.4 → 576.3**	15.2	12.43
	734.4 → 158.0	24.6	
Fexofenadine	**502.4 → 466.2**	25.6	12.24
	502.4 → 484.2	20.3	
Gabapentin	**172.3 → 154.0**	11.8	5.35
	172.3 → 137.1	14.8	
Gabapentin-d_{10}	**182.2 → 163.9**	10.3	5.28
Hydrocodone	**300.2 → 199.0**	29.5	5.35
	300.2 → 171	11.8	
Hydrocodone-d_3	**303.2 → 199.1**	30.4	5.36
Lidocaine	**235.2 → 86.2**	17.3	6.69
	235.2 → 58.3	32.8	
Lidocaine-d_6	**241.3 → 86.2**	18.4	6.66
Loratadine	**383.1 → 336.9**	23.2	15.71
	383.1 → 267.1	42.5	
	383.1 → 259.1	30.7	
Metformin	**130.2 →60.3**	11.7	1.39
	130.2 → 71.3	20.2	
Metformin-d_6	**136.2 → 77.1**	21.0	1.36
Noreistherone	**299.2 → 109.1**	26.5	13.80
	299.2 → 83.2	29.5	
Oseltamivir	**313.2 → 166.1**	18.4	10.51
	313.2 → 225.1	10.25	
Oxazepam	**289.9 → 240.9**	18.4	12.77
	289.9 → 268.9	10.3	
Oxazepam-d_5	**292.2 → 246**	21.4	12.72

Table 7. (continued) The optimised mass spectrometer operating conditions. The bold transition is the quantification transition. When no corresponding internal standard is reported, atrazine-d_5 was used as the IS.

Pharmaceutical	Quantifier and qualifier transitions	Collision energy	Retention time
Paracetamol	**152.0 → 110.1**	14.2	4.04
	152.0 → 93.1	20.3	
Paracetamol-d_4	156.2 → 114.1	17.1	4.01
Propranolol	**260.2 → 116.1**	18.2	9.90
	260.2 → 183.0	17.8	
Propranolol-d_7	**267.2 → 116.2**	17.8	9.84
Raloxifene	**474.2 →112.1**	30.9	10.15
	474.2 → 84.3	46.6	
Raloxifene-d_4	**478.2 → 116.2**	29.4	10.15
Ranitidine	**315.2 → 176.0**	16.8	4.32
	315.2 → 130.0	24.6	
Sertraline	**306.1 →159.0**	20.0	13.33
	306.1 → 129.1	22.1	
	306.1 → 274.9	19.0	
Sertraline-d_3	**308.9 →274.9**	10.3	13.25
Sitagliptin	**408.1 → 235.0**	18.3	7.91
	408.1 → 174.0	26.2	
Sitagliptin-d_4	**412.2 → 239.1**	17.7	7.88
Sulfamethoxazole	**254.1 → 156.0**	17.7	6.82
	254.1 → 108.1	25.2	
Sulfamethoxazole-d_4	**258.2 → 160.0**	16.7	6.78
Temazepam	**301.1 → 255.0**	21.6	13.14
	301.1 → 283.0	13.1	
Temazepam-d_5	**305.8 → 260.1**	22.4	13.09
Tramadol	**264.1 → 58.4**	15.1	7.51
	264.1 → 43.4	50.3	
Triamterene	**254.0 → 237.0**	17.7	7.10
	254 .0→ 104.0	25.2	

Table 7. (continued) The optimised mass spectrometer operating conditions. The bold transition is the quantification transition. When no corresponding internal standard is reported, atrazine-d_5 was used as the IS.

Pharmaceutical	Quantifier and qualifier transitions	Collision energy	Retention time
Triamterene-d_5	**259.2 → 242.1**	27.9	7.03
Trimethoprim	**291.2 → 230.1**	24.0	6.06
	291.2 → 261.1	25.0	
Trimethoprim-d_9	**300.2 → 234.0**	24.2	5.98
Venlafaxine	**278.2 → 260.1**	11.6	9.42
	278.2 → 58.1	20.0	
Venlafaxine-d_6	**284.2 →121.1**	28.1	9.41
Verapamil	**455.3 → 165.0**	28.4	11.18
	455.3 → 150.0	38.4	
Verapamil-d_7	**462.4 → 165.1**	29.0	11.16

4.4.2 Calibration solutions

Calibration solutions were prepared by dilution of an intermediate calibration solution. The intermediate calibration solution contained all pharmaceuticals at a concentration of 0.02 mg/mL in methanol and was diluted to 0.0004 mg/mL. The 15 calibration levels in Table 8 were prepared by dilution of the 0.0004 mg/mL calibration solution. Once calibration solutions were prepared, 20 µL of the appropriate solution was pipetted into 975 µL of HPLC grade water, spiked with 5 µL of ISS and vortexed for at least 5 seconds. A fresh batch of calibration solutions was prepared daily.

4.4.3 Quality control and assurance samples

4.4.3.1 Laboratory blanks

Laboratory reagent blanks (LRB) were 1000 µL of HPLC grade water. The LRB blanks were injected prior to starting the main sample batch to identify whether the system was free of detectable contamination. Laboratory blanks (LB), which contained 995 µL water and 5 µL of ISS were prepared with the same procedure as environmental samples. LB samples were injected after calibration standards and then after every 12 environmental samples or directly after matrix spikes to identify detectable carryover.

4.4.3.2 Field blank

Three field blanks were collected during field visits to assess possible contamination of sampling materials or cross-contamination between sites. HPLC grade water was brought into the field, filtered and frozen alongside and in exactly the same way as the environmental samples. Transported HPLC water itself was also analysed. Both types of field blank were prepared for analysis identically to environmental samples, mixing 995 µL field blank with 5 µL ISS.

4.4.3.3 Continuing Calibration Checks

At least three continuing calibration solutions (CCC) were prepared identically to the calibration solutions at a concentration of 80 ng/L. There was also a limit of quantification check (LOQC) of 4 ng/L prepared with each batch, using calibration level 3 solution and the preparation method described in section 4.4.2.

Table 8. Calibration concentrations used for 15 point calibration in the pharmaceutical quantification method.

Level	Final concentration (ng/L)
15	8000
14	4000
13	2000
12	1400
11	800
10	400
9	200
8	140
7	80
6	40
5	20
4	10
3	4.0
2	2.0
1	1.0

4.4.3.4 Matrix Recovery Checks

Potential matrix effects in all sample matrices were monitored using routinely prepared matrix 'recovery' spikes (MRS). At least three MRS samples from different sampling sites were prepared per analytical batch to monitor analyte response in the sample matrix.[251] Surface water spikes were prepared by spiking 20 µL of level 7 or 9 calibration solution (80 or 200 ng/L) into a sample replicate with 5 µL of ISS. The higher concentration of pharmaceuticals in WWTP influent and effluent required MRS samples to be prepared at a higher concentration, using level 14 (4000 ng/L) calibration solution.

4.5 Quantification of 33 pharmaceuticals using a rapid HPLC-MS/MS method

Quantification of pharmaceutical residues in environmental samples was achieved using HPLC-ESI-MS/MS operating in positive mode. The protocol has been developed with reference to the method published by Furlong et al.[77] The protocol reported has been optimised (Table 7) for application on the York Centre of Excellence in Mass Spectrometry instruments and validated for the study compounds (Chapter 4 Part B).

4.5.1 Instrumental conditions

Prepared samples were placed in the 4°C temperature-controlled autosampler. A 100 µL aliquot was injected onto a column held at 40°C and eluted at a constant flow of 450 µL/min. The reversed phase chromatographic program is summarised in Table 9. All target pharmaceuticals were eluted by 18 minutes, which was followed by 5 minutes at 100% methanol and finally a 7 minute equilibration period prior to the next sample injection, giving a total run time of 30 minutes per sample.

Sample components were ionised by a heated ESI source operating under the following conditions: nebuliser gas (sheath gas) 48 Arb, auxiliary gas 14 Arb, sweep gas 2 Arb, vaporiser temperature 379°C, ion transfer tube temperature 346°C and a static positive ion spray voltage of 3500 V. Detection was achieved with the mass spectrometer operating in MRM. The optimised MRM properties were: a 0.5 second cycle time to ensure at least 15 data points from each eluting peak were collected, collision gas (argon) pressure set to 2 mTorr and quadrupole resolution (Q1, Q3) at full width half maximum

(FWHM) set to 0.7. Two transitions were monitored for each analyte (except internal standards). The *m/z* and collision energy parameters were optimised using the Thermo™ Tune 2.0 software (Table 7).

4.5.2 Peak qualification

For detection, extracted-ion chromatograms (EICs) of both transitions were integrated using the TraceFinder™ Genesis algorithm. Three criteria had to be met in order for target analyte detection. Firstly, the retention time (t_R) at the peak apex for both transitions had to match with the t_R for the corresponding reference standard (t_R within ± 2.5%).[270] Secondly, the ion intensity ratio between the quantifying and qualifying transition needed to match the ion intensity ratio of the corresponding standard. The ion intensity ratio was based on a weighted average of ratios from the calibration standards. The allowable range for ion ratios of unknowns is described in Table 10 according to guidelines established by Commission Decision (2002/657/EC).[271] Quantification was based on the transition with the greater peak area (determined from standards), called the quantification peak. Thirdly, both the quantifier and qualifier peaks needed to have a signal-to-noise ratio of at least 3 to be qualified. Quantification was based on the major transition.

Table 9. Liquid chromatography gradient. Flow and column temperature were maintained at 450 µL/min and 40°C, respectively.

Time (minutes)	% Organic
0	10
1	10
5	40
10	60
15	100
23	100
23.01	10
30	10

Table 10. Maximum permitted tolerances for relative ion intensities using LC-MS/MS. Table reproduced from Commission Decision (2002/657/EC).[271]

Relative ion intensity	Maximum permitted tolerance
> 50 %	± 20 %
> 20 % to 50 %	± 25 %
> 10 % to 20%	± 30 %
≤ 10 %	± 50 %

4.5.3 Calibration

A 15-point internal standard corrected calibration curve covering the range 1 to 8000 ng/L was determined for each analyte. Linear regression analysis was undertaken using the Thermo Scientific TraceFinder 4.0 software. An R^2 of at least 0.98 was considered acceptable[77] and the full calibration range was within the linear response range for all analytes. The broad concentration range was required to encompass the variability between analytes, sampling days and matrices. If the R^2 <0.98 or calibration standards did not meet qualitative peak criteria (4.5.2), points were removed from either the top or bottom of the curve to leave no fewer than 6 points, if necessary to achieve an $R^2 \geq 0.98$. The origin was ignored and a $1/x^2$ calibration weighting was used to improve precision across the wide concentration range. When matrix interferences on an internal standard were suspected, external calibration was evaluated.

4.6 Quality assurance and quality control

A rigorous quality assurance and quality control (QA/QC) plan was followed during method development, sample collection and analysis to produce high quality analytical results. Contamination was minimised by appropriately cleaning reusable glassware (sample bottles and volumetric flasks) by means of a triple methanol rinse after washing and monitoring of procedural and field blanks processed in the same manner as environmental samples. The occurrence of false positives was limited by ensuring all qualitative peak criteria were met. Throughout batch analysis accuracy was monitored using CCC and LOQC samples and required to be within 20% of the true value. To identify

possible column carryover and ensure acceptable calibration across all analyses the following batch order was applied. Two LRB samples followed by an LB sample and the 15-point calibration samples (in random order). After calibration, an additional LB was injected, followed by samples. Each batch included samples, run in a randomised order (three field replicates each); a CCC and LB followed every 10 injections. Two LOQC samples were injected at the end of the batch (4 ng/L and 80 ng/L). Finally, matrix spikes (MRS samples) were evaluated with each batch to assess signal response (suppression or enhancement) during routine analysis. Acceptable matrix 'recovery' was defined as 70 - 120%.[105,272] This monitoring of matrix effects throughout the monitoring campaign is very important for appropriate interpretation of the analytical data obtained over the time course of the experiment. This is because the heterogenous nature of the sample matrix indicates that it may not be the same from one sampling day to the next. Therefore, these routine MRS samples provide an indication of whether the internal standards are compensating well for matrix effects in a particular set of samples or whether these particular data should be interpreted with caution. Finally, environmental sample concentrations falling between the LOD and the LOQ were also flagged as semi-quantitative.

Assessment of blanks was as follows: if the concentration in the LB was less than the LOQC and 10 times less than relevant environmental detections, no action was taken; blank subtraction was not used.[77,273] If the concentration in environmental samples was less than 10 times the blank concentrations, the instrument was cleaned and affected samples re-run. If no improvement was observed, results were considered semi-quantitative.

Field blanks (CF and CNF) were also prepared in triplicate with each batch. Quantitative detections (near or above the LOQ) in these blanks were considered an indication that contamination in the field had occurred. Detections in field blanks were compared to the relevant HPLC water (not-filtered) field blanks to determine if the reagent water was the source of the contamination; if this was the case, no action was taken. If this did not explain the contamination of the field blank (filtered), results for the affected analytes were flagged and considered semi-quantitative, similarly to the procedure with LBs.

Chapter 4

Part B: Analytical method validation

4.7 Introduction

The analytical method described in Chapter 4A was adapted from a fully validated method developed by the US Geological Survey for the detection of 110 pharmaceuticals and wastewater indicators.[77] The Furlong et al.[77] method was used for the Chapter 3 scoping study where it was determined that many of the pharmaceuticals targeted in the original method are used too infrequently in the UK to be detected. Therefore, to save time and cost, 32 of the original pharmaceuticals were included in this new method, along with the addition of a new highly prescribed and potentially ecotoxic anticonvulsant,[274] gabapentin. The decrease in number of study compounds permitted two new methodological goals: to lower detection limits to 10 ng/L, and halve the chromatographic program from 1 hour to 30 minutes.

A partial method validation was deemed sufficient to assess the impact of the chromatographic changes and method performance on different analytical instrumentation from the original Furlong et al.[77] methodology. The validation followed the approach outlined by Commission Decision 2002/657/EC.[271] Therefore, precision (inter- and intra-day), LOD, LOQ, analyte response and blank detection were assessed in blank matrix (HPLC-grade water). In addition, 'recovery' (analyte response) in surface water, WWTP influent and effluent were assessed to estimate method performance in all studied matrices.[275] The aim of the work described in this chapter was to validate the method described in Chapter 4A and demonstrate its suitability for the detection and quantification of all study compounds in sampled matrices.

4.8 Validation methods

4.8.1 Intra-/inter-day precision

Repeatability (intra-day precision) was expressed as the % relative standard deviation (%RSD) resulting from six replicate injections of solutions prepared at concentrations of 10 ng/L, 80 ng/L and 200 ng/L during a single day. Inter-day repeatability consisted of six replicate injections of 10 ng/L, 80 ng/L and 200 ng/L solutions preformed on three separate days and was expressed as %RSD.

4.8.2 Linearity

Linearity is reported as the coefficient of determination (R^2) resulting from the linear regression fitted to the calibration line described in Section 4.5.3 using TraceFinder™ 4.0 Software. The goal was to achieve an R^2 of ≥ 0.98 over the entire 1 – 8000 ng/L range to ensure all analyte responses were within their linear dynamic range.

4.8.3 Limits of detection and quantification

The limit of detection was calculated from the USEPA (2016) approach using Equation 4.1. The standard deviation (SD) of seven replicates at a concentration 2-5 times greater than an estimated LOD (signal-to-noise (S/N) ≤ 3) was multiplied by the Student's t-value for a single-tailed 99th percentile test suitable for the degrees of freedom. To determine the LOQ, a multiplier from 2-5 was applied to the LOD.[245] The suitable multiplier was that which produced the closest value to the lowest calibration level where six replicate injections yielded an RSD ≤ 20% and ±20% recovery.[273]

$$LOD = t_{(n-1,\ \alpha=0.99)} \times SD \qquad \text{Equation 4.1}$$

4.8.4 Blanks

A series of LBs (n=10) was prepared and run prior to injection of environmental samples. These LBs were subject to the same data quantification procedures as all other QC and unknown samples. The LBs were assessed to determine if there was contamination present in the system or reagents prior to environmental analysis. LB samples were also prepared with and run alongside environmental samples in all sample batches run throughout the study. The mean and SD of LBs (n=92) from all analysis batches was calculated to give an indication of regularly-occurring sample contamination. LBs were also processed with respective analysis batches to identify localised contamination of environmental samples. Recurring and localised contamination is reported as well as any action taken such as flagging or re-analysis.

4.8.5 Recovery and accuracy

Due to the cost of certified reference materials, an assessment of trueness was not undertaken. Analyte response – referred to as recovery – from spiked samples was instead used to gauge method accuracy.[271] Method recovery is presented for target analytes in HPLC water, since sampled matrices (surface water, WWTP influent and effluent) contain heterogeneous ambient pharmaceutical concentrations. Five

concentrations (six replicates each) corresponding to the commonly detected pharmaceutical ranges (4 ng/L, 10 ng/L, 20 ng/L, 80 ng/L and 200 ng/L) were prepared by spiking 975 µL HPLC grade water with 5 µL ISS and 20 µL of the relevant calibration solution. The recovery (%) was calculated by dividing the measured concentration by the known spiked concentration and multiplying by 100. Recovery was considered acceptable when it fell between 70 - 120% and had an %RSD≤20,[105,272] however an RSD<30% was considered acceptable near the quantification limit in accordance with.[273]

4.8.5.1 Matrix recovery

To gauge the level of matrix interferences and their potential impact on accuracy and precision in all matrices sampled for this work, an assessment of matrix effects was undertaken. It is important to note that this is not a true assessment of recovery, as no sample pre-treatment (e.g. pre-concentration) which could affect the recovery of target analytes is undertaken. It is termed matrix recovery to be consistent with environmental science nomenclature,[77,273] but is an assessment of signal response, not analyte recovery. To gauge the level of matrix effects across the calibration range and evaluate whether strategies other than internal calibration should be considered, surface water, WWTP influent and effluent were spiked and matrix 'recovery' assessed. Surface water was spiked at 4 ng/L, 20 ng/L, 80 ng/L, 200 ng/L and 800 ng/L, 8 replicates each. Three unspiked replicates were also analysed in order to subtract the ambient concentration. Effluent and influent were spiked at a concentration of 4 ng/L, 20 ng/L, 800 ng/L and 4000 ng/L to reflect the range in concentrations expected in these matrices. Similarly to surface water, ambient concentrations were predetermined and subtracted from spiked results. To calculate matrix 'recovery', ambient concentration-corrected spike concentrations were divided by the concentration spiked and multiplied by 100.

4.8.6 Sample filtration

The filtering of samples in the field is beneficial as it removes particulates; this treatment can extend HPLC column life, reduce instrument maintenance as well as remove bacteria associated with particulates that could facilitate analyte degradation. There is a possibility that analytes could be retained on the filter; however pharmaceutical filtration studies including 26 compounds (acids, bases and amphoteres) ranging in hydrophobicity (logKow -2.3 to 6.3) suggest these losses will be insignificant (<5%),[264]

thus an assessment of filter losses has not been repeated here. While there are benefits to filtering samples, the particulates removed by the filter could have a significant portion of pharmaceuticals bound to them.[276,277] This suspended particulate matter (SPM) is frequently overlooked in aquatic pharmaceutical analysis due to the large sample volume required to analyse it.[277] In the environment, analytes may sorb and desorb from particulates, thus only looking in the dissolved phase could underestimate riverine concentrations for certain compounds. This is important as SPM bound pharmaceuticals could still be available for organism uptake and thus not including the SPM fraction could underestimate risk.[263] Due to the size of the monitoring campaign described in Chapter 5, it was not possible to also sample and analyse the SPM in addition to the dissolved phase and this is therefore an important limitation of the data presented in Chapter 5.

4.9 Results and discussion

4.9.1 Precision

The intra-day repeatability produced a mean %RSD of 12, 5.5 and 5.9 at 10, 80 and 200 ng/L, respectively, with an overall range of 1.9 to 27.3% (Table 11). Inter-day repeatability produced slightly higher mean %RSDs of 18, 7.6 and 11 for 10, 80 and 200 ng/L, respectively, with an overall range of 9.5 to 35%. The LOD and LOQ for several compounds was close to or above10 ng/L (Table 11), so that greater variability between measurements resulting in higher %RSDs can be expected.[278] The precision, expressed as repeatability, was poorer for inter-day measurements than intra-day, indicating that better precision is achieved within analytical batches than between them. This is likely due to the greater variability incorporated in inter-day measurements (i.e. different preparation days and analysis batches). Campos-Mãnas et al.[267] observed increasing precision with increasing concentration and lower intra-day precision than inter-day precision, similarly to that observed here. Overall the results indicate that the method is sufficiently reproducible during and across analysis days according to USEPA[273] and Boix et al.[105] where an RSD≥ 20% is desirable above the LOQ.

Table 11. The repeatability (intra-day) and intermediate precision (inter-day) reported in %RSD for three fortification concentrations (n=6). The linearity is reported as R^2 from a 1-8000 ng/L linear regression.

Pharmaceutical	Linearity		Repeatability (intra-day)			Intermediate precision (inter-day)		
		Range	10 ng/L	80 ng/L	200 ng/L	10 ng/L	80 ng/L	200 ng/L
Amitriptyline	0.999	2-8000	13.6	3.3	6.7	19.0	7.9	4.4
Atenolol	0.993	10-8000	16.0	4.9	6.5	20.8	8.3	12.4
Carbamazepine	0.998	1-8000	4.8	3.5	3.0	17.9	4.4	8.6
Cimetidine	0.994	10-8000	13.2	5.9	4.1	19.5	5.8	14.9
Citalopram	0.998	4-8000	10.4	3.9	3.2	10.8	8.4	10.2
Codeine	0.995	4-8000	13.6	8.7	14.6	19.2	10.2	13.1
Desvenlafaxine	0.997	10-8000	8.2	4.6	4.5	17.4	4.3	6.7
Diazepam	0.998	2-8000	5.4	3.0	2.9	14.5	5.1	6.8
Diltiazem	0.997	2-8000	13.7	8.4	2.8	19.6	9.5	9.7
Diphenhydramine	0.996	2-8000	9.1	3.9	6.3	15.1	6.4	9.7
Erythromycin	0.990	20-8000	<LOD	10.3	19.8	<LOD	10.4	16.4
Fexofenadine	0.999	10-8000	6.4	1.9	2.9	11.8	8.2	9.2
Gabapentin	0.999	20-8000	<LOD	6.6	6.5	<LOD	11.3	13.7
Hydrocodone	0.996	1-8000	10.1	4.7	5.8	14.1	7.8	6.5
Lidocaine	0.996	1-8000	9.7	2.2	4.1	11.7	3.8	10.2
Loratadine	0.998	10-8000	19.8	15.5	6.4	23.4	11.8	16.9
Metformin	0.999	10-8000	6.8	7.0	5.2	9.9	6.3	12.0
Noreistherone	0.998	10-8000	17.3	8.0	2.7	26.6	6.8	7.4
Oseltamivir	0.997	10-1000	17.5	6.7	2.7	19.0	7.1	15.6
Oxazepam	0.998	20-8000	15.6	3.8	4.0	23.0	6.0	9.6
Paracetamol	0.997	20-8000	15.5	5.0	3.8	20.7	11.5	9.4
Propranolol	0.998	10-8000	9.7	4.2	5.7	19.6	5.6	8.5
Raloxifene	0.994	20-1000	12.6	7.7	10.9	23.8	13.1	13.7

Table 11. (continued) The repeatability (intra-day) and intermediate precision (inter-day) reported in %RSD for three fortification concentrations (n=6). The linearity is reported as R^2 from a 1-8000 ng/L linear regression.

Pharmaceutical	Linearity	Range	Repeatability (intra-day)			Intermediate precision (inter-day)		
			10 ng/L	80 ng/L	200 ng/L	10 ng/L	80 ng/L	200 ng/L
Ranitidine	0.999	10-8000	17.8	5.3	6.5	22.8	6.7	14.2
Sertraline	0.995	10-8000	13.8	3.8	4.0	35.2	6.6	15.3
Sitagliptin	0.999	10-8000	10.4	3.6	5.4	14.6	7.5	13.0
Sulfamethoxazole	0.997	10-8000	13.5	6.5	4.8	21.9	6.6	10.4
Temazepam	0.998	4-8000	9.3	4.0	4.0	18.5	6.5	7.4
Tramadol	0.998	4-8000	11.9	2.7	3.5	10.7	6.5	11.7
Triamterene	0.991	20-8000	<LOD	6.5	7.4	<LOD	9.2	10.6
Trimethoprim	0.996	1-8000	6.5	4.6	3.4	16.7	4.2	5.4
Venlafaxine	0.998	2-8000	10.8	4.4	2.5	9.5	7.4	8.0
Verapamil	0.993	20-8000	<LOD	6.9	19.6	<LOD	8.0	16.1

4.9.2 Linearity

The R^2 was used as a measure of goodness-of-fit for linear regressions fitted to calibration points over the 1 - 8000 ng/L (or the range reported for each analyte in Table 10). The R^2 was consistently > 0.99 for all target analytes, indicating linearity is achieved over their dynamic range (Table 11).

4.9.3 Limits of detection and quantification

The average LOD was 4.9 ng/L and ranged from 0.9 ng/L (carbamazepine) to 12.4 ng/L (gabapentin) (Table 12). An LOD <10 ng/L was achieved for 91% of analytes, while LODs for triamterene, erythromycin and gabapentin were slightly greater. An estimated LOD based on an S/N ratio was not possible to calculate for fourteen analytes because of inconsistent or impossible S/N (infinite) at the lowest concentration level or signal disappearing from one concentration level to the next. The average LOQ was 12.0 ng/L and ranged from 1.8 ng/L (carbamazepine) to 37.2 ng/L (gabapentin) (Table 12).

The LODs/LOQs reported here are lower than comparable recent rapid screening methods (Figure 20),[77,106,267] with the exception of those for paracetamol, erythromycin, sulfamethoxazole, atenolol and gabapentin.[105,268] Lower LODs, especially for gabapentin (0.6 ng/L),[42] have been reported, however, these were achieved by methods that employ sample pre-concentration/clean-up.[279–282] Several studies calculated LODs based on an S/N estimation, which is less conservative.[278] LODs estimated using this approach where possible were also lower in this study (Table 12); however the evaluation was severely limited by impossible or irreproducible S/N ratios for almost half of the analytes. A mass spectrometer operating in MRM mode can produce little to no background signal, especially when analysing standards.[283,284] The S/N approach, however, is limited to methods that produce baseline noise, especially in the absence of target analytes.[285] Therefore the S/N approach may not be best suited for the evaluation of detection limits for MRM trace analyses. Overall, almost half of the analytes (40%) had an LOQ below 10 ng/L, while none exceeded 40 ng/L (Table 12); this demonstrates the analytical method to be reproducible at low ng/L concentrations and comparable to both recently published rapid determination and extended sample preparation analytical methods (Figure 20).

Table 12. Results from the LOD/LOQ determinations, seven replicates were used in the S/N and calibration level approaches.

Compound	LOD determination		LOQ determination	
	LOD[a]	LOD[b]	Test level	LOQ[b]
	(S/N) = 3	= SD x t-stat$_{(n-1,\ \alpha=0.99)}$ (n=21)	Calibration level = %RSD ≤ 20	LOD x (2 to 5)
Amitriptyline	2.1	1.1	4.0	2.2
Atenolol	13.3	8.9	20.0	17.7
Carbamazepine	0.5	0.9	2.0	1.8
Cimetidine	1.4	2.0	10.0	10.2
Citalopram	0.4	2.1	4.0	4.3
Codeine	-	2.6	10.0	7.8
Desvenlafaxine	1.0	2.2	10.0	10.7

Table 12. (continued) Results from the LOD/LOQ determinations, seven replicates were used in the S/N and calibration level approaches.

Compound	LOD determination		LOQ determination	
	LOD[a]	LOD[b]	Test level	LOQ[b]
	(S/N) = 3	= SD x t-stat$_{(n-1, \alpha=0.99)}$ (n=21)	Calibration level = %RSD ≤ 20	LOD x (2 to 5)
Diazepam	2.0	1.4	4.0	2.8
Diltiazem	-	1.1	2.0	2.2
Diphenhydramine	-	1.2	2.0	2.3
Erythromycin	-	11.2	20.0	22.3
Fexofenadine	-	2.0	10.0	10.3
Gabapentin	20.6	12.4	40.0	37.2
Hydrocodone	-	1.0	2.0	2.0
Lidocaine	2.3	1.4	4.0	2.8
Loratadine	-	5.0	10.0	10.1
Metformin	2.3	4.2	10.0	12.6
Noreistherone	0.5	7.3	10.0	14.5
Oseltamivir	4.7	6.7	10.0	13.3
Oxazepam	-	5.4	20.0	21.5
Paracetamol	11.3	7.1	20.0	21.3
Propranolol	1.0	6.5	10.0	13.0
Raloxifene	-	6.3	10.0	19.2
Ranitidine	4.1	6.2	20.0	18.7
Sertraline	8.6	9.1	20.0	18.3
Sitagliptin	-	7.1	10.0	14.1
Sulfamethoxazole	24.9	9.1	20.0	18.2
Temazepam	5.0	3.6	10.0	7.2
Tramadol	4.0	3.6	10.0	10.6
Triamterene	-	10.8	20.0	21.6

Compound	LOD determination		LOQ determination	
	LOD[a]	LOD[b]	Test level	LOQ[b]
	(S/N) = 3	= SD x t-stat$_{(n-1,\ \alpha=0.99)}$ (n=21)	Calibration level = %RSD ≤ 20	LOD x (2 to 5)
Trimethoprim	0.7	1.3	2.0	2.6
Venlafaxine	1.6	1.5	4.0	3.1
Verapamil	-	10.1	20.0	20.2

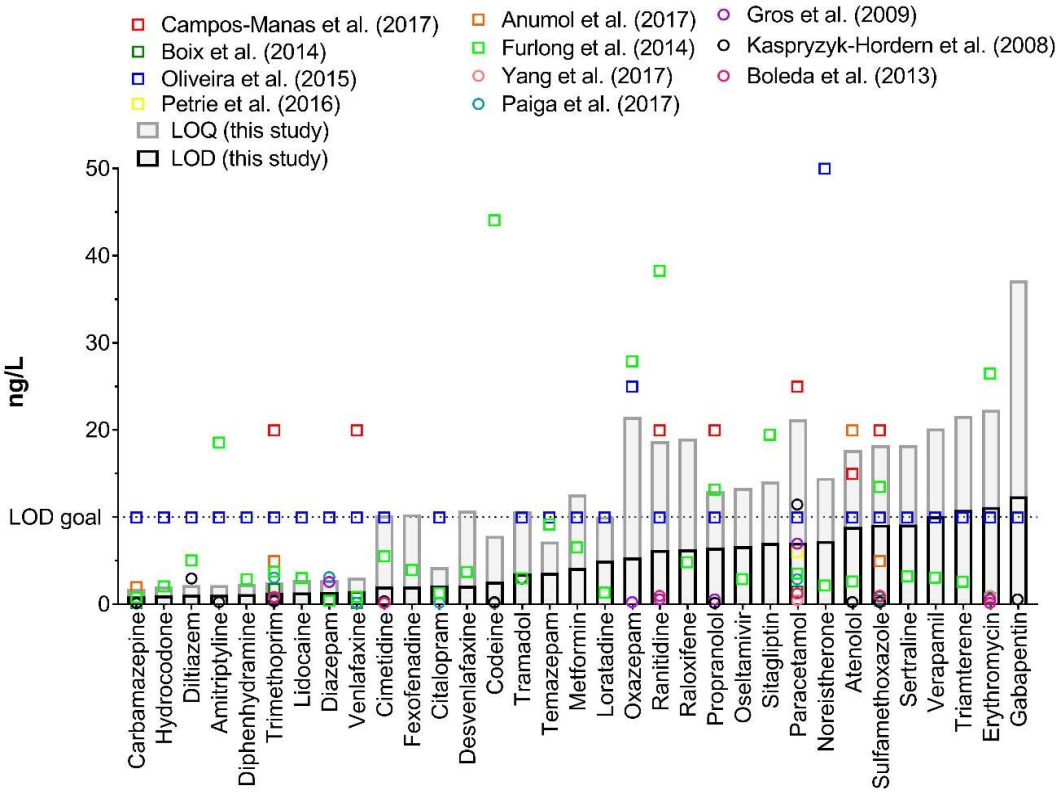


Figure 20. LODs and LOQs determined in reagent water for all analytes. LOQs from published methods quantifying similar compounds are also presented. A circle symbol indicates the method uses a sample pre-concentration/clean-up step, while squares represent similar rapid determination methods. The bars represent the LODs (black) and LOQs (grey) reported in this study.

4.9.4 Assessment of blanks

A series of 10 LB replicates was analysed prior to any environmental samples. No target analytes were quantifiable according to the assessment criteria outlined in Section 4.6. Therefore contamination stemming from laboratory reagents and analysis prior to the introduction of environmental samples is reported as negligible.

4.9.5 Recovery from HPLC water

The concentrations chosen to evaluate matrix recovery from HPLC water (4 to 200 ng/L) for the validation process were based on results from the scoping study where the average river concentration was determined to be 53 ng/L (Chapter 3). Recovery was considered acceptable when it fell between 70-120% and had an %RSD≤20,[77,105,272] the area bound by the x-axis and the dotted line in Figure 21. Acceptable recovery and %RSD were achieved for all compounds at each concentration in HPLC-grade water, Figure 21. Analytes with LODs greater than spiked concentrations were not included in respective box plots as they were not detected (i.e. 4 ng/L, n=13). Overall, these results provide sufficient evidence that matrix recovery (in HPLC water) is acceptable across the low to mid-range of the calibration curve. Higher levels were not assessed due to the decreasing trend in %RSD and recovery moving closer to 100% with increasing concentration, a similar trend observed by Furlong et al.[77] Higher concentrations were also less likely to be observed during the environmental monitoring campaigns for the majority of analytes.

4.9.6 Recovery from environmental matrices

The assessment of matrix recovery (i.e. signal response) is critical to LC-MS/MS quantitative analysis in complex environmental samples.[275] This is due to the occurrence of matrix effects arising from the presence of background interferences that can co-elute with target analytes, which can impair quantification past the point of suitability.[246] Matrix inference occurs in the MS source where analytes are competing for charge.[286] Unionized species are removed from the system. "Dirty" matrices, like surface water, could contain many species which can enhance or suppress the ionization of a target analyte.[96] Therefore the signal at the detector can be reduced or increased, affecting measurement accuracy.[286] Matrix interferences can be unpredictable as surface water or WWTP influent and effluent are heterogeneous; consequently replicates can be impacted differently, diminishing precision.[287]

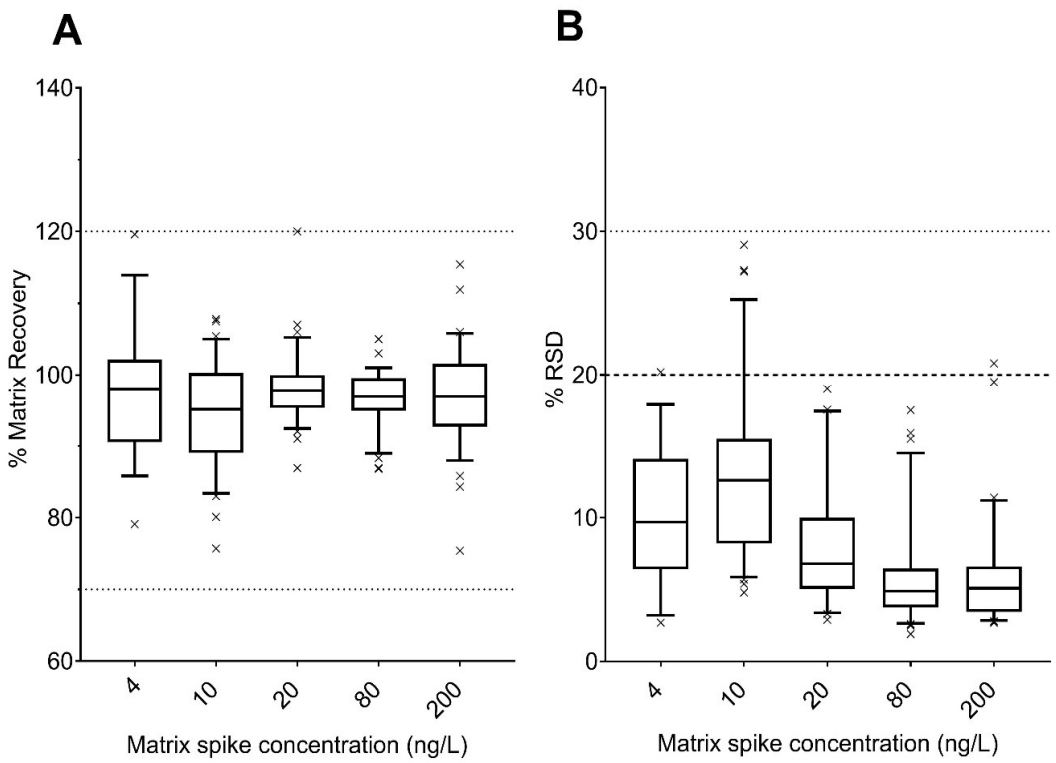


Figure 21. (A) the summary matrix recovery (%) of all target analytes in HPLC-grade water based on 6 replicates at 5 concentration levels, dotted lines represent the desirable 70 – 120% recovery. (B) The respective %RSD calculated from recovery (%) replicates at each concentration level. The dotted line is the desirable 20% RSD and near the LOQ, 30% RSD. Box plots depict the median and interquartile range, while whiskers are the 10^{th} to 90^{th} percentile.

Sample pre-concentration and clean-up steps can help isolate target pharmaceuticals.[288] This is a time consuming process and difficult to apply effectively to a range of physico-chemically diverse pharmaceuticals such as those in the present study. For example, pre-concentration is difficult to optimise and may also concentrate interfering analytes increasing matrix effects,[289] which due to physicochemical similarities may not be removable in the clean-up step, which can also result in analyte losses.[290] Calibration approaches such as matrix-matched, standard addition or internal calibration are other strategies to tackle matrix interferences.[291] A single representative matrix for environmental samples is difficult to find and standard addition requires a great deal of sample and is a tedious and lengthy process. Internal standards, when they co-elute with and behave similarly to target analytes provide good compensation for matrix effects.[291] This was therefore the method of choice to tackle matrix interferences in this study. This

is not a perfect solution because isotopically labelled internal standards can chromatographically separate from the target analyte thereby experiencing a different level of matrix effects than the target analyte,[77] again impacting method accuracy.[292] Therefore it is critical to assess matrix interference in all studied matrices and monitor this performance throughout environmental analysis.[275]

4.9.6.1 Recovery from Surface Water

Median recovery from surface water fell between the range of 70 - 120% at all tested concentrations (Figure 22A). The whiskers, especially for recoveries calculated for 4 ng/L and 20 ng/L, fell outside the 70-120% range and overall recovery is poorer than observed in HPLC-water (Figure 21). Similarly to HPLC water, recovery is again improved with increasing spiked concentration. The %RSD resulting from recoveries from surface water (Figure 22B) is much greater than the %RSD calculated from recoveries from HPLC-grade water Figure 22B. This is likely due to the heterogeneous nature of surface water, where river constituents are likely to be highly variable spatially and temporally than in HPLC-grade water, which should not change significantly in composition. The impact of matrix effects could vary sample to sample due to the heterogeneity of surface water, and the greater variability in % recovery shown by %RSD for surface water over HPLC-grade water could be evidence of this. Overall, the poorer recoveries and higher %RSD for each matrix spike concentration level in Figure 22 compared to Figure 21, are evidence that matrix effects are occurring to a greater degree in surface water than HPLC-grade water.

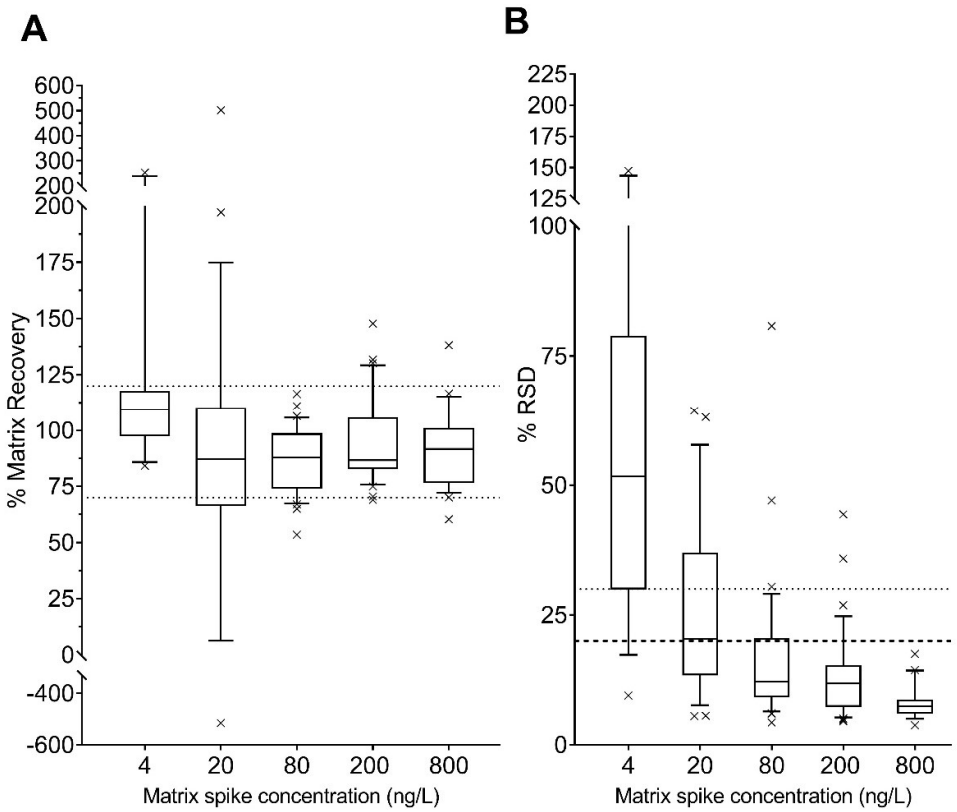


Figure 22. (A) Recovery (%) of target analytes in surface water based on 8 replicates (spiked with 4 ng/L, 20 ng/L, 80 ng/L, 200 ng/L and 800 ng/L), dotted lines represent the desirable 70 – 120% recovery. (B) The respective %RSD calculated from matrix recovery (%). The dotted line is the desirable 20% RSD and near the LOQ, 30% RSD. Box plots depict the median and interquartile range, while whiskers are the 10th Box plots depict the median and interquartile range, while whiskers are the 10th to 90th percentile.

These results also indicate that the matrix effects experienced are sample dependent and even changed from replicate to replicate (evidenced by the high %RSD). This result suggests a standard addition approach to correct for matrix effects would be inappropriate in this matrix, as the magnitude of signal enhancement/suppression observed here is non-linear. Furthermore the tolerance in the measurement of matrix effects is lower at lower concentrations. For example, 95% matrix recovery of 4 ng/L would require measurement to be within ± 0.2 ng/L, while measurements can be ± 4 ng/L to achieve 95% recovery for the 200 ng/L concentration. Figure 22 illustrates this point; the matrix recovery is closer to 100% and the variability in recovery (assessed by %RSD) is reduced at higher concentrations, likely a result of the increasing tolerance in measurement variability. These data indicate that a one-time validation of matrix recovery (e.g. signal response) at several concentrations prior to sampling may not be

the best way to ensure that subsequent quantification is not significantly impacted by matrix effects. Therefore in this study, isotopically labelled internal standards were the most suitable strategy to compensate for matrix effects due to the heterogeneity of the sample matrix. This is not a perfect solution as matrix recovery is not always within the acceptable range (Figure 22A), due to the heterogeneity of surface water. The use of internal standards accompanied by routine checks of matrix recovery at a mid-calibration concentration (80 ng/L or 200 ng/L) to ensure quantification is not significantly impacted (e.g. matrix recovery falls within the 70 – 120% range) for a particular batch of samples was thus the approach chosen.

4.9.6.2 Recovery from WWTP Influent and Effluent

The recoveries from WWTP influent and effluent were much poorer than from HPLC water and surface water (Figure 21 and 22), especially for the 4 ng/L and 20 ng/L matrix spike concentrations (Figure 23 and Figure 24), which is consistent with recoveries from these matrices reported by others.[13,77] The majority of analytes had matrix recoveries between 70 – 120% from both WWTP influent and effluent at the higher spiked concentrations of 800 ng/L and 4000 ng/L. Matrix recoveries across all target analytes was slightly better in effluent than influent, a phenomenon also reported by others.[12,91,105,293] Boix et al.[105] suggested that this was due to WWTP influent being a more complex matrix than effluent, consisting of a greater proportion and diversity of chemical species that can affect ionisation.

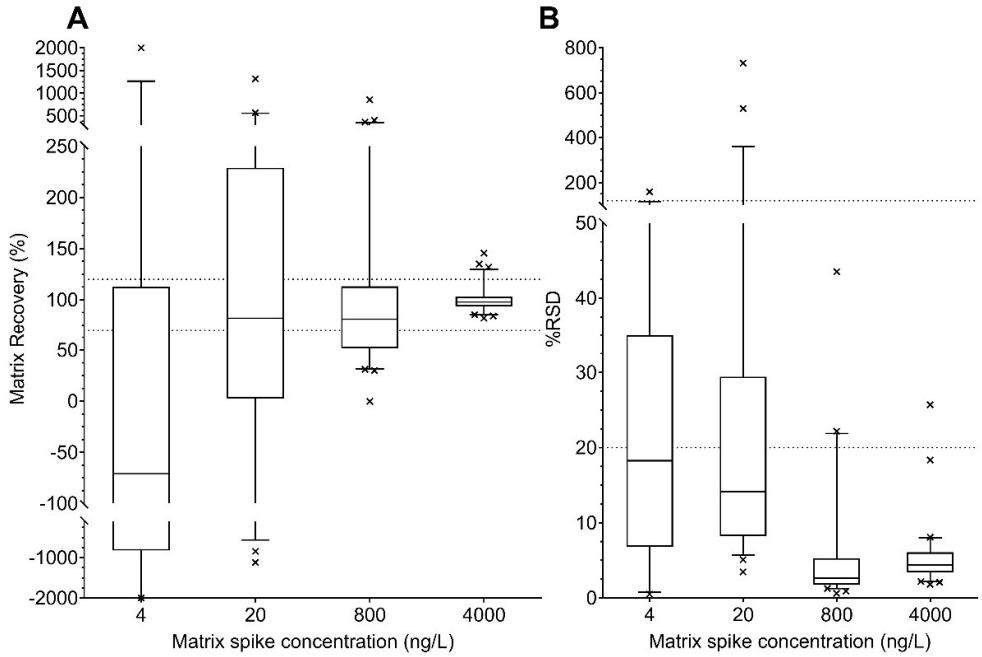


Figure 23. (A) Recovery (%) of target analytes in WWTP influent based on 8 replicates (spiked with 4 ng/L, 20 ng/L, 800 ng/L and 4000 ng/L), dotted lines represent the desirable 70 – 120% recovery. (B) The respective %RSD calculated from matrix recovery (%). The dotted line is the desirable 20% RSD and near the LOQ, 30% RSD. Box plots depict the median and interquartile range, while whiskers are the 10th Box plots depict the median and interquartile range, while whiskers are the 10th to 90th percentile.

WWTP influent and effluent matrix recoveries were similar to those of other rapid quantification methods (i.e. no sample pre-concentration/clean-up).[105,268] Oliveira et al.[268] reported influent matrix recovery for codeine (120%), hydrocodone (115%), trimethoprim (82%), carbamazepine (86%), atenolol (30%), propranolol (110%), diltiazem (121%) and venlafaxine (114%) compared with 66%, 107%, 98%, 86%, 92%, 104%, 113% and 105% observed here for these compounds, respectively. In effluent, Boix et al.[105] reported matrix recovery for venlafaxine (100%), trimethoprim (104%), erythromycin (94%), carbamazepine (94%), sulfamethoxazole (106%) and paracetamol (130%) compared with 105%, 108%, 90%, 98%, 103% and 130% observed here for these compounds, respectively. Overall signal enhancement was observed in both influent and effluent, consistent with the results of others.[268]

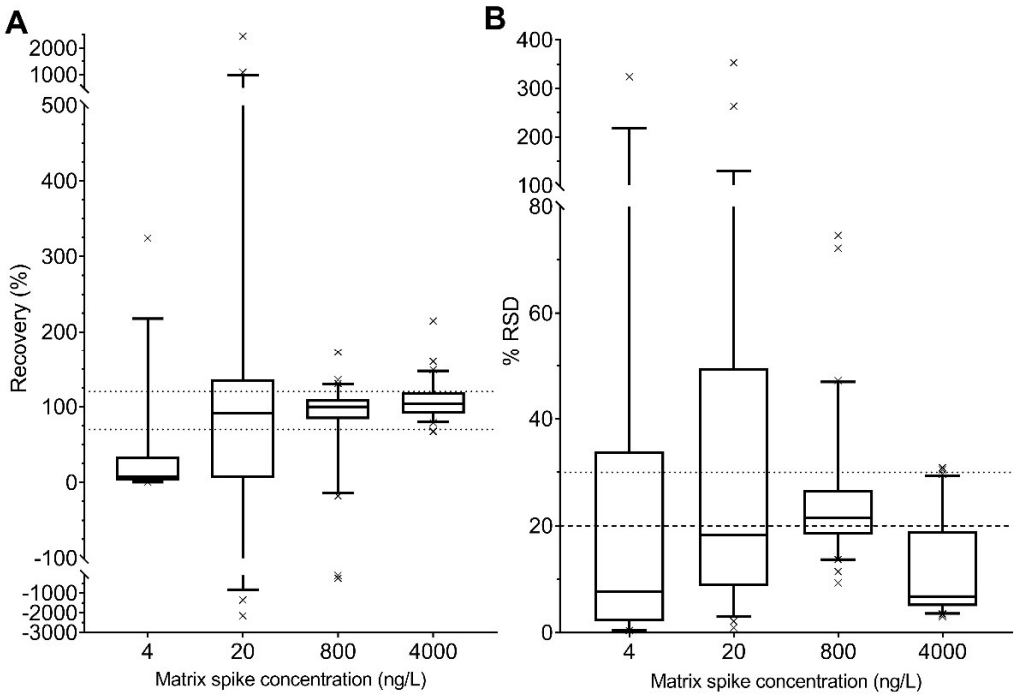


Figure 24. (A) Recovery (%) of target analytes in WWTP effluent based on 8 replicates (spiked with 4 ng/L, 20 ng/L, 800 ng/L and 4000 ng/L), dotted lines represent the desirable 70 – 120% recovery. (B) The respective %RSD calculated from matrix recovery (%). The dotted line is the desirable 20% RSD and near the LOQ, 30% RSD. Box plots depict the median and interquartile range, while whiskers are the 10th Box plots depict the median and interquartile range, while whiskers are the 10th to 90th percentile.

Matrix effects reported varied substantially from study to study, however a general trend of increasing ion suppression/enhancement from surface water to WWTP effluent to WWTP influent was observed.[13] Schlüsener et al.[294] reported ion suppression in WWTP samples was too severe to quantitatively assess burproprion, despite using solid-phase extraction and internal calibration. Similarly, Lajeunesse et al.[91] also did sample pre-concentration and internal calibration and reported average pharmaceutical recoveries from effluent of -31% and from influent, -44%. Moreover, Kosma et al.[295] observed significant signal suppression/enhancement of up to 75% in influent and effluent despite using matrix-matched calibration and sample pre-concentration. Anumol et al.[106] also used a rapid quantification method for a different set of pharmaceuticals than in this study and reported that matrix effects were <30% and significantly lower than other studies using SPE-LC-MS/MS for the same compounds. Others have corrected data based on the magnitude of observed matrix effects;[62,296] however the variability in matrix recovery even from one replicate to the next suggests the level of

suppression/enhancement can be highly variable, evidenced by the large %RSD (>20%) reported for recoveries in WWTP influent (8 analytes) and effluent (9 analytes) (Figure 23 and 24). This was also observed by Oliveira et al.[268] where the %RSD calculated from matrix recoveries in influent and effluent was near 50% for 94% of analytes. This makes correcting for calculated matrix effects difficult, with the best solution likely being to compensate with internal standards and to routinely monitor recoveries using matrix spikes, highlighting exceptionally poor recoveries and interpreting affected data semi-quantitatively. Overall, WWTP influent and effluent recoveries indicate that caution is needed when interpreting quantitative results and estimating WWTP removal efficiencies (Chapter 5).

4.10 Conclusion

The described rapid screening HPLC-MS/MS method can reliably quantify 33 pharmaceuticals over a substantial concentration range in surface water. The method can also be applied to WWTP influent and effluent, although careful monitoring of analyte matrix recovery is required. The validation followed the approach outlined by Commission Decision 2002/657/EC,[271] with further specific guidance from the USEPA[273] and Childress et al.[245] The sample dependent matrix effects observed in the validation of this study indicate that a correction method such as standard addition would be inappropriate as signal suppression/ionisation is non-linear. Therefore, possible matrix interferences require close monitoring of matrix spikes to ensure accurate quantification for each sample batch. A trade-off between lower LODs/LOQs obtained using more intensive sample handling approaches and this rapid sample preparation was observed; however, the reduced chromatographic run time paired with limited sample preparation was highly beneficial for the large sampling campaign described in the following Chapter.

CHAPTER 5

Temporal and spatial variations of pharmaceutical residues in an urban river system

5.0 Introduction

To adequately characterise the temporal and spatial exposure of pharmaceuticals in the environment, robust monitoring campaigns which include seasonal or year-long sampling covering a range of compounds at a reasonable spatial resolution are required. However, only a small number of spatiotemporal exposure studies have been performed that meet these criteria.[39,46,297,298] These exposure studies are extremely valuable as they provide detailed information which can be related back to the myriad of factors (many varying both seasonally and temporally) which influence environmental concentrations of pharmaceuticals including hydrology,[39] WWTP removal efficiency,[299] pharmaceutical usage,[300] and in-stream removal processes (e.g. biodegradation and sorption to sediment).[297,301,302] In combination, the impact of these processes on pharmaceutical exposure and fate is largely unknown but, if better defined, could improve exposure prediction approaches and offer greater confidence, in terms of exposure, when evaluating risks that pharmaceuticals may pose to the environment.

WWTPs are significant sources of pharmaceuticals to the environment.[303] Removal rates are highly variable between treatment types,[20,43] seasons,[62] and even within treatment plants themselves.[51] Moreover, removal rates have only been estimated for a small fraction of the total number of pharmaceuticals in use[304] and only a few studies have reported WWTP removals in the UK specifically.[39,43,305] WWTP removal rates are valuable parameters, and their inclusion in occurrence modelling substantially improves the accuracy of pharmaceutical exposure predictions (Chapter 3).[7]

In this study, the rapid determination aqueous HPLC-MS/MS method for the quantification of 33 physico-chemically diverse pharmaceuticals (Chapter 4) is applied to a year-long surface water exposure campaign conducted during 2016 at 11 sites along the urbanised and larger River Ouse and smaller, more rural River Foss , York, UK (Figure 25). The monthly sampling design provided good temporal resolution, while unparalleled spatial resolution was achieved in the two contrasting river systems. In addition, influent and effluent samples from two of the WWTPs that serve the city were collected when possible and removal efficiencies estimated. The robust data set produced through the monitoring efforts was evaluated for temporal and spatial exposure patterns and

compared with monthly prescription volumes and flow dynamics to identify key exposure drivers in the contrasting river systems.

5.1 Methods

5.1.1 Study area, sampling and quantification

The River Ouse and River Foss were chosen for the study, as they flow through the city of York, UK, and converge downstream of the city centre and were previously studied in Chapter 3 (Figure 25). The two rivers represent differing levels of urbanisation and size. Grab water samples were collected from the network of 11 sampling sites in the same order and on approximately the same day and time each month from January to December 2016 to minimise variability. Site locations were strategically chosen based on their ease of access and position in relation to WWTP outfalls. Both rivers were sampled with sufficient spatial resolution to build concentration profiles and increase the probability of detecting transient pharmaceuticals in the absence of composite sampling techniques. Three WWTPs serve the city within the sampling network (Figure 25). WWTP A is a trickling filter plant and serves a population of 18 600, WWTP B is conventional activated sludge (CAS) facility serving a population of 27 900, while WWTP C is a secondary activated sludge (SAS) plant serving a population of 180 500. WWTP characteristics along with dates of sampling are detailed in Table 13.

5.1.1.1 Sample collection and pharmaceutical quantification

Full details of sample collection, filtration, storage and quantification using the HPLC-MS/MS method are provided in Chapter 4. All samples (surface water or WWTP influent/effluent) were subject to the same filtration and storage protocol. Three field replicates (1 L) were collected from the centroid of flow, sites have been previously determined to be well-mixed, therefore sampling in a single location was deemed appropriate (Appendix 12). All samples were analysed were analysed within seven days of field collection. The concentration reported for each sample per site is the median of the three field replicates collected. A rigorous quality control plan was followed during environmental sample analysis using a series of matrix recovery spikes, calibration solution spikes, field blanks and laboratory blanks randomly dispersed throughout analytical batches (Chapter 4).

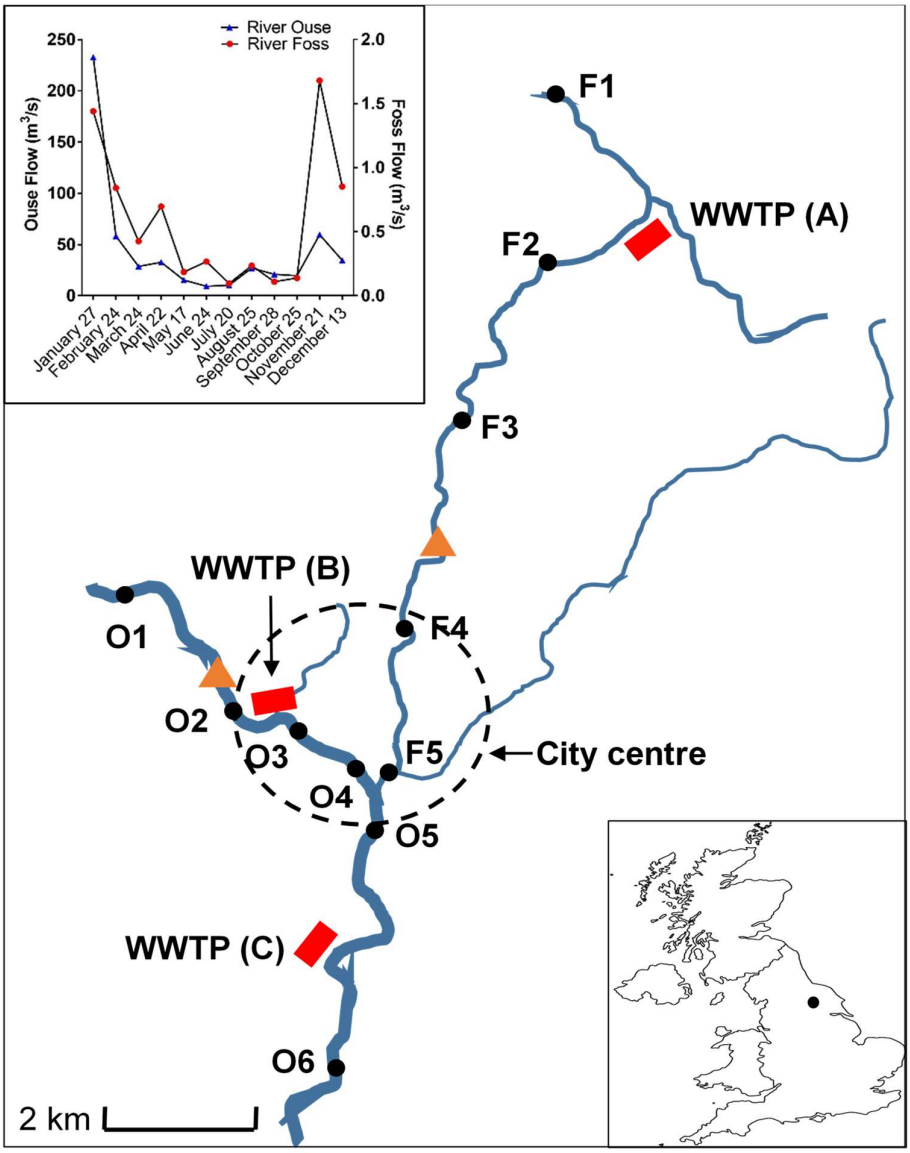


Figure 25. Sampling sites within the sampling network. River flows recorded from a gauge in each river (orange triangle) from each sampling day (m^3/s) are pictured top left. WWTPs that serve the city (3) are represented by the red rectangles. Sites F1-F5 are along the smaller River Foss, while sites O1-O6 are along the larger River Ouse.

5.1.2 Validity of sampling approach

The monitoring data was collected using a grab sampling approach, however this method of assessing pharmaceuticals concentrations has been criticised as it represents a snapshot in time and may not be representative of daily variations in concentration.[60] Due to safety and accessibility concerns, composite samples could not be collected at many of the sampling sites. To ensure the grab samples collected were suitable to estimate annual MECs, paired time-proportional 24 h composite samples and daily grab

samples were collected and compared using pairwise t-tests for pharmaceuticals frequently detected. Paired grab and 24 h composite samples were collected for 7 days in each month during June, July, August and September at the F3 site in the River Foss for a total of 28 paired replicates. Paired samples were also collected over a 7-day period in July downstream of the O5 site in the River Ouse.

5.1.3 WWTP removal efficiency

Due to access restrictions, 24 h composite samples for influent and effluent could only be collected once from WWTP A and B during summer 2016. Only grab samples unsuitable for estimating removals could be collected from WWTP C. WWTP removal efficiency was estimated, when appropriate, for WWTP A and B based on mean influent and effluent concentrations according to Equation 5.1. In this context 'removal' is the change in concentration between influent and effluent which does not represent true removal, but rather partitioning to the solid phase and/or the formation of transformation products. Negative removals can occur, potentially due to sampling limitations (e.g. longer than 24 h hydraulic/sludge retention time)[60] from the conversion of conjugated metabolites back to the parent compound during treatment,[7] or desorption from sludge during secondary treament.[306] The removal efficiency calculations do not account for the hydraulic residence time (HRT) of the sampled WWTPs. Yorkshire Water personnel stated the HRT at WWTP A is approximately 20 hours and approximately eight and 26 hours at WWTP B and C, respectively. The composite samples collected at each WWTP could not be altered to match the HRT. This is a limitation and indicates that removal efficiencies reported need to be interpreted with caution as matching influent and effluent loads may not have been sampled.

$$\% \text{ Removal} = \left(1 - \frac{C_{Effluent}}{C_{Influent}}\right) \times 100 \qquad \text{Equation 5.1}$$

Table 13. Characteristics of WWTPs operating in the sampling area. Composite samples (24 h) were collected in triplicate.

WWTP	Treatment type	Population served	River	Dry weather flow m^3/s	Samples collected (2016)	
					Influent	Effluent
WWTP A	Trickling filter with biological aerated filtration option	18 600	Foss	0.0575	Aug. 23	Aug. 23
WWTP B	Carbon activated sludge with nitrifying filters	27 900	Ouse	0.0452	June 13	June 13
WWTP C	Carbon activated sludge	180 500	Ouse	0.5192	June 27*	June 27*

*Collected sample was a grab sample instead of a 24 h composite.

5.1.4 Statistical analysis

Data analysis was performed using Graphpad Prism.[307] To use statistical tests when non-detects were present, data substitution according to Equation 5.2 was undertaken. This approach was suggested to be appropriate for left censoring of up to 40% of a dataset.[308] If the non-detect frequency for a compound was greater than 40%, it was not included in statistical testing. To determine whether significant spatial differences existed between sites, pairwise t-tests were conducted based on the monthly concentrations.[309] To determine whether any analytes were seasonally variable in each river, concentrations

and mass loads from sites F3-F4 and O3-O4 were grouped by season and a Friedman's Test followed by Dunn's multiple comparisons post hoc test was undertaken. These sites were used in the seasonality test due to their downstream location in relation to WWTP A and B, as well as their location in relation to Environment Agency flow gauges (Figure 1) as the flow recorded at these gauges was not representative of flow conditions at the remaining study sites.[243]

$$\text{Substitution} = \frac{\sqrt{2}}{2} * \text{LOD} \qquad \text{Equation 5.2}$$

Flow data from all sampling days was obtained from the flow gauges maintained by the Environment Agency in both rivers.[243] Prescription data was obtained from the NHS practice-level prescribing data.[310] All relevant medical practices in the local area were identified (Chapter 3) to provide a total monthly prescribed mass (mg) for York (Appendix 13).

5.2 Results and discussion

5.2.1 Quality control results

There were no quantifiable concentrations of any of the target pharmaceuticals in field blanks collected routinely throughout the monitoring campaign. In reagent blanks, no target analytes were detected with the exception of loratadine and raloxifene. Relevant environmental concentrations of loratadine were >10 times greater than the blank concentration which was also less than the LOQ and therefore no action was taken. The laboratory blank detection of raloxifene was 12.6 ng/L and bracketed a single environmental detection of 12.5 ng/L, therefore it could not be distinguished from the blank concentration and a quantitative detection was not reported.

Routine matrix spikes in surface water fell within the acceptable 70 – 120% recovery range, indicating that throughout the sample analysis quantification was not unacceptably impaired due to matrix effects (Figure 26). Matrix effects were observed in effluent and influent, a phenomenon also reported by others suggested to be due the presence a greater proportion of chemical species that can affect consistent ionisation in comparison to surface water.[105,268] In effluent 13% and in influent 19% of analytes fell outside the acceptable matrix signal response, identified in Figure 26. Signal

enhancement was most prominent for diphenhydramine in both influent and effluent (375% and 442%, respectively), while metformin (214%) and tramadol (156%) also exhibited significant signal enhancement in influent. In this study, a slight shift in retention time (t_R) was observed in WWTP influent and effluent in comparison to surface water, which, in addition to a greater number of chemical constituents, could help explain why matrix effects were not well compensated for all analytes using isotopically labelled internal standards.[292] WWTP influent and effluent matrix spikes indicate that caution is needed when interpreting quantitative results and removal efficiencies due to significant matrix effects, while matrix spikes in surface water indicate that matrix effects are sufficiently compensated for by the internal standards.

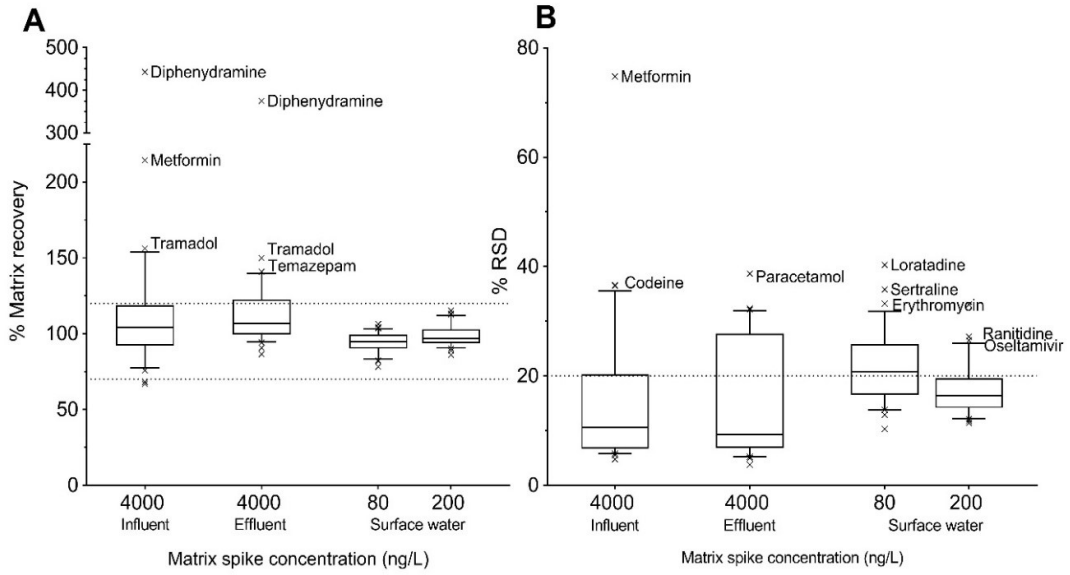


Figure 26. Summary of matrix recovery from routine matrix spikes run alongside each analytical batch of samples during the 2016 monitoring campaign. A) Matrix recovery from spikes in influent and effluent (n=9) and surface water at 80 ng/L (n=34) and 200 ng/L (n=27). The acceptable matrix recovery range is 70 to 120% (bound by the lines on the x-axis). B) The respective %RSD from matrix recovery %, where the line along the x-axis indicates 20%, which was considered ideal. Box plots depict the median and interquartile range, while whiskers are the 10th to 90th percentile.

5.2.2 Validity of sampling approach

In the River Foss, only amitriptyline and lidocaine had significantly different (p<0.05) concentrations between the two sampling approaches. In the River Ouse, only desvenlafaxine had significantly different concentrations between the two sampling approaches. All results are reported in Appendix 14. There are limitations with this comparison of sampling approaches as the validation and potential degradation of samples in the composite sampler was not investigated. The composite samples remain unfiltered and at river temperature for 24 hours and therefore could be subject to substantial microbial degradation, which needs to be confirmed. Several compounds are environmentally persistent (carbamazepine and gabapentin[274,302]) and significant differences were not found between the two sampling approaches for these compounds, suggesting the comparison is suitable. Therefore, based on the current dataset, the small number of significant differences found between sampling approaches does suggest that in this river network the monthly grab sampling approach is suitable for assessing spatial and temporal concentrations trends and for calculating robust annual average MECs to use for model validation (Chapter 6), however a validation of the composite sampling approach used is required to confirm this.

5.2.3 Pharmaceuticals in WWTPs

The highest summed pharmaceutical concentrations in influent were observed in samples from WWTP B, while highest summed concentrations in effluent were observed in samples taken at WWTP A. Paracetamol had the highest concentration in all WWTP influents, 282, 186 and 117 µg/L at WWTP B, A and C, respectively. In effluent, metformin had the highest concentration (6111 ng/L) at WWTP A, while fexofenadine (4770 ng/L) and gabapentin (8451 ng/L) had the highest concentration in effluent at WWTP B and C, respectively. Seven pharmaceuticals (diphenhydramine, norethisterone, oseltamivir, raloxifene, sertraline, triamterene and verapamil) were not detected in any WWTP sample. Average concentration and standard deviation (SD) of WWTP influent and effluent samples are reported in the Table 14.

In a global review of pharmaceuticals in WWTPs, Verlicchi et al.[51] reported influent concentrations for many compounds also observed in WWTP samples in this study. Codeine, paracetamol, gabapentin, hydrocodone, tramadol, erythromycin, trimethoprim,

diltiazem, atenolol, propranolol, carbamazepine, gabapentin, cimetidine and ranitidine influent concentrations all fell within the ranges reported by Verlicchi et al.,[51] while concentrations of amitriptyline were an order of magnitude lower. A study of effluents in the European Union (EU) reported average concentrations an order of magnitude lower than those determined here for tramadol, codeine, citalopram, fexofenadine, diltiazem, ranitidine and amitriptyline, while effluent concentrations were similar for venlafaxine, trimethoprim, carbamazepine and sulfamethoxazole in the York samples.[311]

The estimated removal efficiency in each WWTP is presented for all detected analytes in Figure 27. The median removal efficiency was estimated to be 62% in WWTP A and 37% in WWTP B. Paracetamol was the analyte most efficiently removed at both treatment plants (>99%), while removals greater than 75% were reported for gabapentin, ranitidine, atenolol, sulfamethoxazole, metformin and codeine. Despite being a trickling filter plant and expected to have poorer pharmaceutical removal than CAS systems,[43] WWTP A had similar and even greater removals for select compounds (i.e. carbamazepine, diltiazem, gabapentin and venlafaxine). In the UK specifically, similar removals were reported previously[43] for trimethoprim, amitriptyline, diltiazem, cimetidine, gabapentin and paracetamol, while sulfamethoxazole, erythromycin, codeine, tramadol, carbamazepine, propranolol and ranitidine were, in general, more efficiently removed for this study. WWTPs with similar treatment capabilities were also studied previously in the UK.[43] In comparison with results reported here, WWTP removal rates were highly variable despite operating in the same region and employing similar treatments, a conclusion also observed in other regions.[51] The single sampling event in the WWTPs and composite samples not matching the HRT of sampled WWTPs are major limitations, however these estimates are still useful for comparative purposes. For example, sitagliptin removal efficiency (25 - 40%) has not been previously reported to the authors' knowledge.

Negative removals were observed in both WWTPs. There are several possible reasons for this: firstly influent and effluent are particularly complex matrices and many of these concentrations were detected at ~100 ng/L levels where analytical precision and recovery is poorer, therefore increases could be due to analytical error.[61] Secondly, the hydraulic retention time (HRT) may not be well represented by the 24 h composite

sampling approach.[60,295] Thirdly, the results may be explained by the conversion of glucuronide or sulfato-conjugates back to their parent compounds during treatment.[248,295] Negative removals greater than -200% were observed for hydrocodone at WWTP A and B and this has not been observed by others.[268,312,313] The biotransformation of codeine, dihydrocodine or other synthetic opiates to hydrocodone may be facilitated by bacteria present during water treatment.[314] This could be why large hydrocodone negative removals were observed, however it also could be an artefact of sampling, particle desorption or sludge retention times in the studied WWTPs, more data would be required to determine this.

While WWTPs are significant sources of pharmaceuticals entering the environment, analysis of WWTP removal efficiencies (i.e. reduction in parent pharmaceutical concentration from influent to effluent) as documented in this and previously published studies, demonstrate that WWTPs are significantly decreasing the aquatic environmental burden of select pharmaceuticals by reducing parent pharmaceutical concentrations (not considering degradates or transformation products) for many of the compounds studied.

Table 14. Average influent and effluent concentrations (ng/L) with standard deviation (SD) from 24 h composite samples collected from the two WWTPs that discharge within the sampling area, WWTP A (trickling filter), WWTP B (carbon activated sludge). Samples from WWTP C (secondary activated sludge) are grab samples.

Compound	WWTP A				WWTP B				WWTP C			
	Influent		Effluent		Influent		Effluent		Influent		Effluent	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Amitriptyline	300	14	113	13	163	20	53.9	7.4	15.3	1.2	31.0	0.2
Atenolol	3868	376	359	3.7	2934	69	152	25	1084	97	147	4.3
Carbamazepine	564	19	423	0.6	725	14	722	40	1983	84	544	26
Cimetidine	280	2.3	82.7	9.7	127	14	79.3	11	15.2	0.7	17.7	0.7
Citalopram	313	14	218	18	203	11	218	12	12.0	1.6	158	7.8
Codeine	4315	153	196	14	2935	118	191	13	260	20	408	23
Desvenlafaxine[b]	969	61	545	66	722	12	848	26	231	20	646	25
Diazepam	1.5*	0.2	2.1*	0.2	2.2*	0.4	2.1*	0.3	2.8*	0.4	2.9*	0.2
Diltiazem	626	18	154	3.6	268	21	168	8.2	77.5	8.7	95.8	6.0
Diphenhydramine	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	
Erythromycin	974	84	257	37	924	51	775	62	400	38	394	88
Fexofenadine[a]	4795	122	4770	32	2714	115	2094	113	445	12	2302	47
Gabapentin[b]	30483	2819	3841	397	29244	1265	623	21	31163	612	8451	796
Hydrocodone[b]	49.2	5.9	199	10	44.0	0.9	306	10	2.8	0.2	123	8.1

Table 14. (continued) Average influent and effluent concentrations (ng/L) with standard deviation (SD) from 24 h composite samples collected from the two WWTPs that discharge within the sampling area, WWTP A (trickling filter), WWTP B (carbon activated sludge). Samples from WWTP C (secondary activated sludge) are grab samples.

Compound	WWTP A				WWTP B				WWTP C			
	Influent		Effluent		Influent		Effluent		Influent		Effluent	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lidocaine	100	10	96.6	2.4	124	5.7	90.1	9.2	70.9	5.7	81.2	2.9
Loratadine	5.2*	1.5	n.d		n.d.		6.6*	8.3	n.d		n.d	
Metformin[a]	85875	2352	6111	554	76418	2029	1150	67	13774	1358	976	54
Norethisterone	n.d		n.d		n.d		n.d		n.d		n.d	
Oseltamivir	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	
Oxazepam	26.8	5.7	15.7	1.6	22.3	1.1	28.3	7.1	37.2	4.5	32.8	7.7
Paracetamol[b]	185878	6314	197	2.2	282319	25971	33.3	1.2	116810	7683	27.1	0.9
Propranolol	283	8.4	164	8.8	204	0.6	169	11	28.5	2.9	132	3.8
Raloxifene	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	
Ranitidine[a,b]	746	80	74.2	26	1047	68	846	70	56.7	4.0	102	6.1
Sertraline	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	
Sitagliptin	494	56	273	9.2	742	25	558	40	187	23	263	3.3
Sulfamethoxazole	129	12	5.6	2.4	91.2	1.8	57.4	29	28.2	1.7	11.1	3.2

Table 14. (continued) Average influent and effluent concentrations (ng/L) with standard deviation (SD) from 24 h composite samples collected from the two WWTPs that discharge within the sampling area, WWTP A (trickling filter), WWTP B (carbon activated sludge). Samples from WWTP C (secondary activated sludge) are grab samples.

Compound	WWTP A				WWTP B				WWTP C			
	Influent		Effluent		Influent		Effluent		Influent		Effluent	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Temazepam[a,b]	88.3	4.9	66.4	2.1	58.2	4.2	62.0	2.5	27.6	2.1	48.9	6.9
Tramadol[a,b]	2429	32	1465	54	1474	79	1111	66	562	17	768	12
Triamterene	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	
Trimethoprim	710	2.3	180	1.3	580	56	326	15	47.1	1.9	433	51
Venlafaxine	207	5.9	172	11	1809	68	609	39	18.1	0.7	123	8.7
Verapamil	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	

Removal efficiency was not estimated for WWTP C because collected samples were grab samples.
[a]Matrix recovery fell outside of the 70 – 120% range in influent.
[b]Matrix recovery fell outside of the 70 – 120% range in effluent.

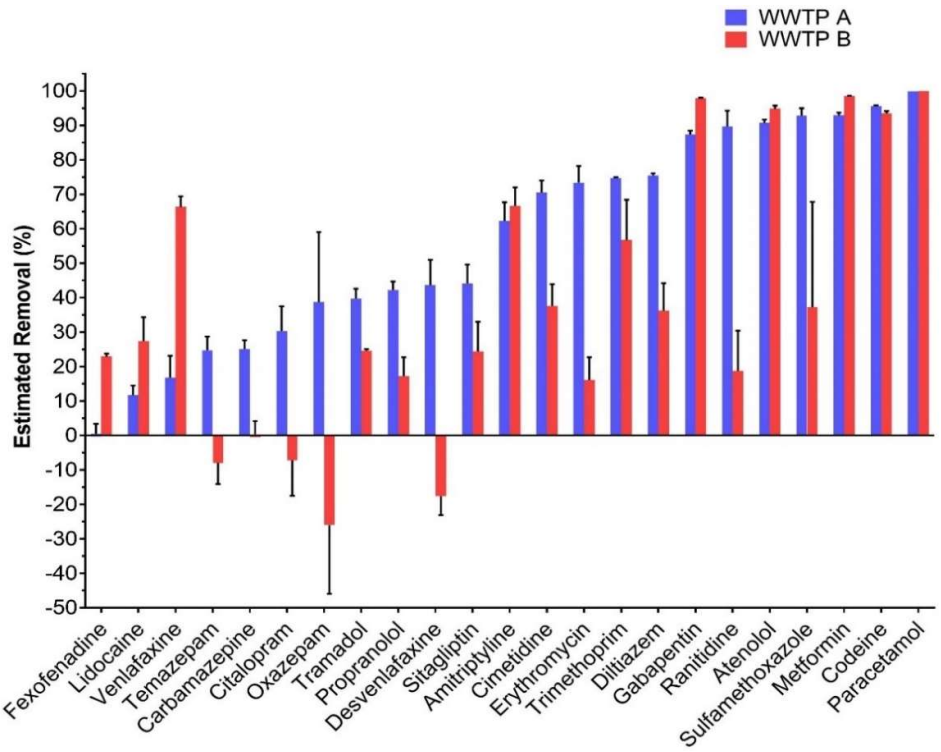


Figure 27. Estimated % removal in WWTP A (trickling filter), WWTP B (carbon activated sludge). Hydrocodone not show, estimated removal in WWTP A -307% and in WWTP -597%.

5.2.2 Pharmaceuticals in surface waters

Of the 33 pharmaceuticals monitored, 21 were detected in all 12 months in samples from the River Foss. Three compounds, oxazepam, verapamil and triamterene, were not detected in any Foss sample. The remaining nine study compounds, diazepam, diphenhydramine, loratadine, norethisterone, oseltamivir, raloxifene, sulfamethoxazole, sertraline and temazepam, were sporadically detected from month to month in this river. In comparison, ten compounds (carbamazepine, codeine, fexofenadine, gabapentin, hydrocodone, lidocaine, metformin, paracetamol, tramadol and trimethoprim); were detected in all 12 months in the River Ouse samples. Eight compounds were not detected in any Ouse sample: diazepam, loratadine, oseltamivir, oxazepam, raloxifene, sulfamethoxazole, triamterene, and verapamil. The highest five annual median concentrations followed the same trend in both rivers: metformin>gabapentin>paracetamol>fexofenadine>tramadol, indicating that usage patterns, WWTP removal and environmental fate for the most prevalent pharmaceuticals are similar in these two systems. The range, detection frequency and annual median for each pharmaceutical in both river systems is reported in Tables 15 and 16.

Table 15. Summary results (ng/L) for the River Foss from the January to December 2016 monitoring campaign. The annual median, concentration range and frequency of detection (n=12) for each sampling site are reported.

Compound	F1		F2		F3		F4		F5	
	Range (Med)	%	Range (Med)	%	Range (Med)	%	Range (Med)	%	Range (Med)	%
Amitriptyline	n.d.	0	n.d. – 25.7 (10.3)	92	1.2* – 12.2 (5.7)	100	n.d. – 11.2 (2.6)	83	n.d. – 6.4 (2.0*)	75
Atenolol	n.d.	0	18.9 – 100 (55.4)	100	12.3* – 98.2 (43.6)	100	13.7* – 97.8 (34.8)	100	10.1* – 67.0 (21.8)	100
Carbamazepine	n.d. – 11.8 (4.5)	67	19.0 –195 (45.2)	100	8.7 – 194 (66.0)	100	12.5 – 175 (61.6)	100	11.4 – 193 (36.8)	100
Cimetidine	n.d. – 49.6 (19.8)	83	n.d. – 44.0 (19.9)	92	3.0* - 40.5 (10.6)	100	2.1* - 16.9 (7.3*)	100	n.d. – 11.8 (7.2*)	67
Citalopram	n.d.	0	5.0 – 71.4 (15.4)	100	3.8* - 31.0 (15.3)	100	3.1* - 13.5 (7.8)	100	n.d. – 11.4 (5.9)	83
Codeine	n.d. – 10.8 (5.9*)	83	8.0 – 101 (59.2)	100	11.5 – 84.2 (57.3)	100	12.9 – 97.7 (44.0)	100	12.0 – 64.7 (29.1)	100
Desvenlafaxine	n.d. – 55.8 (16.8)	83	25.8 – 268 (70.0)	100	4.6* - 195 (86.2)	100	11.7 – 170 (77.3)	100	8.5* - 96.4 (44.5)	100
Diazepam	n.d.	0	n.d. – 1.6* (n.d.)	8.3	n.d. - 1.6* (n.d.)	8.3	n.d. - 1.8* (n.d.)	8.3	n.d. - 2.3* (n.d.)	8.3
Diltiazem	n.d. – 4.1 (1.2*)	75	4.7 – 48.7 (16.4)	100	4.7 – 36.0 (14.5)	100	4.4 – 25.0 (10.6)	100	n.d. – 12.7 (5.8)	92
Diphenhydramine	n.d.	0	n.d. -12.7 (9.5)	67	n.d. – 3.8 (n.d.)	25	n.d. – 1.6* (n.d.)	17	n.d. – 3.4 (n.d.)	8.3
Erythromycin	n.d. – 34.5 (20.2*)	58	26.8 – 242 (90.0)	100	15.0* - 263 (88.8)	100	18.8* - 142 (80.5)	100	14.4 – 116 (45.9)	100
Fexofenadine[1]	n.d. – 104 (24.9)	83	43.8 – 1144 (177)	100	17.2 – 956 (253)	100	27.5 – 638 (166)	100	26.4 – 268 (92.5)	100
Gabapentin	17.4* – 229 (82.7)	100	476 – 1429 (789)	100	260 – 1445 (843)	100	404 – 1183 (768)	100	223 – 1341 (544)	100

Table 15. (continued) Summary results (ng/L) for the River Foss from the January to December 2016 monitoring campaign. The annual median, concentration range and frequency of detection (n=12) for each sampling site are reported.

Compound	F1		F2		F3		F4		F5	
	Range (Med)	%	Range (Med)	%	Range (Med)	%	Range (Med)	%	Range (Med)	%
Hydrocodone	n.d. – 5.7 (n.d.)	43	11.2 – 91.8 (21.6)	100	6.4 – 60.3 (25.0)	100	6.8 – 43.5 (20.6)	100	5.2 – 22.2 (11.1)	100
Lidocaine	n.d. – 3.9 (2.6*)	58	4.6 – 40.4 (8.2)	100	1.7* - 39.7 (11.8)	100	3.1 – 36.9 (10.4)	100	n.d. – 16.0 (6.1)	92
Loratadine	n.d.	0	n.d.	0	n.d. – 6.46 (n.d.)	8.3	n.d.	0	n.d.	0
Metformin	45.2 – 291 (121)	100	246 -1783 (856)	100	266 – 2339 (1117)	100	340 – 2595 (888)	100	263 – 1750 (664)	100
Norethisterone	n.d.	0	n.d. – 7.4* (n.d.)	8.3	n.d.	0	n.d.	0	n.d.	0
Oseltamivir	n.d.	0	n.d. – 8.8* (n.d)	8.3	n.d.	0	n.d.	0	n.d.	0
Oxazepam	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Paracetamol	n.d. – 119 (60.0)	67	14.3* - 749 (74.4)	100	n.d. – 9822 (97.2)	92	32.0 – 9676 (209)	100	25.0 – 5445 (180)	100
Propranolol	n.d.	0	n.d. – 64.9 (17.8)	92	n.d. – 29.9 (20.1)	92	n.d. – 20.6 (10.0*)	92	n.d. – 18.3 (10.4*)	50
Raloxifene	n.d.	0	n.d.	0	n.d. -7.2*	8.3	n.d. – 7.2*	8.3	n.d.	0
Ranitidine	n.d. – 10.8* (n.d.)	17	n.d. – 69.6 (53.4)	83	6.6* – 74.0 (27.9)	100	n.d. – 60.6 (22.2)	92	n.d. – 30.0 (13.6*)	92
Sertraline	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d. - 21.2 (n.d)	8.3
Sitagliptin	n.d.	0	16.5 – 121 (35.2)	100	9.3* - 103 (46.5)	100	15.2 – 85.7 (36.9)	100	12.2* – 33.9 (19.5)	100
Sulfamethoxazole	n.d.	0	n.d. – 10.2* (n.d.)	33	n.d. – 33.0 (n.d.)	50	n.d. – 27.5 (n.d.	42	n.d. – 18.1* (n.d.)	17
Temazepam	n.d.	0	n.d. – 38.2 (12.1)	67	n.d. – 25.0 (16.7)	75	n.d. – 27.8 (15.9)	67	n.d. – 12.6 (7.1*)	58

Table 15. (continued) Summary results (ng/L) for the River Foss from the January to December 2016 monitoring campaign. The annual median, concentration range and frequency of detection (n=12) for each sampling site are reported.

Compound	F1		F2		F3		F4		F5	
	Range (Med)	%	Range (Med)	%	Range (Med)	%	Range (Med)	%	Range (Med)	%
Tramadol	n.d. – 48.1 (31.2)	75	54.4 – 650 (117)	100	21.0 – 456 (177)	100	34.0 – 368 (169)	100	29.2 – 201 (84.7)	100
Triamterene	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Trimethoprim	n.d. – 9.8 (2.5*)	75	13.2 - 76.0 (30.3)	100	10.1- 60.3 (26.4)	100	15.2 – 49.4 (19.8)	100	5.3 – 38.0 (13.8)	100
Venlafaxine	n.d. – 4.3 (2.2*)	42	9.2 – 102 (16.2)	100	2.4* – 82.6 (20.6)	100	5.9 – 37.9 (17.6)	100	2.3* -17.8 (9.2)	100
Verapamil	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0

*Below LOQ

[1] data for 11 months only available (April 2016 missing).

n.d. No detect

(Med) Median

% Detection frequency (100% = 12 months)

Table 16. Summary results (ng/L) for the River Ouse from the January to December 2016 monitoring campaign. The annual median, concentration range and frequency detection (n=12) for each sampling site are reported.

Compound	O1		O2		O3		O4		O5		O6	
	Range (med)	%	Range (med)	%	Range (med)	%	Range (med)	%	Range (med)	%	Range (med)	%
Amitriptyline	n.d.	0	n.d.	0	n.d. – 2.7 (n.d.)	17	n.d. -1.2* (n.d.)	17	n.d. – 1.5* (n.d.)	8	n.d. -2.5 (n.d.)	17
Atenolol	n.d.	0	n.d. – 22.0 (11.1*)	58	n.d. – 19.5 (10.7*)	67	n.d. – 16.9* (10.2*)	75	n.d. – 20.4 (10.4*)	67	n.d. – 18.8 (13.6*)	92
Carbamazepine	1.0* – 14.0 (5.8)	100	1.1* - 34.8 (9.2)	100	1.4* - 54.4 (19.2)	100	1.1* - 31.4 (12.1)	100	1.7* - 33.9 (15.0)	100	7.9 – 48.0 (23.4)	100
Cimetidine	n.d. – 2.3* (n.d.)	8	n.d. – 2.4* (n.d.)	8	n.d. - 5.7* (n.d.)	33	n.d. – 2.9* (n.d.)	17	n.d.	0	n.d. – 3.7 (n.d.)	42
Citalopram	n.d. - 3.3* (n.d.)	8	n.d. – 3.7* (n.d.)	33	n.d. – 7.0 (4.0*)	75	n.d. – 3.2* (n.d.)	50	n.d. – 4.0* (2.2*)	67	n.d. – 7.2 (4.8)	83
Codeine	n.d. – 13.5 (10.5*)	92	3.3 – 17.1 (10.7)	100	3.0* – 20.5 (14.3)	100	3.5* – 17.5 (13.8)	100	4.5* – 17.4 (14.9)	100	6.4* - 17.8 (8.8)	100
Desvenlafaxine	n.d. – 14.8 (n.d.)	50	n.d. – 27.5 (11.3)	75	n.d. – 46.8 (21.5)	83	n.d. -31.0 (14.2)	83	n.d. – 28.8 (15.2)	75	12.3 – 40.1 (26.8)	100
Diazepam	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Diltiazem	n.d. – 1.6* (n.d.)	25	n.d. – 2.5 (n.d.)	50	n.d. – 8.0 (3.6)	92	n.d. – 6.4 (1.8*)	67	n.d. – 3.7 (1.8*)	75	n.d. – 4.3 (3.7)	92
Diphenhydramine	n.d.	0	n.d. – 1.7* (n.d.)	8	n.d.- 2.9 (n.d.)	25	n.d.	0	n.d.- 4.8 (n.d.)	8	n.d. - 2.2* (n.d.)	8
Erythromycin	n.d.	0	n.d. – 17.3* (n.d.)	33	n.d. – 31.1 (21.3*)	92	n.d. – 20.3* (15.3*)	67	n.d. – 21.7* (n.d.)	50	n.d. – 33.9 (21.3*)	83
Fexofenadine[1]	n.d. – 41.7 (17.9)	83	n.d. – 48.7 (24.1)	83	n.d. – 77.8 (46.1)	92	n.d. – 68.2 (25.8)	83	n.d. – 44.0 (29.2)	92	7.4* – 98.5 (33.4)	100
Gabapentin	28.1* -242 (130)	100	39.4 – 351 (191)	100	24.5* - 429 (230)	100	30.0* - 369 (202)	100	33.8* - 364 (192)	100	39.5 – 450 (208)	100
Hydrocodone	n.d. – 2.9 (n.d.)	50	n.d. – 5.7 (3.6)	83	n.d. – 14.9 (7.8)	92	n.d. – 8.0 (4.0)	92	n.d. – 6.9 (4.0)	92	2.2 – 10.7 (6.0)	100

Table 16. (continued) Summary results (ng/L) for the River Ouse from the January to December 2016 monitoring campaign. The annual median, concentration range and frequency detection (n=12) for each sampling site are reported.

Compound	O1		O2		O3		O4		O5		O6	
	Range (med)	%	Range (med)	%	Range (med)	%	Range (med)	%	Range (med)	%	Range (med)	%
Lidocaine	n.d. – 4.1 (n.d.)	50	n.d. – 5.0 (2.7*)	83	n.d. – 6.5 (3.7)	92	n.d. – 5.4 (2.8)	83	n.d. – 5.6 (3.1)	83	1.6* – 8.8 (4.1)	100
Loratadine	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Metformin	52.5 – 323 (180)	100	63.4 – 431 (223)	100	60.6 – 422 (237)	100	60.2 – 422 (237)	100	73.6 – 445 (233)	100	142 – 483 (276)	100
Norethisterone	n.d.	0	n.d. -7.7 (n.d.)	8	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Oseltamivir	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Oxazepam	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Paracetamol	22.3* – 191 (46.4)	100	15.4* - 202 (51.7)	100	16.8* – 186 (54.5)	100	20.1* – 186 (54.3)	100	22.7 – 369 (77.6)	100	21.2 – 226 (66.9)	100
Propranolol	n.d.	0	n.d.	0	n.d. – 8.3* (n.d.)	33	n.d.	0	n.d.	0	n.d. – 7.6* (n.d.)	8
Raloxifene	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Ranitidine	n.d. -10.3* (n.d.)	25	n.d. – 10.5* (n.d.)	25	n.d. – 30.6 (15.1*)	75	n.d. - 13.3* (n.d.)	42	n.d. – 12.0* (n.d.)	25	n.d. – 15.5* (9.2*)	75
Sertraline	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Sitagliptin	n.d. – 10.7 (n.d.)	33	n.d. – 16.2 (9.3*)	75	n.d. – 32.5 (15.0)	92	n.d. – 16.9 (12.0*)	83	n.d. – 15.8 (10.4*)	83	n.d. – 26.5 (18.2)	92
Sulfamethoxazole	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Temazepam	n.d.	0	n.d.	0	n.d. – 7.2* (n.d.)	8	n.d.	0	n.d. – 4.4* (n.d.)	8	n.d. – 4.7* (n.d.)	8
Tramadol	n.d. – 27.0 (19.6)	83	3.9* - 39.9 (19.8)	100	n.d. – 57.2 (34.6)	92	n.d. – 44.8 (28.9)	92	n.d. – 47.4 (27.4)	92	20.7 – 52.4 (40.5)	100

Table 16. (continued) Summary results (ng/L) for the River Ouse from the January to December 2016 monitoring campaign. The annual median, concentration range and frequency detection (n=12) for each sampling site are reported.

Compound	O1		O2		O3		O4		O5		O6	
	Range (med)	%	Range (med)	%	Range (med)	%	Range (med)	%	Range (med)	%	Range (med)	%
Triamterene	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Tramadol	n.d. – 27.0 (19.6)	83	3.9* - 39.9 (19.8)	100	n.d. – 57.2 (34.6)	92	n.d. – 44.8 (28.9)	92	n.d. – 47.4 (27.4)	92	20.7 – 52.4 (40.5)	100
Triamterene	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0
Trimethoprim	n.d. – 19.0 (2.7)	92	2.0* – 8.9 (5.3)	100	2.8* - 19.3 (12.4)	100	n.d. – 11.1 (5.4)	92	2.3* - 12.1 (5.5)	100	7.3 – 22.9 (14.2)	100
Venlafaxine	n.d. – 2.6* (n.d.)	42	n.d. – 5.2 (2.6*)	75	n.d. – 8.5* (4.9)	83	n.d. – 4.3 (2.9*)	75	n.d. – 5.0 (3.1)	75	n.d. – 8.2 (4.5)	83
Verapamil	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0	n.d.	0

*Below LOQ

[1] Only data for 11 months available (April 2016 missing).

No detect (n.d.)

Median (Med)

Monthly total pharmaceutical concentrations at each sampling site are presented in Figures 28 and 29. These concentration maps provide a spatiotemporal overview of the relationship between sampling sites, rivers and WWTPs serving the city. The lowest monthly average concentrations across analytes in the River Foss (70 ng/L, February) was still greater than the highest recorded in the Ouse (26 ng/L, August) despite the WWTPs on the River Ouse serving a larger population. This is due to greater dilution of discharged effluent in the Ouse; for example, flow ranged from 9.2 to 233 m^3/s in the Ouse, compared with 0.0096 to 1.68 m^3/s in the Foss on sampling days (Figure 26). For the sites immediately downstream of the WWTPs (O3, O6 and F2), the months with the lowest flows, July and June, yielded both the most analytes and the highest concentrations. Thus, concentrations appear to be inversely proportional to flow at site F2, similarly to observations reported previously.[315] The trend is not continued moving downstream in the River Foss (sites F3-F5), potentially due to losses from in-stream removal processes such as biodegradation or sorption to sediment,[302] dilution or contributions from domestic septic systems,[316] and/or inputs from combined sewer overflows (CSO).[317] In the Foss, a substantial spike downstream of F2 in paracetamol (9822 ng/L) was detected in the March sampling along with less intense spikes from other pharmaceuticals, such as metformin (2592 ng/L). These observations may be explained by CSOs present immediately upstream of the F3, O2 and O4 sites, which were in operation following heavy rainfall prior to the March sampling period. Paracetamol can be >99% removed and metformin >93%, in conventional water treatment (Figure 27), therefore the spike in March concentrations be explained by CSO releases of untreated wastewater.[49,317] Concentrations in the River Ouse varied less month to month than in the Foss, and a relationship with flow was less clear, with March and May, in general having slightly greater total concentrations. March has also been reported to have the highest monthly concentration in recent temporal studies.[318,319] Sun et al.[319] suggested March coincided with a spike in usage and reduced WWTP removal capacity, however the more plausible explanation for the current study is the operation of CSOs in March, while the spike in May coincides with decreased river flow (Figure 28).

Metformin, a type II diabetes drug, had the highest annual median concentration (1117 and 237 ng/L in the Foss and Ouse, respectively), followed by gabapentin (anti-convulsant) (843 and 230 ng/L, Foss and Ouse, respectively) and paracetamol (analgesic)

(209 and 77.6 ng/L, Foss and Ouse, respectively) This trend is different from those observed in previous temporal exposure campaigns studying similar compounds throughout the world. For example in China, Zhang et al.[320] studied urbanized rivers and found antibiotics the most frequently detected pharmaceuticals. They did, however, report atenolol as having one of the highest annual median concentrations (53 ng/L), which is similar to the median concentration for this compound reported at site F2 (55.4 ng/L) in the current study. In Spain, Camacho-Munoz et al.[301] reported propranolol most frequently detected in surface water, with a higher average concentration (80 ng/L) than observed in this study (20.1 ng/L). In Portugal, Paíga et al.[298] reported carbamazepine the most frequently detected pharmaceutical with an annual median of 31.7 ng/L, while other similarly studied compounds, citalopram and venlafaxine had annual median concentrations of 0.86 and 40.1 ng/L, respectively and trimethoprim was not detected. In the River Foss, the highest annual median concentrations for carbamazepine, citalopram and venlafaxine was 66 ng/L, 15.4 and 21 ng/L, respectively while trimethoprim was detected in 100% of samples with an annual median of 30 ng/L. In Sweden, carbamazepine was also most frequently detected and at a higher annual mean than observed in York, 204 ng/L versus 66 ng/L in the River Foss, while atenolol concentration was similar to that reported here (60.2 ng/L, compared to 55.4 ng/L).[297] In a similar temporal study in Wales, tramadol and gabapentin had the highest annual median concentrations (968 ng/L and 227 ng/L, respectively).[39] Several similarly studied compounds in Wales also had higher annual median concentrations than measured in York: gabapentin, tramadol, trimethoprim, paracetamol, carbamazepine, cimetidine and atenolol, while diltiazem, atenolol, sulfamethoxazole and erythromycin concentrations were lower than those observed in the River Foss.[39] These comparisons suggest that annual pharmaceutical exposure in river systems are highly variable regionally, in part due to variability in prescribing practices, hydrogeology, wastewater management and urbanisation. In addition, certain annual median pharmaceutical concentrations observed in this study are higher than those previously observed in the European Union and Asia.

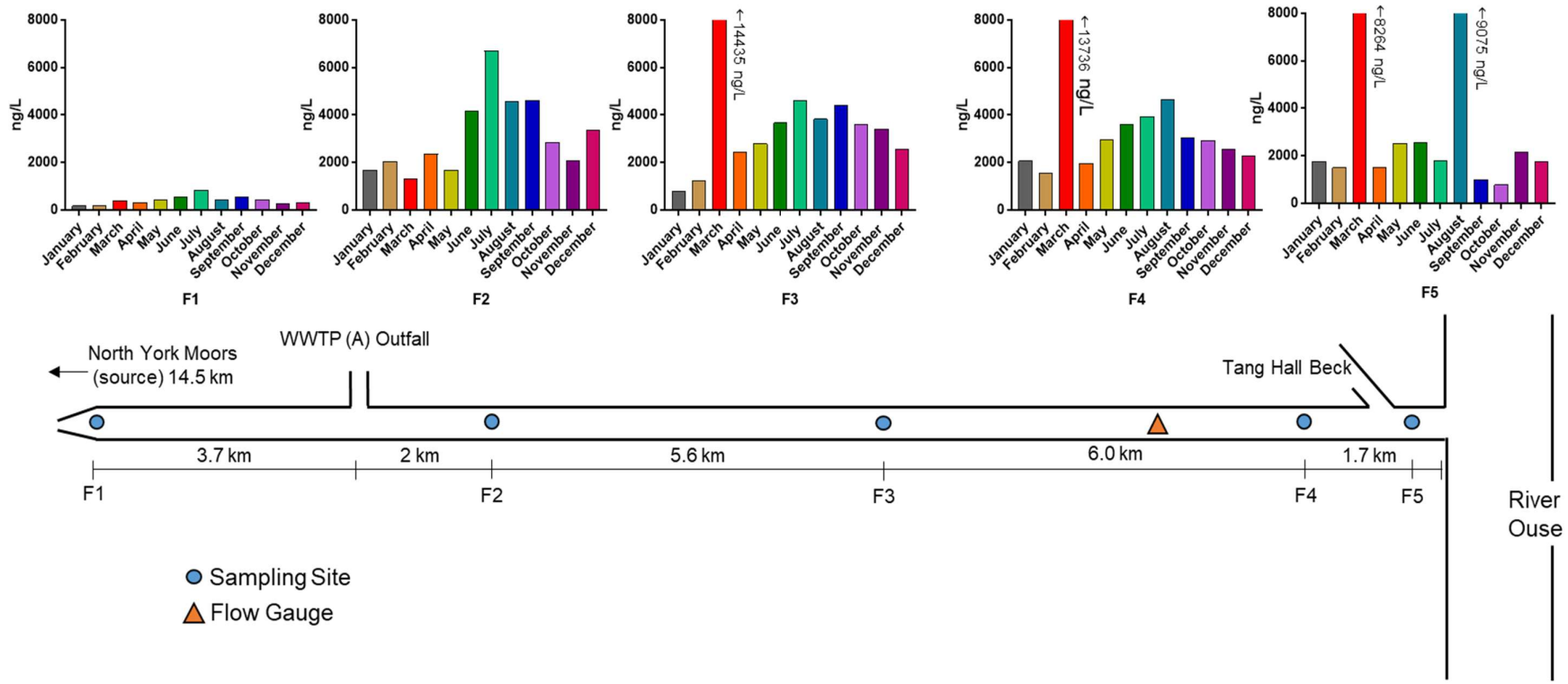


Figure 28. Total pharmaceutical concentration (summed) of all detected analytes at each sampling site from each month during 2016 in the River Foss. Sampling locations (blue circles) in relation to Environment Agency Flow gauges (orange triangles) are depicted along the river.

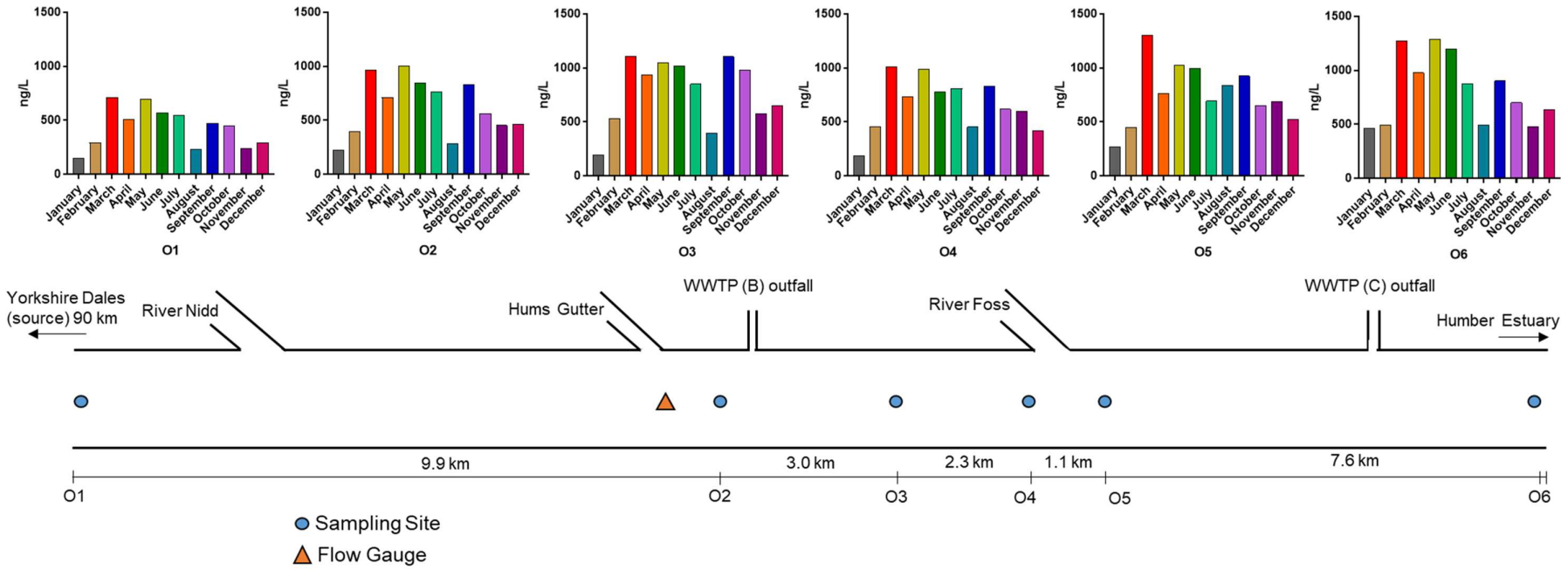


Figure 29. Total pharmaceutical concentration (summed) of all detected analytes at each sampling site from each month during 2016 along the River Ouse. Sampling locations (blue circles) in relation to Environment Agency Flow gauges (orange triangles) are depicted along the river.

5.2.3 Spatial trends in surface water

The spatial trends for both rivers are presented in Figure 31, significant differences between a site and the adjacent downstream site are also noted. Spatial trends are apparent in both rivers, the greatest number of significant differences ($p<0.05$) were found between the sites upstream and downstream of the WWTPs (i.e. F1-F2, O3-O4 and O5-O6) (Figure 30). In addition, significance increases when comparing to sites further downstream. WWTPs make a significant contribution to pharmaceutical concentrations in both river systems, however upstream sources of certain pharmaceuticals exist in both rivers as significance was not achieved for cimetidine in the River Foss and paracetamol, codeine, trimethoprim and atenolol in the River Ouse. There are WWTPs along the River Nidd (Figure 29) and upstream of sites O1 and F1 (>10 km), demonstrating that pharmaceuticals from upstream sources are transported into the city. Concentrations are generally highest immediately downstream of the WWTPs and decrease moving to downstream sites, evidenced by difference in height (i.e. concentration) between the bars from each site (Figure 30), similarly to observations in previous studies.[39] The decrease in concentrations moving downstream is variable between compounds indicating that in-stream attenuation is compound specific. For example, carbamazepine concentrations are similar between sites downstream of the WWTP in the River Foss (i.e. F2-F5) while over the same stretch of river hydrocodone and citalopram decreases by 51% and 38%, respectively (Figure 30). In the Ouse, all concentrations decrease slightly from O3 to O4, however there is a slight increase occurring at O5, likely due to the confluence with the River Foss and again at O6, which is downstream of WWTP C.

In the River Foss, temazepam had no significant downstream spatial differences, while carbamazepine was only significantly different between WWTP A upstream and downstream sites. Temazepam and carbamazepine have been reported to be resistant to biodegradation and stable in the environment.[302,321] In the River Ouse, all pharmaceuticals exhibited spatially significant trends. Carbamazepine was significantly different between each downstream site tested. Since this did not occur in the River Foss over a greater distance, 15.3 versus 13.4 km, and the literature agrees that carbamazepine is resistant to biotransformation, a combination of dilution (e.g. urban drainage/runoff) and other pharmaceutical sources (i.e. River Foss) moving downstream could be a

plausible explanation. Temazepam was not detected frequently enough in the Ouse to draw further conclusions.

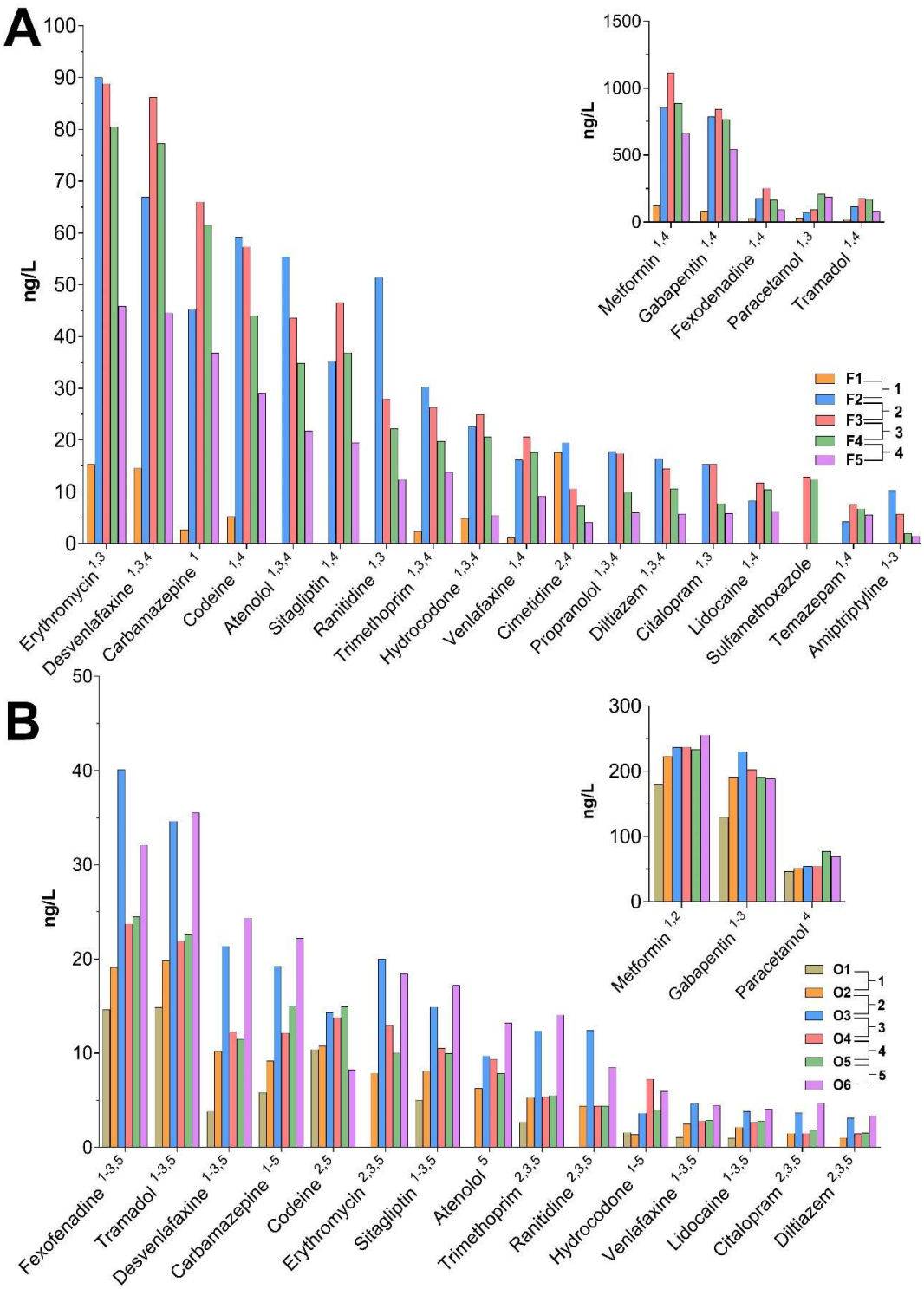


Figure 30. Annual median concentration from all sampled sites in (A) the River Foss and (B) River Ouse. Pairwise t-tests were conducted between neighbouring sites and significant differences are denoted by the corresponding number. Sites F1-F2, O1-O2 =1; F2-F3, O2-O3 =2; F3-F4, O3-O4 =3; F4-F5, O4-O5 =4; O5-O6 =5.

Overall, these results indicate that a wide variety of environmental processes such as dilution and in-stream degradation are operating to differing extents in neighbouring rivers leading to different spatial patterns in pharmaceutical concentrations between sampling sites. For example, the reduction in concentrations moving downstream in the River Foss may be symptomatic of in-stream removal processes such as photolysis or microbial degradation,[297] while fluctuating concentrations in the River Ouse could be due to a complex dynamic between dilution and other pharmaceutical sources (i.e. tributaries, CSOs, septic systems) while natural removal processes potentially operating in the Foss may be masked or occur to a lesser extent in the larger Ouse system.

5.2.4 Temporal trends in surface water

Temporal variability between the seasons (Figure 31) is presented similarly to the approach for displaying spatial variability between sampling sites (Figure 32). Seasonal differences in pharmaceutical concentrations exist in the two river systems, especially in the River Foss. In both rivers, the lowest concentrations correspond with winter, the season which had the highest average flow (2.7 times higher than the next highest season, autumn). Conversely, the highest mass loads occur in winter, 1.4 times higher than the next highest season, spring. Lower concentrations in winter have also been reported previously,[39,46] however several studies report higher concentrations in winter.[303,320,322] In addition, the extent of concentration variability between seasons differs between compounds, which could be due to seasonal patterns in usage[300] seasonal variability in photodegradation or biodegradation, of which both processes can peak in summer, thus having a greater impact on more readily biodegradable compounds.[303] In general, autumn was the season with the second highest median concentrations, except for paracetamol, where highest median values were observed during spring in both rivers. This could be due to increased usage coinciding with symptomatic treatment of illnesses more common in spring such as colds[30] in conjunction with lower flows than winter. To determine whether concentrations between seasons were significant, Friedman's test was used for pharmaceuticals with sufficient detections. Seventeen compounds (86%) were found to vary significantly by season in the River Foss, while amitriptyline, codeine, cimetidine, metformin and ranitidine did not vary seasonally. Nine compounds (50%) had significant seasonal differences in the River Ouse, atenolol,

carbamazepine, codeine, desvenlafaxine, gabapentin, lidocaine, ranitidine, sitagliptin and trimethoprim.

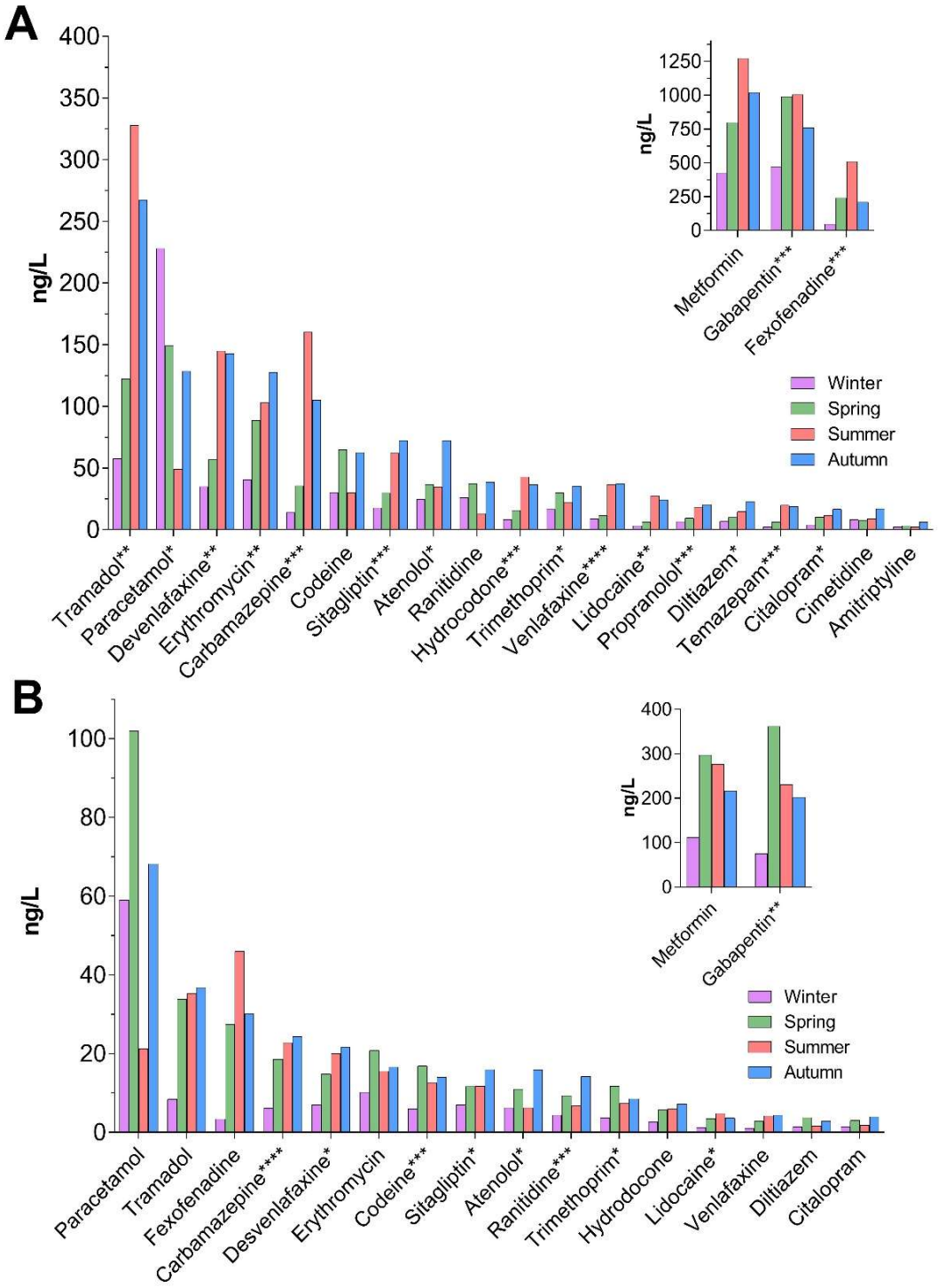


Figure 31. Median seasonal concentration from sites F3-F4 in the River Foss (A) and O3-O4 in the River Ouse (B) for select pharmaceuticals. Temporal variations were tested using Friedman's Test and results are reported for each compound where a significant result was found, $p<0.05$ (*), $p<0.01$ (**), $p<0.0005$ (***), $p<0.0001$ (****).

5.2.4.1 Temporal relationship with pharmaceutical usage and river flow

The reasons for temporal variations in pharmaceutical concentrations have varied between studies with several reporting flow as the major driver, observing higher concentrations during times of low flow.[39,315] Others suggest higher pharmaceutical concentrations in winter months coincide with higher winter usage patterns[300] or decreased biodegradation in winter,[302] while others found no significant differences between sampled seasons.[301]

To explore the temporal relationship between MECs, pharmaceutical usage and flow, MECs were plotted against the monthly pharmaceutical usage divided by flow, Figure 32. Similar plots for all other pharmaceuticals can be found in Appendix 15. Visually, a positive relationship between MECs and prescriptions divided by flow emerges in the River Foss for hydrocodone, desvenlafaxine, metformin and fexofenadine, Figures 32A, C, E and G, respectively. Conversely, only metformin (Figure 32F) and fexofenadine (Figure 32H) visually follow this trend in the River Ouse. The possible influence of septic effluent in March is again apparent, similarly to Figure 28, evidenced by the metformin spike (Figures 32E). Metformin was >90% removed from WWTP A and B, providing further evidence that when wastewater treatment is by-passed by either septic effluent or CSOs, a significant increase in riverine concentration results. A series of correlations (Pearson correlation coefficient) between MECs and flow, prescriptions and prescriptions divided by flow were calculated similarly to previous studies.[323,324] Summary results are reported in Table 17. Few pharmaceutical MECs in either river were correlated with pharmaceutical usage, Table 17. Conversely, a greater proportion of MECs were correlated with flow, particularly in the River Ouse (61%). In agreement with the visual trend observed in Figure 32, the largest proportion of MECs were significantly correlated with pharmaceutical usage divided by flow in the River Foss (71%); however, this trend did not emerge in the River Ouse. In total, five pharmaceuticals were not correlated with prescriptions/flow in the River Foss, three of which are available over-the-counter (OTC): codeine, ranitidine and cimetidine. Therefore usage based on local prescriptions alone is unlikely to be sufficient to describe them.[7] A mixture of both prescription and OTC pharmaceuticals were not correlated with prescription/flow in the River Ouse. This would suggest that factors other than flow and pharmaceutical usage affect pharmaceutical concentrations in the River Ouse.

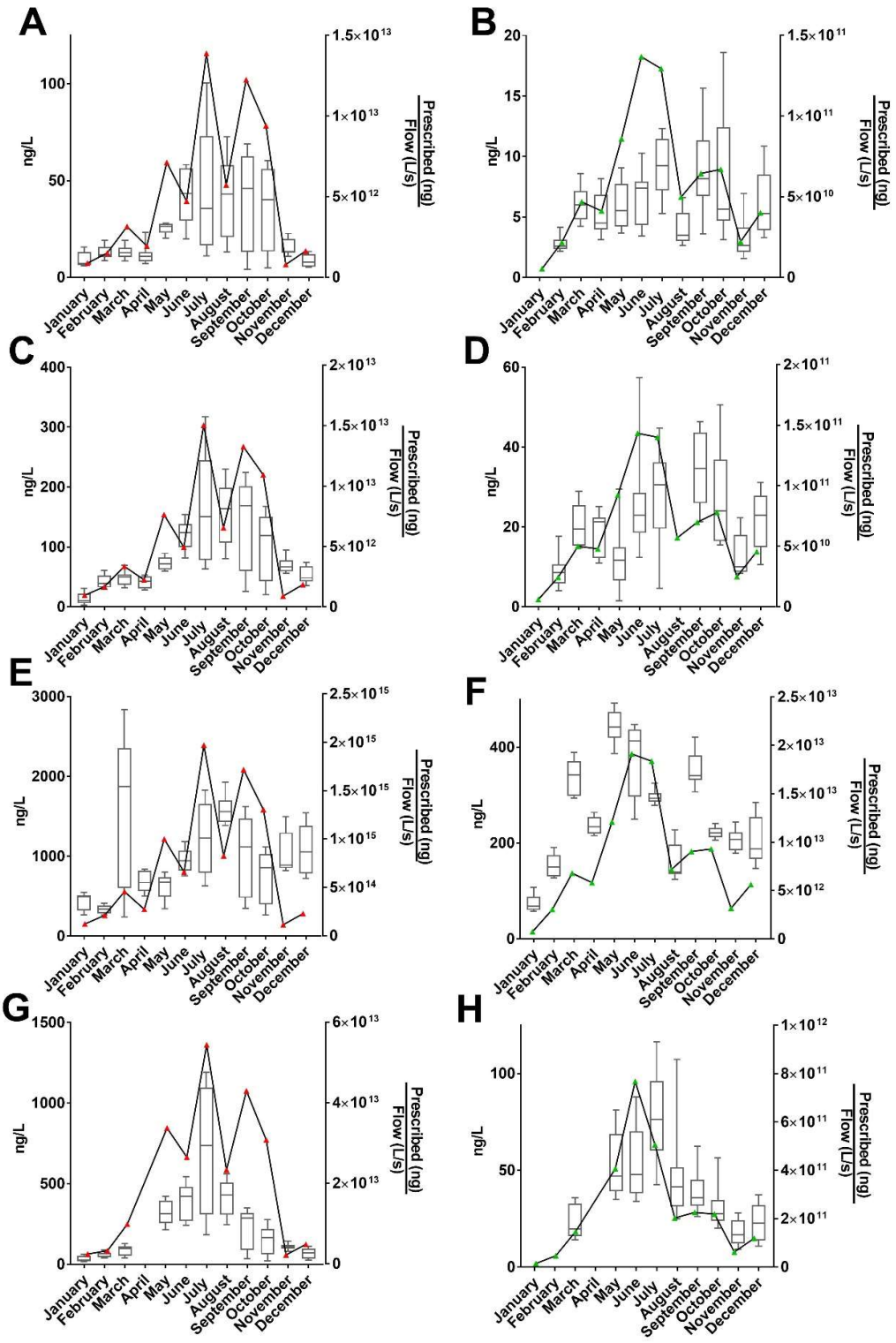


Figure 32. Selected box plots (mean, 25th and 75th percentile) of MECs monthly at sites downstream of WWTP A and B plotted against monthly pharmaceutical usage (ng) divided by flow (L/s) in the River Foss (red symbols) and the River Ouse (green symbols). (A & B) hydrocodone, (C & D) desvenlafaxine, (E & F) metformin and (G & H) fexofenadine.

Table 17. Summary results from Pearson correlations between MECs from site F2 (River Foss) and site O3 (River Ouse) and three scenarios involving monthly flow (L/s) and monthly pharmaceutical usage.

MEC vs.	Pearson correlation	
	River Foss	River Ouse
	Significant (r) (n=24)	Significant (r) (n=18)
Pharmaceutical usage	8%	5%
Flow	54%	61%
$\frac{\text{Pharmaceutical usage}}{\text{Flow}}$	71%	33%

5.2.4.2 River mass loading

A greater percentage of pharmaceutical concentrations exhibited significant temporal trends in the River Foss (86%), than the River Ouse (50%) (Figure 30). When these concentrations were converted to mass loads, only a single compound (ranitidine) exhibited a significant temporal trend in the River Foss, while 83% of compounds exhibit temporally significant trends in the River Ouse. Diltiazem and venlafaxine were the only pharmaceuticals which did not have seasonally significant mass loads trends in the River Ouse. Baker and Kasprzyk-Hordern et al.[46] reported that mass loads did not significantly differ throughout the year in rivers in south Wales, similarly to the River Foss.

River Ouse seasonal mass loads were plotted against seasonal pharmaceutical usage in the River Ouse, Figures 33 and 34. For all plotted pharmaceuticals, apart from carbamazepine (Figure 34C), the highest river mass load was observed in winter which does not coincide with the season where the highest mass of pharmaceutical was prescribed (Figure 33 and 34). The lowest riverine mass load was observed in summer for all pharmaceuticals shown in Figures 33 and 34. Which again, does not correspond with the season in which the lowest mass of pharmaceutical was prescribed, except trimethoprim (Figure 33C).

WWTP removal treatment technology has been demonstrated to impact pharmaceutical removal efficiency. For example, conventional activated sludge (CAS) (i.e. WWTP B and C) has been demonstrated to be more efficient at removing various pharmaceuticals than trickling filter technology (i.e. WWTP A).[43] CAS removal efficiency can also be seasonally affected, with removal efficacy dropping in winter[62,63] and result in temporally variable mass loading.[299] Vieno et al.[63] demonstrated that higher flow rates caused lower hydraulic retentions times (time spent in the biological compartment of the WWTP) and resulted in poorer pharmaceutical removal efficiency. Higher WWTP flow rates could be expected during winter, due to wetter weather during this season. On the other hand, Golovko et al.[62] suggested lower removals in winter could be due to reduced microbial activity, linked to a decrease in temperature.

Biodegradability, indicated by the pseudo-first order biological degradation rate constant (k_{biol}),[325] is also specified in Figures 33 and 34. For simplicity, poor degradation is a k_{biol} <0.1 L/gSS d^{-1}, moderate degradation is a k_{biol} of 0.1 to 10 L/gSS d^{-1} and very good biodegradability is a k_{biol} > 10 L/gSS d^{-1}.[51,306] If WWTP removal efficiency is potentially seasonally affected, the most biodegradable pharmaceuticals should be impacted most. Of the pharmaceuticals studied, only paracetamol has a k_{biol} exceeding 10. The highest mass load of paracetamol does correspond with the season in which it was least prescribed (winter, Figure 33A). Paracetamol however, is a commonly used OTC medicine and higher mass loading in winter could be due to increased usage (not included in the usage estimate) coinciding with cold and flu season.[326] The next highest K_{biol} is metformin (Figure 34B) followed by atenolol (Figure 34A) and ranitidine (Figure 34B). For all three pharmaceuticals, an inverse pattern between prescriptions dispensed and river mass loads is observed. This fits with the trend observed for paracetamol as all three are expected to be moderately biodegradable. Trimethoprim (Figure 33C) mass loading also follows the same trend, however visually, is more closely linked with prescribing rates than the pharmaceuticals with higher k_{biol}. Finally carbamazepine (Figure 34C), which based on the k_{biol} is expected to be poorly degraded in the WWTP, has a positive relationship between mass loads and prescriptions. These comparisons suggest reduced WWTP removal efficiency could be occurring in winter months,

evidenced by both the larger impact on more readily biodegradable pharmaceuticals and higher winter mass loads not correlated with pharmaceutical usage.

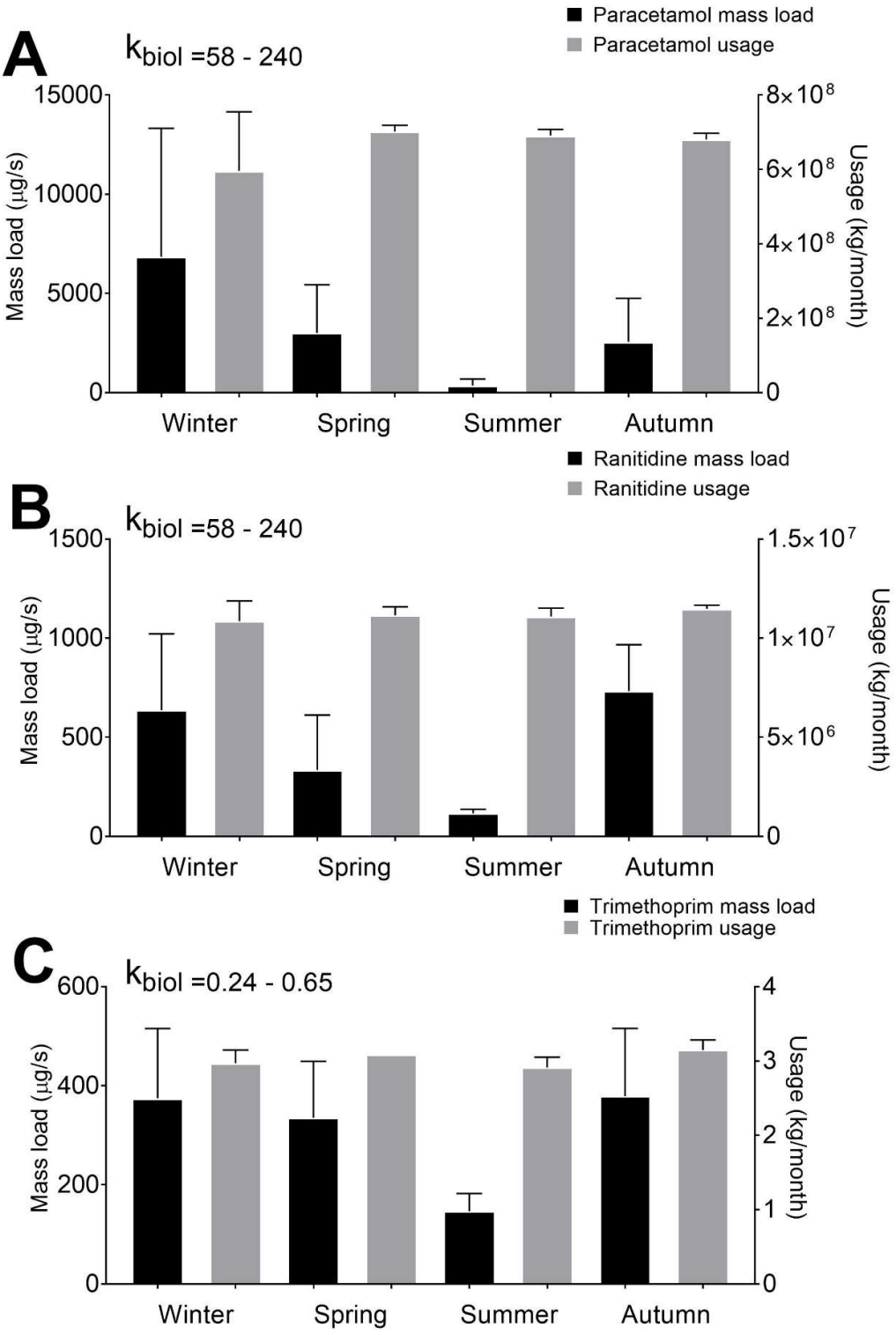


Figure 33. Seasonal mass loads (ng/s) from the River Ouse (site O3) plotted against seasonal pharmaceutical usage for (A) paracetamol, (B) ranitidine and (C) trimethoprim**.** Biodegradability constant (k_{biol}) in L/gSS d^{-1} as reported by Verlicchi et al.[51] and Blair et al.[301]

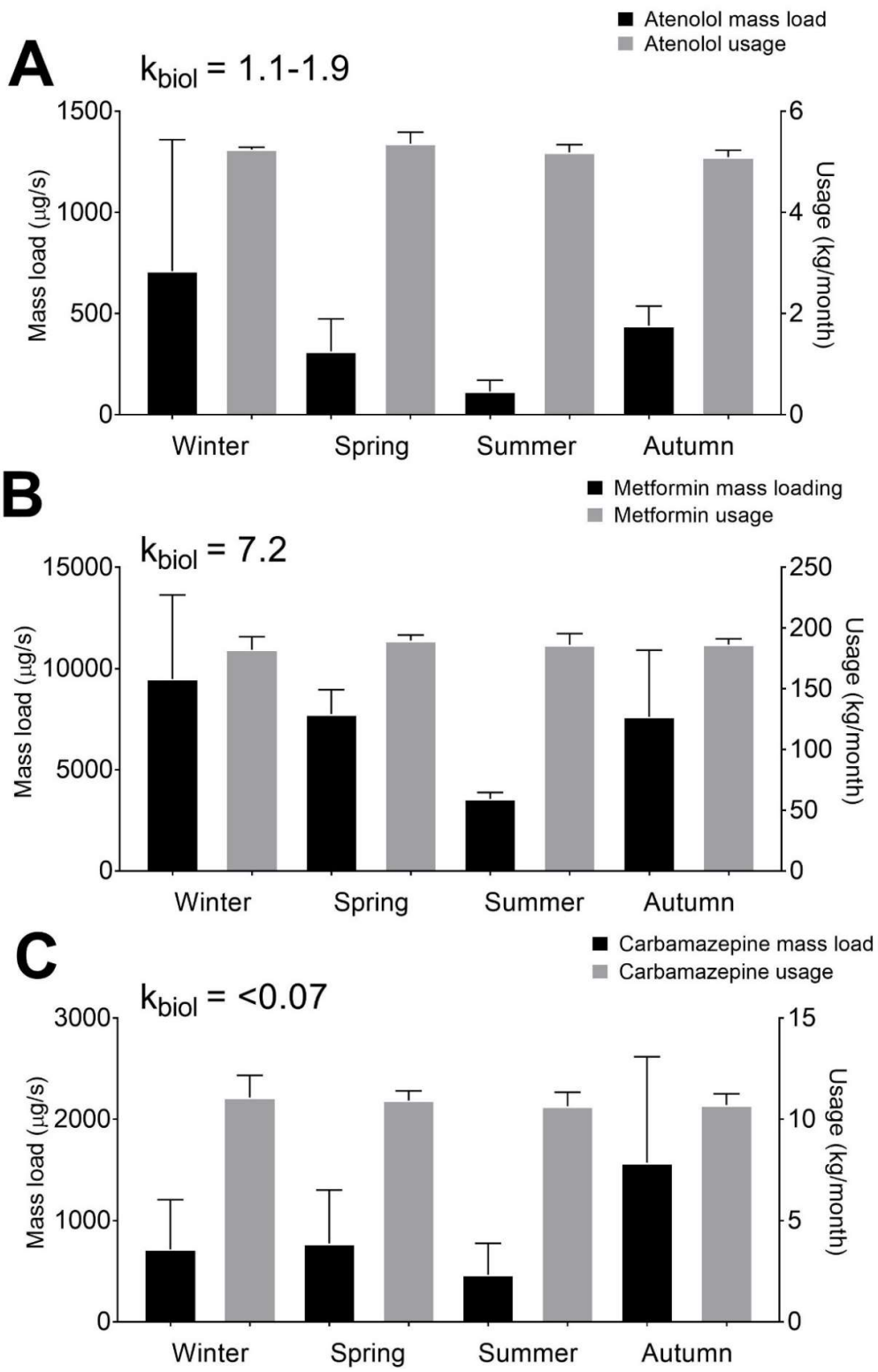


Figure 34. Seasonal mass loads (ng/s) from the River Ouse (site O3) plotted against seasonal pharmaceutical usage for (A) atenolol, (B) metformin and (C) carbamazepine. Biodegradability constant (k_{biol}) in L/gSS d^{-1} as reported by Verlicchi et al.[51] and Blair et al.[301]

It could be expected that if higher flow rates resulting in lower HRTs and lower temperatures affect WWTP removal efficiency, this seasonal trend would also be observed in the River Foss trickling filter WWTP; however, significant seasonal trends in mass loading immediately downstream of the WWTP were not observed. Mass loads in the River Foss are much lower than the River Ouse, which could potentially explain why significant temporal trend was not observed. More research is needed to characterise the conditions within the WWTPs seasonally (e.g. HRT and temperature) in addition to further seasonal sampling to establish a temporal removal efficiency. This is important to determine as pharmaceutical concentrations in the River Ouse were not well correlated with pharmaceutical usage and flow, suggesting another important driver needs to be considered. The evidence provided in Figures 33 and 34 indicate seasonal WWTP removal efficiency could be an important factor to further investigate.

5.3 Conclusion

The rapid determination HPLC-MS/MS quantification method described in Chapter 4 for 33 pharmaceuticals was applied to a 12 month spatiotemporal pharmaceutical monitoring campaign. WWTP removal efficiency was found to be similar between CAS and trickling filter technology for the target pharmaceuticals. Pharmaceutical concentrations in two contrasting rivers that run through the city of York, UK were found to vary significantly spatially and temporally, with the greatest variation observed for paracetamol in the River Foss, ranging from not detected to over 9822 ng/L. Temporal variations in the River Foss were correlated with flow and pharmaceuticals prescribed, suggesting these are the major pharmaceutical concentration drivers in this river. Temporal variations in concentration were less frequently observed in the larger River Ouse, however mass loads differed significantly and were not correlated with pharmaceutical usage suggesting WWTP seasonal removal efficiency could important driver behind pharmaceutical concentrations in that river system. These extensive monitoring results will be instrumental in improving the understanding of temporal pharmaceutical fate and occurrence in river systems. This data will also be useful for estimating average annual concentrations which can be used to validate or improve current exposure models for pharmaceutical prioritisation and risk assessment.

CHAPTER 6

Evaluation of lower and higher tier exposure models for the estimation of pharmaceuticals in urban river systems

6.0 Introduction

In Chapter 3, a scoping study determined that simple exposure models (based on the method suggested by the EMA[112]) may not be suitable for risk assessment and prioritisation. A major drawback of that assessment was the quality of the monitoring data the evaluation was based on. To address this, a robust set of monitoring data was generated (Chapter 5). The EMA-based PEC, or simple PEC, typically calculates a single concentration for the system of interest and is usually derived using default dilution factors and national per capita pharmaceutical usage data.[155] There is no consideration of pharmaceuticals transported from upstream or the convergence with other water bodies, also potentially carrying pharmaceutical residues moving downstream. This may be problematic considering many rivers receive WWTP effluent at various intervals moving downstream[46] and studies have demonstrated that pharmaceuticals can be transported long distances.[327] Furthermore, fate processes such as abiotic or biotic degradation or sorption to sediment, which can also affect riverine pharmaceutical concentrations are not considered.[302] Therefore, the simple PEC is only useful for a single scenario which rarely occurs in the environment, predicting concentrations immediately downstream of a WWTP with no upstream pharmaceutical inputs. Consequently, the simplistic PEC approach is likely to under- or over-estimate pharmaceutical concentrations, mainly as it lacks spatial context.

To overcome this, higher tier exposure assessment tools such as GREAT-ER developed for key catchments in European Union,[328] P*h*ATE or iSTREEM® in the United States[329,330] and LF2000-WQX for the evaluation of smaller catchments in England and Wales[159] have been developed within a GIS framework to predict the concentration of 'down the drain' chemicals in a spatially referenced manner. These models make probabilistic hydrological predictions based on long term flows and point source effluent discharges.[331] These GIS-based approaches have the advantage of identifying pharmaceutical hot spots and have also been shown to impact pharmaceutical risk prioritisation outcomes at the local scale.[203] Previous validation studies of these spatial exposure models have yielded encouraging results with mean predictive values falling within a factor of 2 for select β-blockers and synthetic estrogens when compared to measured environmental concentrations (MECs).[158,167] These previous validation studies

have been limited in terms of the number of compounds considered (less than 10) and the quality of comparative monitoring data. With the number of ecotoxicological endpoints, non-standard or otherwise, observed at environmentally relevant concentrations growing, it is important that the predictive power of these spatial modelling approaches is more thoroughly evaluated to provide greater confidence when assessing the risks of pharmaceuticals, especially in large urbanised systems with multiple pharmaceutical inputs.

In this Chapter, the performance of the simple PEC and a higher-tier spatial exposure model, LF2000-WQX, is evaluated against annually averaged MECs for 29 pharmaceuticals generated from the monitoring study described in Chapter 5. The study pharmaceuticals cover a wide range of physico-chemical characteristics, therapeutic classes and consumption patterns. The best performing model was then used to conduct a risk assessment of the study pharmaceuticals in the York system. This detailed evaluation of higher and lower tier exposure models is highly valuable to further establish the accuracy of these tools for use in prioritisation and risk assessment to ensure risks are not overlooked.

6.1 Methods

6.1.1 Monitoring data

Pharmaceutical monitoring data for surface waters was obtained from the work described in Chapter 5. The monitoring study also included sampling during summer 2016 at two of the WWTPs that discharge into the two rivers (Figure 35) allowing WWTP removal rates to be estimated for the study pharmaceuticals (Table 16) for use in the model parameterisation.

An annual average MEC was calculated from the 12 sampling visits (occurring roughly every four weeks) for each site. If a compound was not detected, data replacement techniques similarly to Chapter 5 (Equation 5.2) were used. If >40% of monthly samples resulted in a non-detect, then the annual average is reported as <LOD.[308] The MECs used in the model evaluation work are reported in Appendix 16.

6.1.2 Exposure modelling

6.1.2.1 Simple PEC modelling

Simple PEC calculations followed the same approach as described in Chapter 3, Equation 3.1. Annual pharmaceutical usage was estimated from localised prescription data reported for each month in 2016 by the NHS,[310] similarly to Chapter 5. The fraction of drug excreted unchanged was based on values reported in the peer-reviewed literature complied in Chapter 3 (Appendix 10). Experimental WWTP removal (Chapter 5) was used when possible (Table 18) supplemented by values from the peer-reviewed literature when no experimental data were available, collated in Chapter 3. A dilution factor was calculated from the average river flow (L/s) across all 12 monthly sampling visits, divided by estimated wastewater generated (200 L/day·person) in WWTP A and B.

6.1.2.2 LF2000-WQX PEC modelling

Low Flows 2000 (LF2000) is a probabilistic hydrological model developed as a decision support tool to predict flow at ungauged sites within England and Wales.[332] It is a spatially referenced river network comprised of interconnected reaches. The reaches are usually defined by a feature which will affect flow and thus needs to be incorporated into the model, for example abstraction or discharge points, tributaries, and confluences. Model output is generated at the bottom of each reach, where mixing indicated by the feature is assumed to be complete. Reaches can also be user-defined, to provide model output at specific locations. Therefore additional reaches were incorporated to accommodate the sampling locations. The bottom of these newly defined reaches coincided with the 11 sampling locations to obtain model output spatially matching sampling site locations (Figure 35).

The WQX (Water Quality eXtension) incorporates point source chemical inputs to the LF2000 river network through a Monte Carlo mass balance framework.[333] WWTP discharge locations, populations served, dry weather flows (DWF) and treatment type have been complied and incorporated with the relevant reaches previously.[159] For pharmaceuticals, the amount expected to enter treatment plants is calculated according to Equation 6.1, similar to the simple PEC. The mass (P_{mass}) of pharmaceutical consumed and excreted per capita (µg/person/day) and Pop. is the population served by the WWTP and the associated dry weather flow (DWF).

$$C_{influent} = \frac{P_{mass} * Pop.}{DWF}$$ Equation 6.1

The model has the option to set a different removal rate for primary, secondary and tertiary treatment, however in this case a constant global removal rate was applied based on experimental removals determined in Chapter 5. Effluent concentrations are then calculated according to Equation 6.2 where R is the global WWTP removal rate.[304]

$$C_{effluent} = C_{influent} * (1-R_i)$$ Equation 6.2

The point source effluent load is then combined with reach-specific dilution in addition to mixing with pharmaceuticals transported from upstream and if applicable, degradation according to first-order decay kinetics.[159] In this case, due to the lack of fate knowledge for many pharmaceuticals or decay not adhering to first order kinetics for several pharmaceuticals modelled, in-stream degradation was not included, similarly to a spatial exposure modelling exercise in Switzerland.[168] LF2000-WQX also has the option of defining upstream pharmaceutical concentrations transported to the modelled area. The model was not simulated from the River Ouse source (>90 km upstream of sampling site O1) or the source of the River Foss (>15 km upstream of sampling site O2) to reduce analysis time. To compensate for this, model concentrations were defined based on the annual average MECs at sites O1 and F1 (Figure 35) prior to simulation.

Several of the model input parameters are associated with variability and uncertainty. To account for this, each parameter is associated with a user-defined distribution. In this study, the following distributions were assumed: pharmaceutical consumption, upstream contributions of pharmaceuticals and effluent discharge were all normally distributed, river flows were log-normally distributed and WWTP removal rates were assumed to be constant. A Monte Carlo simulation was used to propagate the variance of all input parameters by discrete sampling of each distribution, in this case 2000 times (or shots). Results were output as a mean, standard deviation, 90th and 95th percentile concentration for each river reach considered in the simulation.

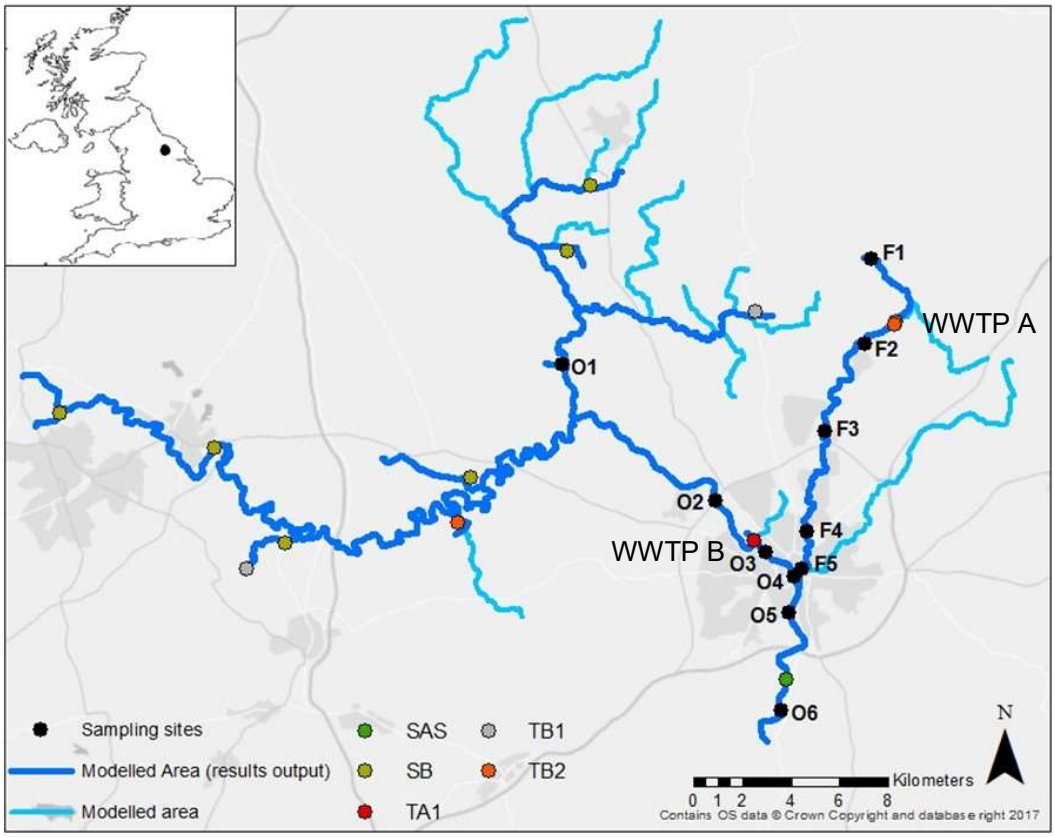


Figure 35. The section of Ouse Catchment modelled with LF2000-WQX. Sampling points along the River Ouse and Foss (black) are shown along with the location of WWTPs within the modelled area. Experimental WWTP removals were estimated at WWTP A and WWTP B. General WWTP classifications are given, SB – secondary biological filter; SAS – secondary activated sludge; TA – Activated sludge with tertiary treatment; TB – biological filter with tertiary treatment.

6.1.3 Model evaluation

6.1.3.1 Semi-quantitative analysis

Five and 11 pharmaceuticals were not detected frequently enough in the River Foss and Ouse, respectively, to calculate an annual average MEC. A quantitative comparison against PECs for these compounds is not possible. Therefore, a semi-quantitative analysis based on expected and not expected detections was conducted, similarly to Chapter 3. Firstly, pharmaceuticals were sorted by predictions, either expected to be detected (i.e. PEC is above the LOD) and not expected to be detected (i.e. PEC less than LOD). Secondly, pharmaceuticals were further sorted based on monitoring results into detected or not detected categories. A prediction was correctly confirmed when the same classification occurred for both monitoring and predicted data.

Table 18. Study pharmaceuticals and modelling parameters. WWTP 1 removals were used for SAS and TA treatment plants, while WWTP 2 removals were used for SB and TB treatment plants (Figure 36) in the modelling exercise.

Pharmaceutical	Therapeutic use	2016 usage (kg/yr)	% Excreted	Experimental Fraction remaining after WWTP removal (%)	
				WWTP 1	WWTP 2
Amitriptyline	Antidepressant	44.1	5	38	33
Atenolol	β-blocker	57.4	50	9.3	5.2
Carbamazepine	Anticonvulsant	118	5	75	99.6
Cimetidine[a]	H_2-receptor antagonist	4.4	87	29.5	62.4
Citalopram	Antidepressant	25.6	26	69.7	107
Codeine[a]	Opioid	131	20	4.5	6.5
Desvenlafaxine	Antidepressant	16.0	55	56.4	118
Diazepam	Benzodiazepine	1.3	3	16	83
Diltiazem	Ca-channel blocker	85.0	5	24.6	63.8
Diphenhydramine[a,b]	Antihistamine	0.06	13	83	83
Erythromycin	Antibiotic	60.3	20	26.6	83.9
Fexofenadine	Antihistamine	52.2	80	99.5	77.1
Gabapentin	Anticonvulsant	645	100	12.6	2.1
Hydrocodone	Opioid	14.4	11	300	600
Lidocaine[a]	Topical anaesthetic	12.2	10	88.3	72.6
Loratadine[a,b]	Antihistamine	3.31	2	85	85
Metformin	Antidiabetic	2040	90	7.1	1.5
Noreistherone[b]	Oral contraceptive	0.78	55	86	86
Oxazepam	Benzodiazepine	0.07	33	61.3	126
Paracetamol[a]	Analgesic	7310	80	0.1	0.02
Propranolol	β-blocker	37.0	25.5	57.8	82.8
Ranitidine[a]	Acid inhibitor	122	70	10.3	81.3
Sitagliptin	Antihyperglycemic	13.7	80	55.9	75.6
Sulfamethoxazole	Antibiotic	4.4	30	7.2	62.8
Temazepam	Benzodiazepine	1.0	75	75.3	108
Tramadol	Opioid	99.1	32	60.3	75.4
Trimethoprim	Antibiotic	33.5	90	25.3	43.3
Venlafaxine	Antidepressant	29.0	10	83.2	33.7
Verapamil[b]	Ca-channel blocker	10.6	5	80	80

[a]Pharmaceutical available over-the-counter in the UK
[b]Experimental WWTP values based on those reported in Chapter 5.

6.1.3.2 Goodness-of-fit

Scatter plots of MECs versus PECs were plotted against a 1:1 line bound by a factor of 10, similarly to a previous assessment of spatially referenced antibiotic PECs and MECs in Europe.[203] Goodness-of-fit was assessed using a modification of the Nash-Sutcliffe

model efficiency (E_1) measure.[334,335] The adaption calculates absolute error instead of squared, diminishing the impact of outliers on assessment, where P are predicted values, O are measured values and $\bar{O}$ is the mean measured value, Equation 6.3.

$$E_1 = 1 - \frac{\sum_{i=1}^{n} |P_i - O_i|}{\sum_{i=1}^{n} |O_i - \bar{O}|} \qquad \text{Equation 6.3}$$

The interpretation of E_1 is dissimilar to other correlation measures such as the coefficient of determination (R^2) as E_1 ranges from 1 (perfect-model-fit) with no lower bounds.[334] For example, an $E_1=0$ indicates that the measured mean is just as good a predictor as the model, while an $E_1=0.6$ indicates that the difference between measured and predicted values accounts for 40% of the variance in the observed data.[334] For our purposes, the closer to one, the better the fit due to a smaller difference between predicted and measured values. Predictive factors (i.e. the ratio of PEC to MEC) are also calculated to express the over/underestimation of predictions for individual pharmaceuticals.

6.1.3.3 In-stream losses

In-stream decay was not included in the model, but based on modelling and monitoring data an assessment of in-stream decay could be made (Equation 6.4). A dilution factor based on the modelled concentrations was first calculated between sites F2 (P_{F5}) and F5 (P_{F2}) in the River Foss (Equation 6.5). This dilution factor was then applied to downstream concentrations (C_{F2}) and any remaining losses between site F2 and F5 (C_{F5}) were assumed to be due to environmental degradation. In-stream losses could not be calculated using this approach in the River Ouse due to the multiple pharmaceutical inputs moving downstream.

$$\text{Fraction remaining in-stream} = \left(\frac{C_{F5} * \text{Dilution factor}}{C_{F2}}\right) * 100 \qquad \text{Equation 6.4}$$

$$\text{Dilution factor} = \frac{P_{F2}}{P_{F5}} \qquad \text{Equation 6.5}$$

6.1.4 Risk Assessment

A risk assessment using LF2000-WQX model output for the portion of the Ouse catchment studied was conducted. PECs of mean model output from the 42 river reaches for the 29 study compounds were used to calculate risk ratios (RQs) in two ways, Equation

6.7 and 6.8. Firstly, RQs were calculated using the Fish Plasma Model (FPM)[118] and secondly, RQs were calculated based on the most sensitive non-standard ecotoxicological endpoint including both no-observed effect concentrations (NOECs) and lowest observed effect concentrations (LOECs) found in the literature (Table 19). Parameters for the FPM, such as the bioconcentration factor (BCF) and the peak serum concentration (C_{max}) have been compiled and calculated previously (Chapter 3). The RQs obtained from each river reach are presented as a distribution. An assessment factor was not applied in either RQ assessment. An RQ near or greater than one indicates risks may be present.

$$RQ = \frac{PEC * BCF}{Cmax} \qquad \text{Equation 6.7}$$

$$RQ = \frac{PEC}{\text{Non-standard NOEC/LOEC}} \qquad \text{Equation 6.8}$$

Table 19. Ecotoxicity endpoints collected from the peer-reviewed literature to evaluate risk against the mean LF2000-WQX output.

Compound	NOEC/ LOEC	Concentration (ng/L)	Species	Endpoint	Reference
Amitriptyline	LOEC 120 h	10	*Danio rerio* (embryo)	Alteration of adrenocorticotropic hormone (ACTH level decrease).	Yang et al.[336]
Atenolol	LOEC 21 d	1000	*Oncorhynchus mykiss*	Higher lactate content and reduced haemoglobin.	Steinbach et al.[337]
Carbamazepine	LOEC 48 hr	10	*Daphnia magna*	Decreased negative photoactive behaviour.	Rivetti et al.[338]
Cimetidine	LOEC 28 d	70	*Gammarus fasciatus*	Biomass reduction.	Hoppe et al.[339]
Citalopram	LOEC 4 h	0.405	*Leptoxis carinata*	Foot detachment.	Fong and Hoy[340]
Diazepam	LOEC 48 hr	100	*Daphnia magna*	Decreased negative photoactive behaviour.	Rivetti et al.[338]
Diltiazem	LOEC 21 d	30	*Oncorhynchus mykiss*	Antioxidant enzyme activity in liver (CAT activity, SOD activity significantly reduced).	Steinbach et al.[341]
Diphenhydramine	NOEC 21 d	120	*Daphnia magna*	Total number of young produced.	Meinertz et al.[342]

Table 19. (continued) Ecotoxicity endpoints collected from the peer-reviewed literature to evaluate risk against the mean LF2000-WQX output.

Compound	NOEC/ LOEC	Concentration (ng/L)	Species	Endpoint	Reference
Erythromycin	LOEC 28 d	50	*Oncorhynchus mykiss*	Antioxidant responses (gill catalase activity) Genotoxicity (genetic damage index – comet assay).	Rodrigues et al.[343]
Fexofenadine	LOEC 7 d	2200	*Zygoptera (Damselfly)*	Increased boldness.	Jonsson et al.[344]
Metformin	LOEC 360 d	40000	*Pimephales promelas*	Development of intersex gonads, size reduction, reduced fecundity.	Niemuth and Klaper[238]
Noreistherone	LOEC 21 d	1.2	*Pimephales promelas*	Decrease in egg production. Presence of dorsal fin spot (male secondary sexual characteristic).	Paulos et al.[345]
Oxazepam	LOEC 7 d	840	*Rutilus rutilus*	Increased boldness, more active.	Brodin et al.[346]
Paracetamol	LOEC 10 d	100	*Lemna minor*	Decrease in photosynthetic pigments, glutathion-S-transferase activity elevated.	Kummerová et al.[347]
Propranolol	LOEC 7 d	0.3	*Mytillus galloprivincialis*	Content of cAMP in digestive gland and mantle/gonads. PKA activity decrease.	Franzellitti et al.[348]
Ranitidine	LOEC 14 d	245	*Danio rerio*	Comet test-DNA fragmentation.	Rocco et al.[349]

Table 19. (continued) Ecotoxicity endpoints collected from the peer-reviewed literature to evaluate risk against the mean LF2000-WQX output.

Compound	NOEC/ LOEC	Concentration (ng/L)	Species	Endpoint	Reference
Tramadol	LOEC 14 d	10000	*Cyprinus carpio*	Changes in antioxidant enzyme activity.	Sehonova et al.[350]
Trimethoprim	LOEC 7 d	440	*Ruditapes philippinarum*	CAT activity in digestive glands and gills increased.	Matozzo et al.[351]
Venlafaxine	LOEC 4 h	0.313	*Leptoxis carinata*	Foot detachment.	Fong and Hoy[340]
Verapamil	NOEC 31 d	4630	*Cyprinus Carpio*	Malformations and edemas.	Steinbach et al.[352]

Ecotoxicity data could not be obtained for codeine, gabapentin, hydrocodone, lidocaine, sitagliptin, sulfamethoxazole and temazepam.

6.2 Results and Discussion

6.2.1 Overall evaluation of exposure models with monitoring data

LF2000-WQX Modelled mean flow was 0.78 m^3/s (SD=1.08 m^3/s) and 42.6 m^3/s (SD=55.3 m^3/s) for the River Foss and Ouse, respectively. In comparison, the measured mean flow from the sampling days was 0.87 m^3/s (SD=0.53 m^3/s) and 45.7 m^3/s (SD= 61 m^3/s) in the River Foss and Ouse, respectively. This indicates that flow from the sampling days is well represented by the model output and simulated flow adjustments were not required prior to comparisons.[158]

6.2.1.1 Semi-quantitative analysis

The semi-quantitative assessment of the simple PEC identified that 90% and 65% of predictions were correct in the River Foss and Ouse, respectively when compared against annually averaged MECs (Figure 36A). In both rivers, all compounds expected to be detected were detected. PEC underestimations occurred in both rivers; however to a greater extent in the River Ouse, evidenced by the detected, not expected bar. Overall, the simple PEC model performed much better in the River Foss, which could be expected as this is a simpler river system with limited upstream pharmaceutical input.

The semi-quantitative assessment of the LF2000-WQX PEC revealed that 93% and 87% of predictions were correctly confirmed in the River Foss and Ouse, respectively (Figure 36B). Similarly to the simple PEC model, all pharmaceuticals expected to be detected were in the River Foss; however, 10% of compounds expected in the River Ouse were not detected. Semi-quantitatively, the LF2000-WQX preforms better than the simple PEC in both rivers, evidenced by a greater percentage of correctly confirmed predictions. Furthermore, fewer underestimates were made by LF2000-WQX indicated by the detected not expected bars, particularly in the River Ouse.

The semi-quantitative performance of both models was similar in the River Foss, which could be expected as river system has limited upstream pharmaceutical input and dilution moving downstream. The advantage of the LF2000-WQX model is highlighted in the River Ouse, with 87% of predictions correct while this value was 65% for the simple PEC model. Moreover 10% of the incorrect predictions were overestimated PECs (i.e. expected not detected) which, in terms of risk assessment is preferable to an

underestimate. In addition, several of the pharmaceuticals expected to be present, had PECs near the LOD where greater analytical uncertainty exists, thus performance could be slightly better than it appears in the River Ouse for LF2000-WQX.

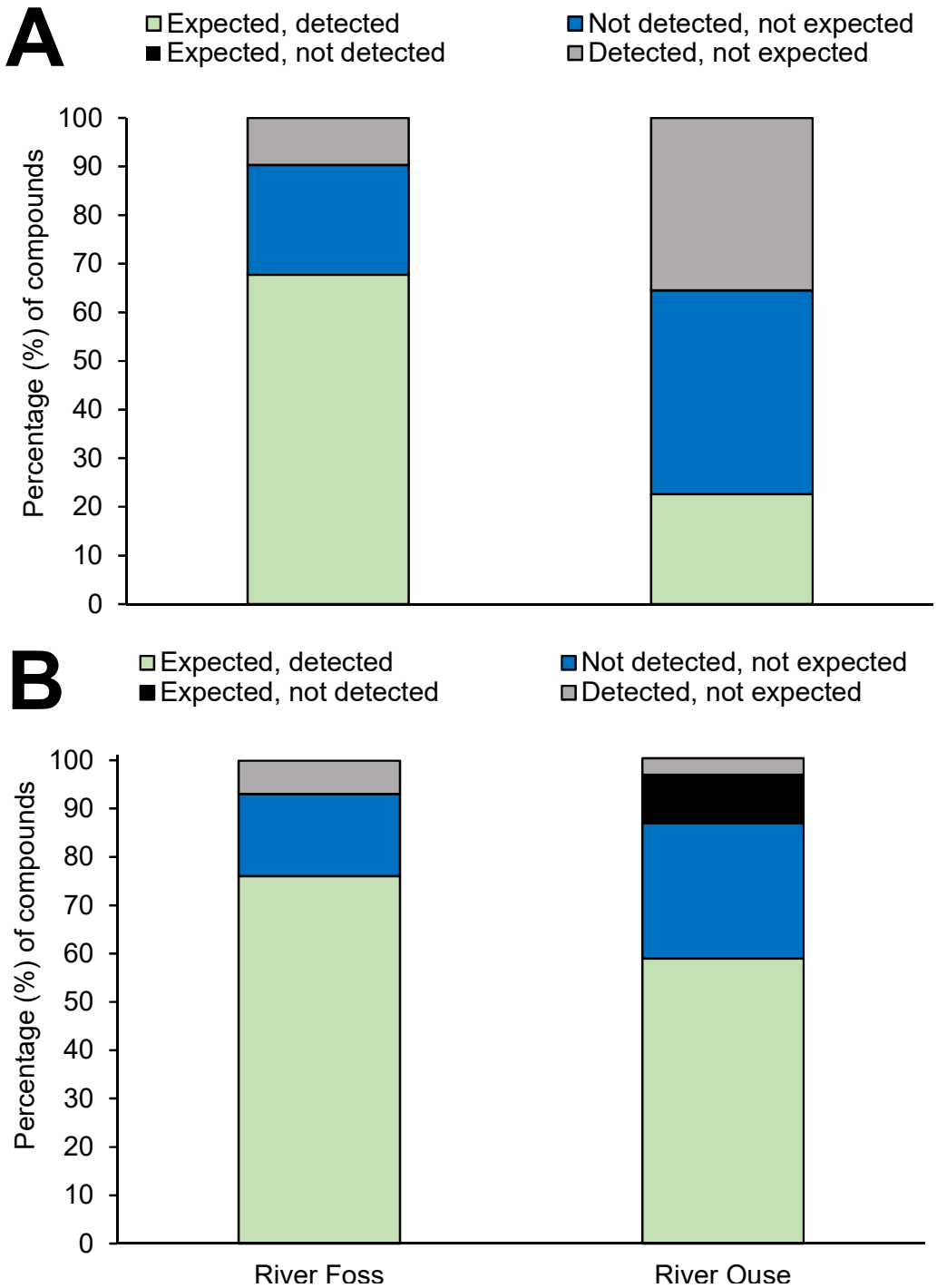


Figure 36. A semi-quantitative analysis of simple PEC (A) and LF2000-WQX PEC (B) performance in the rivers based on the annually averaged measured environmental concentrations (n=12) from site F2 (River Foss) and O3 (River Ouse). A compound is expected to be detected when the PEC is greater than the respective analytical method detection limit.

6.2.1.2 Quantitative analysis

To gauge the overall predictive power of the simple PEC (Figure 37) and LF2000-WQX (Figure 38), all PECs were plotted against their respective MEC (n=172) from all 9 study sites. The simple PEC model performance is poor, with the majority of points falling below the 1:1 line indicating concentrations were underestimated by the simple model (Figure 38). The overall model efficiency (E_1) was found to be 0.32 and the mean predictive factor (i.e. PEC/MEC) across all pharmaceuticals was 0.22 (SD=41) (Table 19). Eight pharmaceuticals predictions were underestimated by greater than a factor of 10 including, gabapentin, metformin, paracetamol, codeine, atenolol, carbamazepine temazepam and venlafaxine, indicating poor model performance (Figure 38).

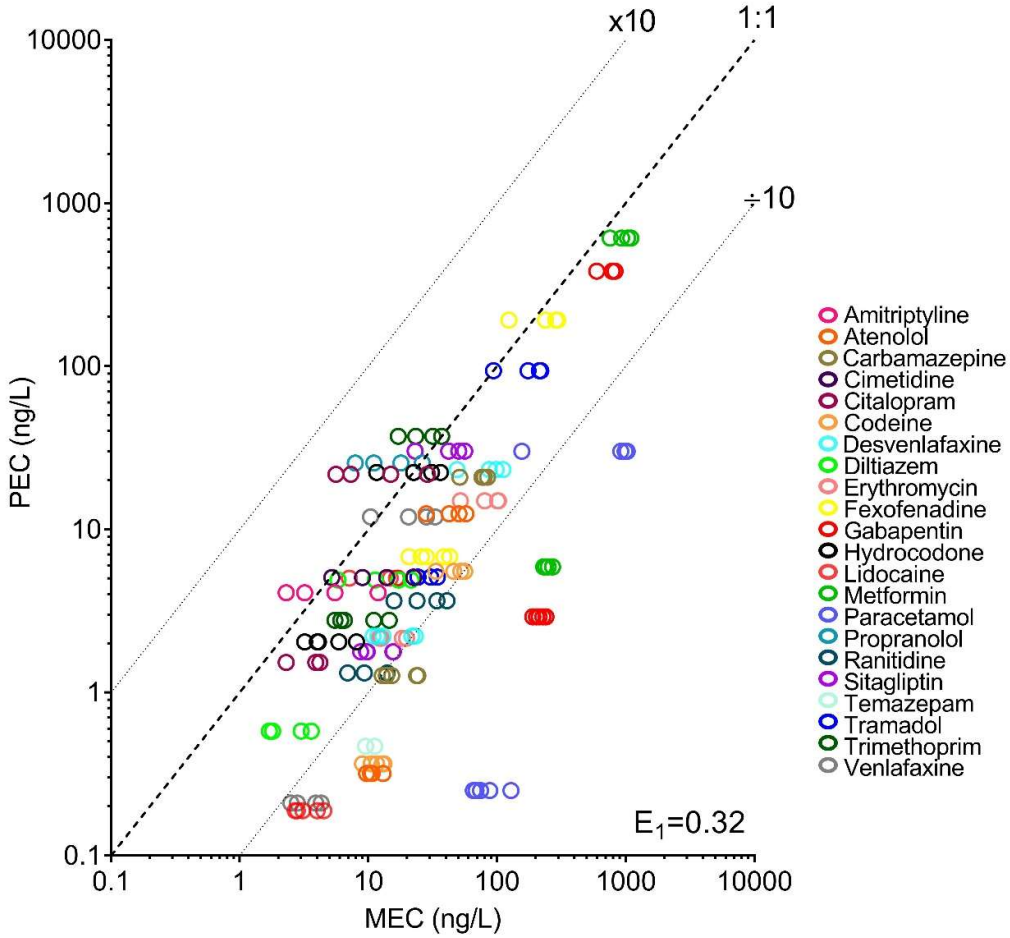


Figure 37. All simple PECs plotted against the corresponding MECs from all nine sampling sites. The goodness-of-fit modified Nash-Sutcliffe model efficiency value (E_1) is also presented.

The overall predictive power of LF2000-WQX was much improved over the simple PEC model, evidenced by an E_1 of 0.57 and with many points falling near the 1:1 line (Figure 38). The mean predictive factor across all compounds was 0.83 (SD=0.77) and

ranged from 0.29 (ranitidine) to 3.18 (propranolol) (Table 20). Only a single compound, paracetamol had a LF2000-WQX PEC underestimated by a factor of 10 (Figure 38). These points correspond to sites F3 to F5 along the River Foss, where exceedingly high values (8 times higher) of paracetamol were found in March and these concentration outliers impacted the annual average MEC. These high concentrations are likely explained by septic effluent entering downstream of the F2 site (Chapter 5) captured in the March sampling. Concentrations of paracetamol may have been disproportionately affected compared to the other determinands due to its high use and high removal capacity in the studied WWTPs (>99%), which when by-passed resulted in a substantial concentration spike in receiving systems downstream of the CSO discharge. This discharge route is not considered in the LF2000-WQX model.

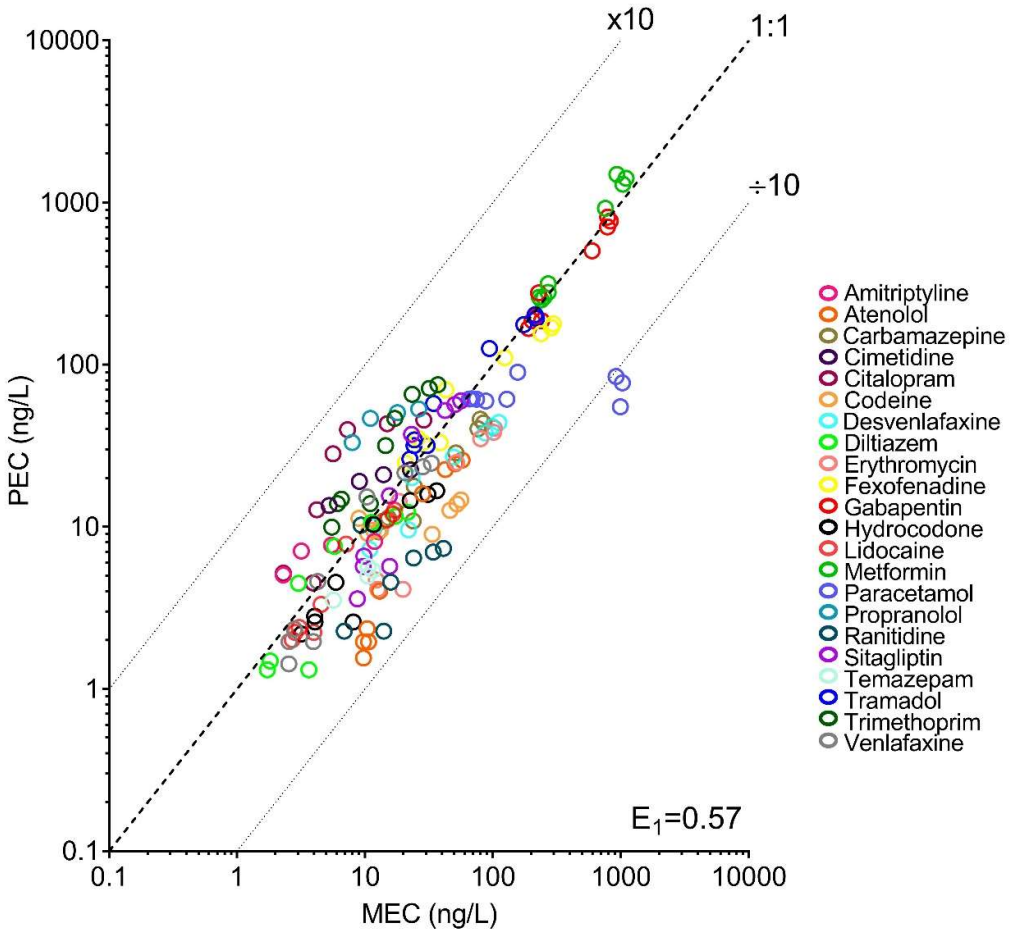


Figure 38. All LF2000-WQX PECs data plotted against the corresponding MECs from all nine sampling sites. The goodness-of-fit modified Nash-Sutcliffe model efficiency value (E_1) is also presented.

Table 20. Average predictive factor (PEC/MEC) for each pharmaceutical from all sites possible to evaluate (i.e. detected) with a maximum of 9 sites. The range of predictive factors is also provided.

Compounds	Predictive factor (PEC/MEC)				Number of sites
	Simple PEC		LF2000-WQX		
	Mean	Range	Mean	Range	
Amitriptyline	0.87	0.34 - 1.78	1.48	0.68 - 2.24	4
Atenolol	0.08	0.02 - 0.44	0.31	0.16 - 0.57	9
Carbamazepine	0.14	0.05 - 0.41	0.62	0.46 - 0.79	9
Cimetidine	0.46	0.22 - 0.97	1.69	0.99 - 2.59	4
Citalopram	1.03	0.36 - 3.88	2.68	1.15 - 5.45	7
Codeine	0.06	0.03 - 0.17	0.51	0.26 - 1.26	9
Desvenlafaxine	0.19	0.10 - 0.48	0.57	0.4 - 0.86	9
Diltiazem	0.31	0.16 - 0.84	0.80	0.36 - 1.49	8
Erythromycin	0.16	0.11 - 0.29	0.40	0.21 - 0.78	8
Fexofenadine	0.41	0.16 - 1.55	0.94	0.59 - 1.64	9
Gabapentin	0.07	0.01 - 0.64	0.93	0.78 - 1.22	9
Hydrocodone	0.61	0.25 - 1.92	0.60	0.32 - 0.89	9
Lidocaine	0.13	0.04 - 0.71	0.76	0.56 - 1.10	9
Metformin	0.10	0.02 - 0.81	1.19	1.04 - 1.6	9
Paracetamol	0.01	0.001 - 0.19	0.33	0.06 - 0.94	9
Propranolol	1.79	0.97 - 3.23	3.18	2.03 - 4.23	4
Ranitidine	0.14	0.09 - 0.23	0.29	0.16 - 1.1	7
Sitagliptin	0.31	0.11 - 1.31	0.81	0.37 - 1.61	9
Temazepam	0.31	0.25 - 0.50	0.52	0.47 - 0.63	4
Tramadol	0.31	0.15 - 1.00	1.18	0.88 - 1.68	9
Trimethoprim	0.64	0.19 - 2.17	2.13	1.27 - 2.81	9
Venlafaxine	0.17	0.05 - 1.15	0.83	0.50 - 1.48	9

Boxall et al.[304] employed an inverse LF2000-WQX modelling approach and compared predictions with annual measured data for atenolol, carbamazepine and trimethoprim, reporting median predictive values of 0.66, 3.2 and 1.6, respectively. This is slightly better than achieved here where median predictive factors for atenolol, carbamazepine and trimethoprim were 0.31, 0.58 and 2.2, respectively. GREAT-ER was simulated for the Glatt river (Switzerland)[158] and overestimated the presence of atenolol by a mean factor of 1.4 and propranolol by a factor of 1.8, both more accurate estimates than determined here (0.31 and 3.18, respectively); however, fewer (two) monitoring points were included in their assessment.[158] Another spatial model developed for Switzerland assessed 12 pharmaceuticals and select metabolites and achieved a mean predictive factor of 1.6 and individual compound predictive factors ranged from 0.8 to 3.4, indicating model under-predictions were less prevalent that in this study.[168] Despite this, the predictive factor mean reported here was slightly more accurate, 0.83 (i.e. closer to 1).[168] These comparisons indicate LF2000-WQX predictions from both rivers perform

similarly to previous spatial pharmaceutical exposure modelling evaluations, however the spatial resolution of the comparative monitoring data in this study permits a more detailed evaluation of each river. The overall assessment also indicates that LF2000-WQX outperforms the simple PEC. The rivers differ in terms of size, depth, WWTP discharge points and treatment technology, upstream pharmaceutical sources and level of urbanisation, therefore a more detailed evaluation of the performance of LF2000-WQX and the simple PEC in these contrasting scenarios was undertaken.

6.2.2 Model evaluation in the River Ouse

6.2.2.1 Simple PEC

Scatter plots of MECs and simple PECs, (i.e. a constant PEC value for all sites) are provided for sampling points O2 to O6, which span 14 km along the River Ouse (Figure 39B). Normally, upstream monitoring data is not available, however to make the comparison with LF2000-WQX more reasonable, upstream concentrations (i.e. site O2) were subtracted from Sites O3 to O6 prior to evaluation. The goodness-of-fit indicated by the Nash-Sutcliffe model efficiency, E_1, ranged from -0.21 to 0.36. The mean predictive factor (across all compounds) for sites O3 to O6 was 0.3, 0.3, 0.15 and 0.24, respectively

Visually, there is a large departure from the 1:1 line due to underestimated PECs at site O3 and O6. The improved simple PEC performance at site O4 (E_1=0.17) could be due to dilution between sites O3 and O4. The significant reduction of MECs observed between these sites provides further evidence of this (Chapter 5). Site O5 is downstream of the confluence with the River Foss, which would have diluted the Ouse further thus further reducing pharmaceutical concentrations at site O5. This could explain the higher E_1 observed at site O5. Site O6 on the other hand, is downstream of another large WWTP (180 500 people) which would contribute pharmaceuticals to the river and would explain the drop in model performance at this site, evidenced by the greater number of points falling below the 1:1 line (Figure 39B).

6.2.2.2 LF2000-WQX

Scatter plots of MECs and PECs, obtained using the LF2000-WQX model are presented for comparison with simple PECs (Figure 39A). Goodness-of-fit indicated by E_1 ranged from 0.81 to 0.89 with visually, limited deviation from the 1:1 line (Figure 39A). The LF2000-WQX mean predictive factors for were: 0.75, 0.53, 0.74, 0.84, 1.07 for sites O2

to O6, respectively. While slightly underestimated, the majority of these values are closer to parity with monitoring data than reported in spatial pharmaceutical model evaluations in the UK and mainland Europe.[158,167,168] The model does have a tendency to underestimate the concentrations of several pharmaceuticals when compared with MECs, which is opposite to a previous LF2000-WQX evaluation exercise using synthetic estrogens where overestimates were more common.[167]

Similarly to the simple PEC, a slightly improved model performance is observed at site O4 in comparison to site O3. Since the LF2000-WQX incorporates the dilution between these two sites, this is not likely the reason for slightly improved model performance at site O4. The LF2000-WQX model assumes complete mixing of effluent with the river by the time it reaches the O3 site, which may not have been the case. The across the channel experiments (Chapter 5, Appendix 12) determined that the river was well mixed when it reaches site O4, however for safety reasons site O3 could not be evaluated. The slightly poorer model performance observed at site O3 may be due to incomplete mixing with effluent and therefore sampling bias, rather than model underperformance.[60,353]

Several WWTPs are present upstream of the city (i.e. site O2), which based on the monitoring results, contribute to concentrations of pharmaceuticals observed in the city. LF2000-WQX can be run from the catchment source, however because upstream measurements were available (Site O1), the simulation was initiated from this point using measured values. Eleven pharmaceuticals were detected at Site O1, and fourteen at Site O2, just upstream of the city. The E_1 for the LF2000-WQX model and the mean predictive factor at site O2 indicate that the model accurately incorporated the contributions from the River Nidd, which was simulated from the source and joins the River Ouse just downstream of site O1 (Figure 35). This suggests that if LF2000-WQX had been run from the source of the River Ouse, good agreement with measured values would likely still be obtained.

The comparative performance of the two modelling approaches help identify several aspects of the simple PEC which make it poorly suited to predicting pharmaceutical concentrations in rivers. Firstly, subtraction of upstream concentrations is likely an oversimplification of mixing in the system and subject to sampling bias (e.g.

the same packet of water was unlikely to be sampled at all sites), which could have contributed to the large PEC underestimations at Site O3 and poor model performance. The incorporation of upstream contributions has been previously suggested to improve the simple PEC estimates,[331] however monitoring data would not normally be available and even if present, such as here, a simple subtraction is likely not sufficient. Secondly, over the stretch of river considered (~ 14 km) significant changes in volume occur due to the merging of tributaries or urban drainage. These influences significantly affect pharmaceutical concentrations, but cannot be accounted for in the simple PEC approach. Thirdly, within this stretch of river pharmaceuticals are introduced via the River Foss and another WWTP, neither of which can be incorporated into the simple PEC approach.

This demonstrates the clear limitations of the simple PEC in the River Ouse, all of which can be accounted for using higher tier spatial modelling approach. This includes changes in river volume due to the convergence with other water bodies, multiple WWTP inputs and mixing with upstream pharmaceutical contributions. Incorporation of these factors are highly important as evidenced by the significantly improved performance of LF2000-WQX at all River Ouse sampling sites over the simple PEC (Figure 39).

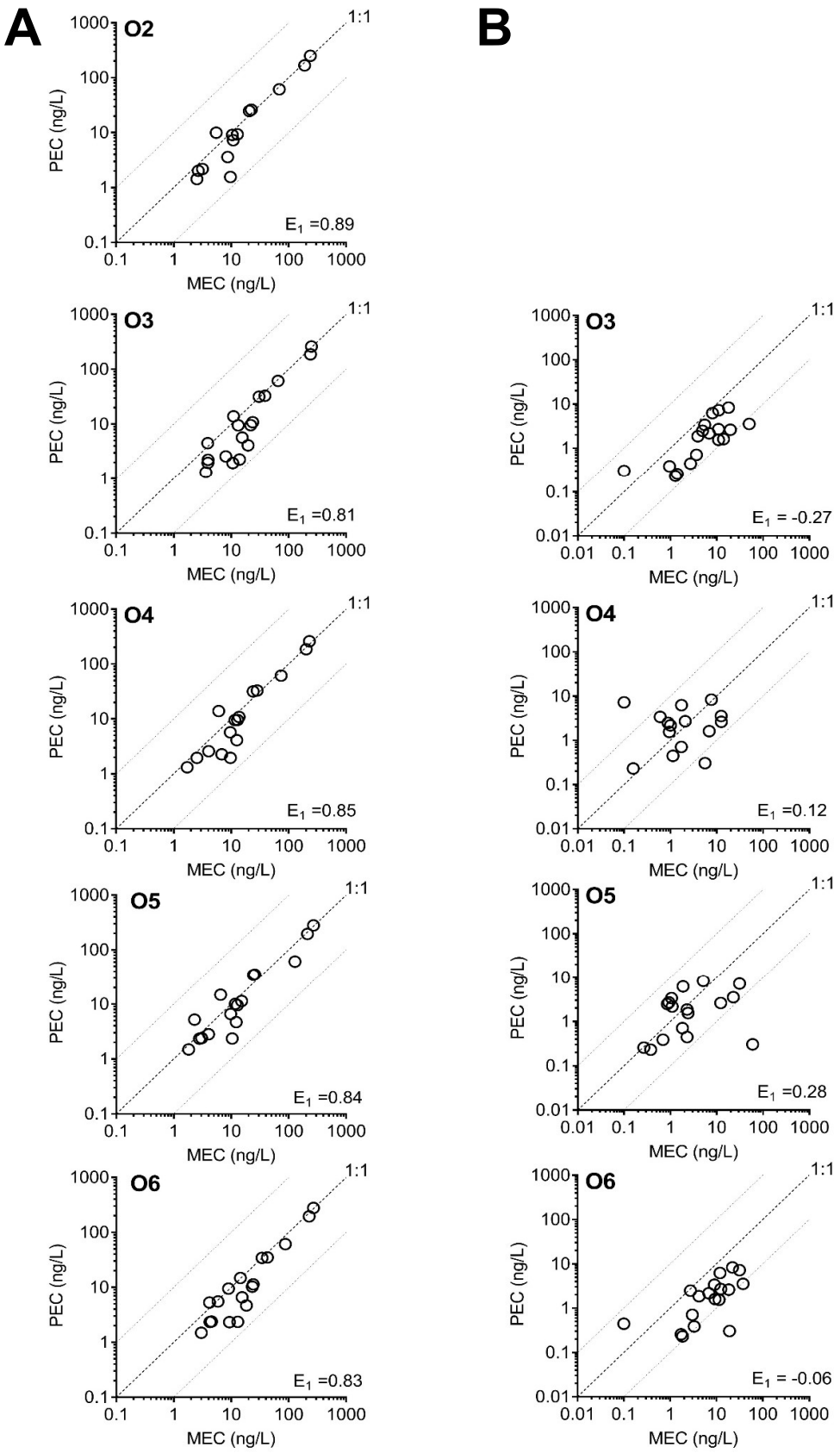


Figure 39. LF-2000-WQX (A) and simple static PECs (B) plotted again MECS from sites O2 to O6 in the River Ouse (continued on next page).

Figure 40. (continued) Site O2 is not plotted for the simple PEC because this data was used for the upstream of the WWTP subtraction. The simple PEC is the same for each site, while the LF2000-WQX output is location specific. The goodness-of-fit is indicated by the modified Nash Sutcliffe model efficiency value (E_1). Paracetamol outliers were excluded from the model evaluation.

6.2.3 Model evaluation in the River Foss

6.2.3.1 Simple PEC

The smaller more rural River Foss, with a single WWTP input and four monitoring sites (F2 to F5) spanning 13 km within the modelled area, produced E_1 values ranging from 0.57 to 0.70 at Sites F2 to F5 (Figure 40B). Model performance was slightly better at site F2 (immediately downstream of the WWTP) than at sites F3 and F4 and the highest E_1 value was observed at site F5. Mean predictive factors were 0.36, 0.37, 0.44 and 0.77 at sites F2 to F5, respectively.

The improved performance of the simple PEC in the River Foss is consistent with the characteristics of this river. There a single WWTP, limited upstream inputs and dilution along its length is significantly less than in the River Ouse. In theory, this is the ideal scenario for using the simple PEC, and this was observed in the model performance at site F2 (E_1=0.6). The limited influence of flow in this river is demonstrated by the minimal change in E_1 values at site F3 and F4 (Figure 40B). In contrast, the model performance improves at the most downstream site, F5 (E_1=0.7). Similarly to the River Ouse O3 site, many pharmaceutical predictions were underestimates at site F2 (immediately downstream of the WWTP discharge), Figure 40B. Dilution of the River Foss, which would reduce concentrations prior to the F5 site could potentially explain the improved model performance at this site.

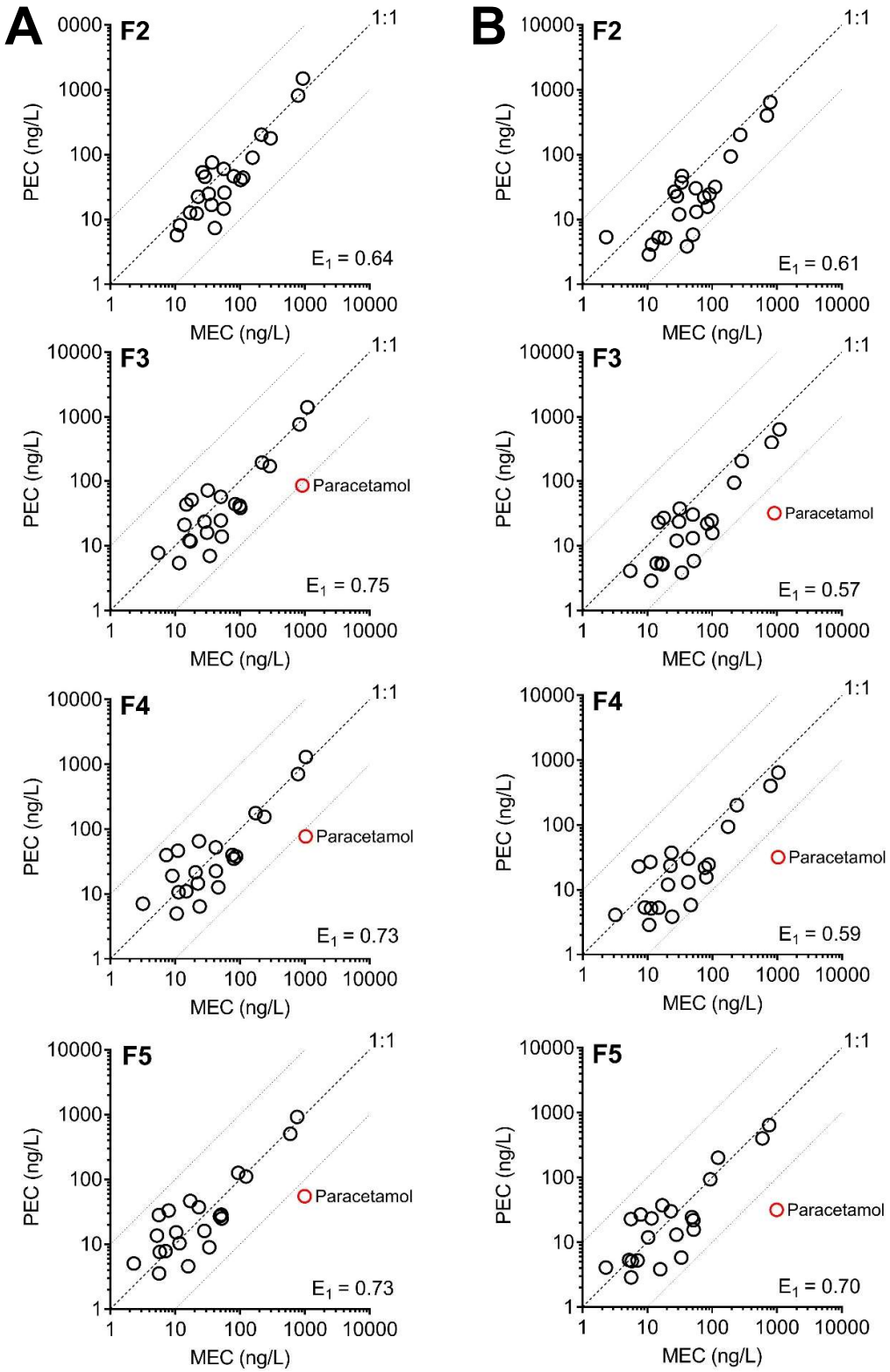


Figure 40. LF-2000-WQX (A) and simple static PECs (B) plotted again MECS from sites F2 to F5 in the River Foss. The simple static PEC is the same for each site, while the LF2000-WQX output is location specific. The goodness-of-fit is indicated by the modified Nash-Sutcliffe model efficiency value (E_1). Paracetamol outliers were excluded from the model evaluations.

6.2.3.2 LF2000-WQX

The LF2000-WQX model performance in the River Foss was more closely related to the simple PEC model than in the River Ouse, with model efficiency values E_1 ranging from 0.62 to 0.74 (Figure 40A). The LF2000-WQX mean predictive factor for sites F2 to F5 was 0.70, 0.71, 0.84 and 0.94, indicating that similarly to the River Ouse, LF2000-WQX slightly underestimated concentrations, but on average, predictions were much more similar to the MECs than the simple PEC. The higher E_1 values, predictive factors closer to one and visually, points more equally scatter around the 1:1 line (Figure 40A) indicate that this spatial model is superior to the simple PEC spatially, even in this more simplistic scenario.

The goodness-of-fit and mean predictive factor were poorest at the site closest to WWTP discharge (site F2), improving at site F5, furthest downstream. Out of the 22 pharmaceuticals, LF2000-WQX underestimated concentrations of 16 compounds at site F2, overestimating only citalopram, gabapentin, metformin, propranolol, sitagliptin and trimethoprim. Interestingly, only 11 pharmaceuticals were underestimated at the downstream F5 site (atenolol, carbamazepine, codeine, desvenlafaxine, erythromycin, fexofenadine, gabapentin, hydrocodone, paracetamol, ranitidine and temazepam), where improved mean predictive factors and model performance was observed.

The largest number of model underestimations occurred at site F2, closest to the pharmaceutical source, indicating that the amount of drug predicted to enter the river was likely underestimated. The underestimate could have arisen from the predicted pharmaceutical usage, however high quality local prescription data was used and paired with the highest fraction excreted unchanged reported in the literature. On the other hand, several of the study compounds are available over-the-counter (OTC), a usage pathway that was not accounted for, which has been identified previously as causing a systematic underestimate of PECs.[7] Compounds available OTC are identified in Table 18, all of which were underestimated at Site F2, however this only explains five of the total 16 underestimated PECs at Site F2.

Another possible reason for the underestimations, could be the WWTP experimental removal estimate used in the model simulation. It has been demonstrated pharmaceutical WWTP removal capacity can exhibit both short term and seasonal

fluctuations.[51,354,355] The experimental WWTP estimate was calculated from samples collected during the summer months (Chapter 5), during which time WWTP removal efficiency is expected to be greater, compared to colder months.[62] Therefore, the removal estimate may not have been comparable to removal conditions occurring throughout the year when the monitoring data was collected. WWTP removal capacity may need to be approached differently, a constant removal rate based on a single or few estimations, may not be appropriate as this could be a factor behind the number of underestimated pharmaceutical concentrations.

The improved model performance at site F5 using both modelling approaches is unlikely to be explained by dilution, as this would affect all points equally, which visually, does not quite match (Figure 40) and is accounted for in the LF2000-WQX model. To investigate further, an assessment of in-stream losses was undertaken.

6.2.3.3 In-stream losses

To investigate the spatial trends in the River Foss, MECs and PECs (LF2000-WQX only) from site F2 to F5 are plotted together (Figure 41). The open symbols represent PECs, while the closed symbols are MECs. It is immediately clear that both PECs and MECs decrease moving downstream from the WWTP. PECs follow a consistent loss trend, synonymous with dilution of the river moving downstream. Conversely, the MEC losses are pharmaceutical specific, with certain compounds suffering steep losses for example, diltiazem and cimetidine (Figure 41H), while others are less severe such as codeine (Figure 41B), trimethoprim (Figure 41C) or gabapentin (Figure 41A). Certain pharmaceuticals suffer the greatest loss between site F2 and F3, for example amitriptyline (Figure 41J) or citalopram (Figure 41D), while others have the largest loss between site F4 and F5, for example fexofenadine (Figure 41E) or desvenlafaxine (Figure 41B). Many downstream loss trends are present and for the majority of MECs and this decrease is at a faster rate than the respective PECs in the River Foss.

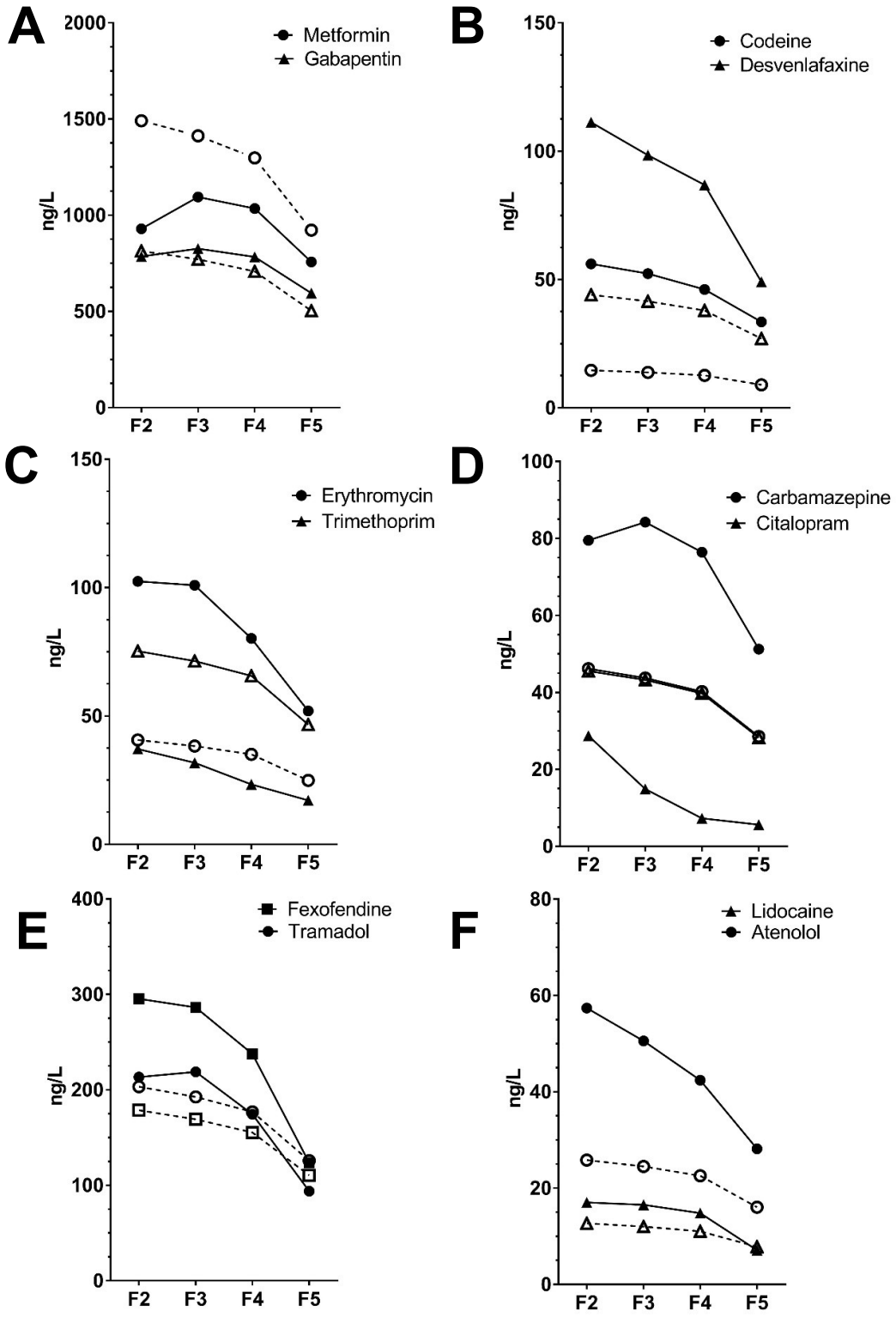


Figure 41. Spatial concentrations trends in the River Foss sites F2 to F5 for MECs (solid symbols) and PECs (open symbols) calculated with LF2000-WQX.

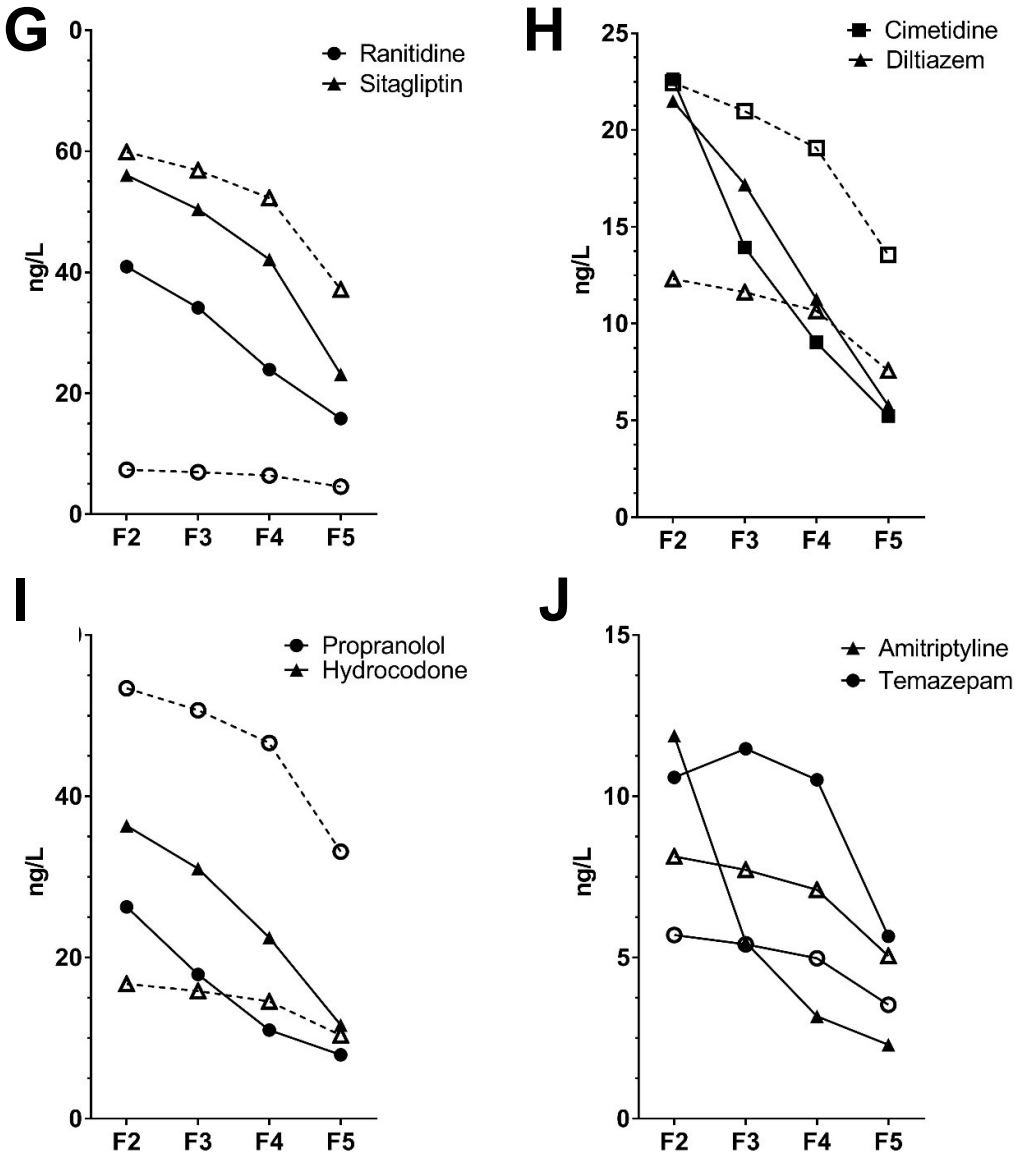


Figure 41. (continued) Spatial concentrations trends in the River Foss sites F2 to F5 for MECs (solid symbols) and PECs (open symbols) calculated with LF2000-WQX.

In several cases PECs which were dissimilar from MECs at site F2 become similar to MECs by site F5 for example, fexofenadine (Figure 41E), lidocaine (Figure 41F) or hydrocodone (Figure 41I). On the other hand, pharmaceuticals which had PECs similar to MECs at site F2, such as cimetidine (Figure 41H), sitagliptin (Figure 41G) or tramadol (Figure 41E) were no longer similar at site F5. The improvement of LF2000-WQX model and simple PEC goodness-of-fit to the monitoring data moving downstream, could therefore be due to in-stream attenuation processes such as photolysis, hydrolysis,

microbial degradation and sorption to sediment operating within the River Foss, which were not included in the model simulation.[302]

The in-stream losses (after accounting for in-stream dilution) between site F2 and F5 were estimated (Figure 42) and range from no observed losses (gabapentin, carbamazepine and metformin) up to 65% for amitriptyline and citalopram. Several of the pharmaceuticals studied have been previously reported as readily photodegradable. Half-lives less than a day have been reported for amitriptyline, citalopram, desvenlafaxine, codeine, cimetidine, propranolol and ranitidine,[356–359] which is consistent with these pharmaceuticals experiencing greater in-stream losses (Figure 42). Conversely, longer photodegradation half-lives (e.g. >3 days) have been reported for fexofenadine, venlafaxine, trimethoprim, atenolol, erythromycin, lidocaine, hydrocodone and temazepam.[67,356–358,360,361] It is probable that abiotic degredation (i.e. photolysis) will be dominant over microbial degredation due to exposure to sunlight,[362] as many pharmaceuticals contain fuctional groups (e.g. aromatic rings or heteroatoms) which can absorb solar radiation (direct photolysis) or react with photo-excited species in the water (indirect photolysis).[73] The in-stream losses presented here are only estimates (Figure 42), likely to be biased by sampling times and subject to multiple in-stream attenuation processes operating simultaneously (e.g. photodegredation, microbial degredation, hydrolysis or sorption to sediment), indicating that quantitative comparison with fate data generated in the laboratory is likely innapropirate.[357] What can be concluded, is that significant in-stream losses are likely occuring for many of the pharmacuticals studied in the River Foss, which affects the preformance of the model.

This in-stream attenuation demonstrated in the River Foss could also be occurring in the River Ouse; however the consistent model performance moving downstream in the River Ouse suggests it may not be as important for obtaining good predictions in this river. This could be investigated by using the loss rates derived here and simulating the LF2000-WQX model again, to identify whether this improves predictions in the River Ouse. Regardless, the decision not to incorporate in-stream decay in the simulation actually resulted in improved model goodness-of-fit at downstream sites in the River Foss. This is because many PECs were initially underestimated at site F2 (due to underestimated WWTP emissions to the river) and experienced no attenuation moving

downstream other than dilution. The result was unintentionally more accurate predictions at site F5. While it is important to recognise these fate processes are occurring and do affect model accuracy, priority should be given to first ensuring that river input concentrations from the WWTP are not underestimates due to inappropriate usage data or WWTP removal estimates.

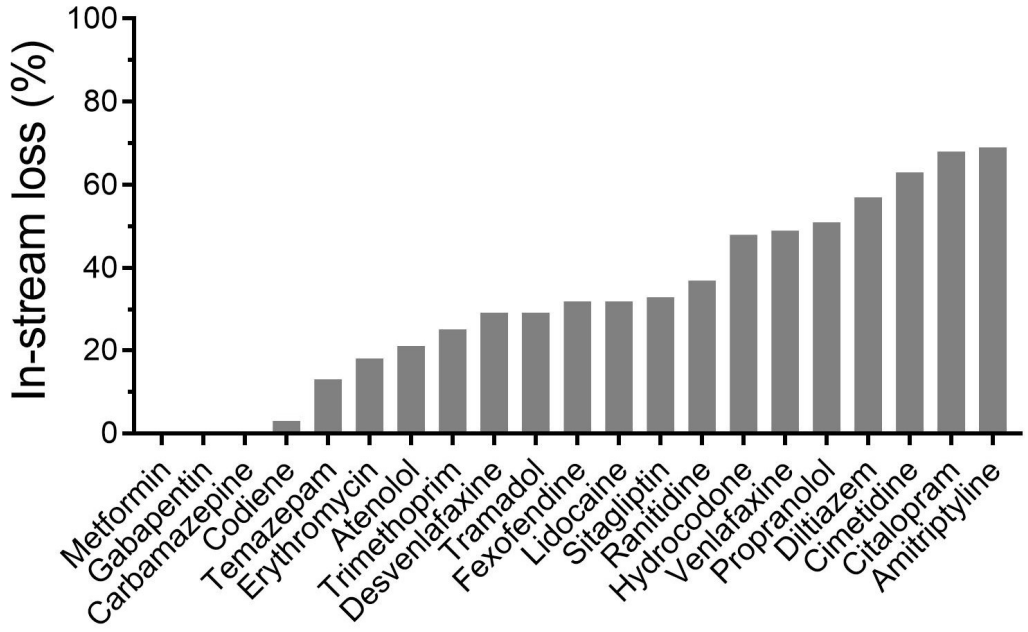


Figure 42. Estimated downstream annual losses between site F2 and F5 based on monitoring data after accounting for losses due to dilution indicated by LF2000-WQX (38%).

6.2.4 Limitations with the current model evaluation

There are several limitations to consider with the LF2000-WQX model evaluation presented. The parameterisation of the model was consistent with practice in the literature,[158,167,168] however, this parameterisation approach could be the reason for discrepancy between MECs and PECs, not the model itself. Firstly, the consideration of WWTP removal as a single value is likely to produce errors, as removal rates are highly variable and analytical accuracy in these matrices is lower.[61] Therefore, including error with this removal value could improve modelling results and could be an important factor in why many PECs were underestimated. Secondly, while many of the PECs were underestimated using the highest fraction of pharmaceutical excreted unchanged in the literature, consideration of this parameter in this way could be another important source

of error. For example, metabolism is variable amongst different age groups and ethnicities therefore applying a single value to define metabolism in model is likely inappropriate. Adaptation of the model to consider error alongside the metabolism estimation should be included. Furthermore, using the highest value found in the literature gave the best results here, but when other factors affecting model performance are addressed this practice could lead to an overestimation of pharmaceutical concentrations. This is a limitation of the current study and in future the quality of the metabolism data reported in the literature needs to be assessed. This could include evaluating the size, age range and ethnicity of the cohort tested in the metabolism study. A focus should be put on collecting data from large studies based on diverse sets of subjects as they are more likely to reflect the general excretion trend of a drug. Furthermore, studies for each pharmaceutical should be pooled and a weighted average calculated based on data quality. Factoring in error and using weighted averages for metabolism and WWTP removal needs to be included in future model simulations and the performance of LF2000-WQX re-evaluated.

Analytical error is another limitation which could affect how the model preformed against MECs in this study. For example, a few compounds in the method did not have their own isotopically labelled internal standard (ILIS). This indicates that for certain compounds, matrix effects may not have been appropriately compensated. Cimetidine and ranitidine did not possess an ILIS and were both significantly underestimated by the model. It is possible that matrix effects caused signal enhancement for these compounds, which would result in higher measured concentrations. In future, a robust evaluation of the model will require each compound to be fully quantitative as results can only be semi-quantitative when an ILIS is not used to compensate for matrix effects.

6.2.5 Catchment risk assessment

Risk quotients (RQ) for the LF2000-WQX modelled river reaches are depicted by box plots (Figure 43). An RQ was calculated using the mean model output for each reach within the modelled area to create the RQ distributions plotted (Figure 43). The FPM approach (Figure 43A) estimates risk based on predicted environmental concentrations and subsequent uptake into a fish (bioconcentration factor). A ratio of the theoretic uptake and the human therapeutic concentration (peak plasma concentration (C_{max}) is

calculated and when this ratio is 1 or greater, a risk could be present. This is because the FPM operates on the assumption that if the therapeutic concentration in a human (i.e. concentration that elicits an effect) is achieved within a fish, an effect is possible. The FPM was an approach chosen to assess risk because it has been used to prioritise pharmaceutical risks previously.[6,194] The FPM also permits the assessment of all study compounds as the parameters used to calculate the theoretical fish uptake and subsequent risk are commonly derived during drug development.[118] This theoretical 'read-across' risk assessment approach was compared with a risk assessment based on experimental non-standard effect data (Figure 43B). The non-standard endpoints were retrieved from the literature and available for 21 of 29 pharmaceuticals assessed (Table 19). This data was classified by the type of endpoint measured including, behavioural, physiological changes, biochemical changes and histopathological changes. The experimental studies include several fish exposures, but also several invertebrates and a plant (i.e. paracetamol).[347] The relevance of these endpoints to healthy ecosystem functioning, species and population level effects is not yet well understood; however, these effects are observed at orders of magnitude lower than apical ecotoxicological assessment approaches, which could be important.[37]

The two risk assessment approaches produce dissimilar results (Figure 43). The FPM-based approach identified two pharmaceuticals which posed a potential risk to 4% (loratadine) and 18% (desvenlafaxine) of river reaches in the modelled area. Conversely, based on non-standard endpoints available, 12 of the 21 pharmaceuticals evaluated were identified as posing a potential risk (i.e. risk characterisation ratio above 1). Propranolol posed a risk to 100% of the reaches within the modelled catchment, while citalopram and venlafaxine posed a risk to 98% of river reaches and carbamazepine posed a risk to 88%. Noreistherone is a risk to 52% of river reaches, while the remainder of risks identified pertain to less than 50% of reaches within the modelled area (Figure 43B). Of the pharmaceuticals possible to evaluate with both approaches, propranolol, citalopram and noreistherone exhibit some of the highest RQs using both methods relative to other pharmaceuticals, while tramadol in terms of relative risks was much higher using the FPM than when non-standard effects were considered.

An absolute comparison of the risks based on both approaches would be inappropriate. For example, for the highest risk compounds identified by the FPM, desvenlafaxine and loratadine, had no non-standard endpoint available. The non-standard ecotoxicity RQs are biased towards the compounds studied, the endpoints considered, as well as the test concentrations used. What can be concluded is that based on the LF2000-WQX output for the catchments, RQs calculated using the FPM are orders of magnitude lower and therefore less risky than those assessed with select non-standard endpoints. It is also important to note that two higher risk compounds (loratadine and noreistherone) were not detected frequently enough to calculate an annual average MEC, which was expected (Figure 36). This highlights an advantage previously observed for evaluating catchment pharmaceutical risks using modelling approaches, compounds with high analytical detection limits or very low environmental concentrations can be assessed.[353] Finally, the comparison of LF2000-WQX PECs with MECs demonstrated that many predictions were underestimates, which could indicate that in reality, a greater number of river reaches could be at risk.

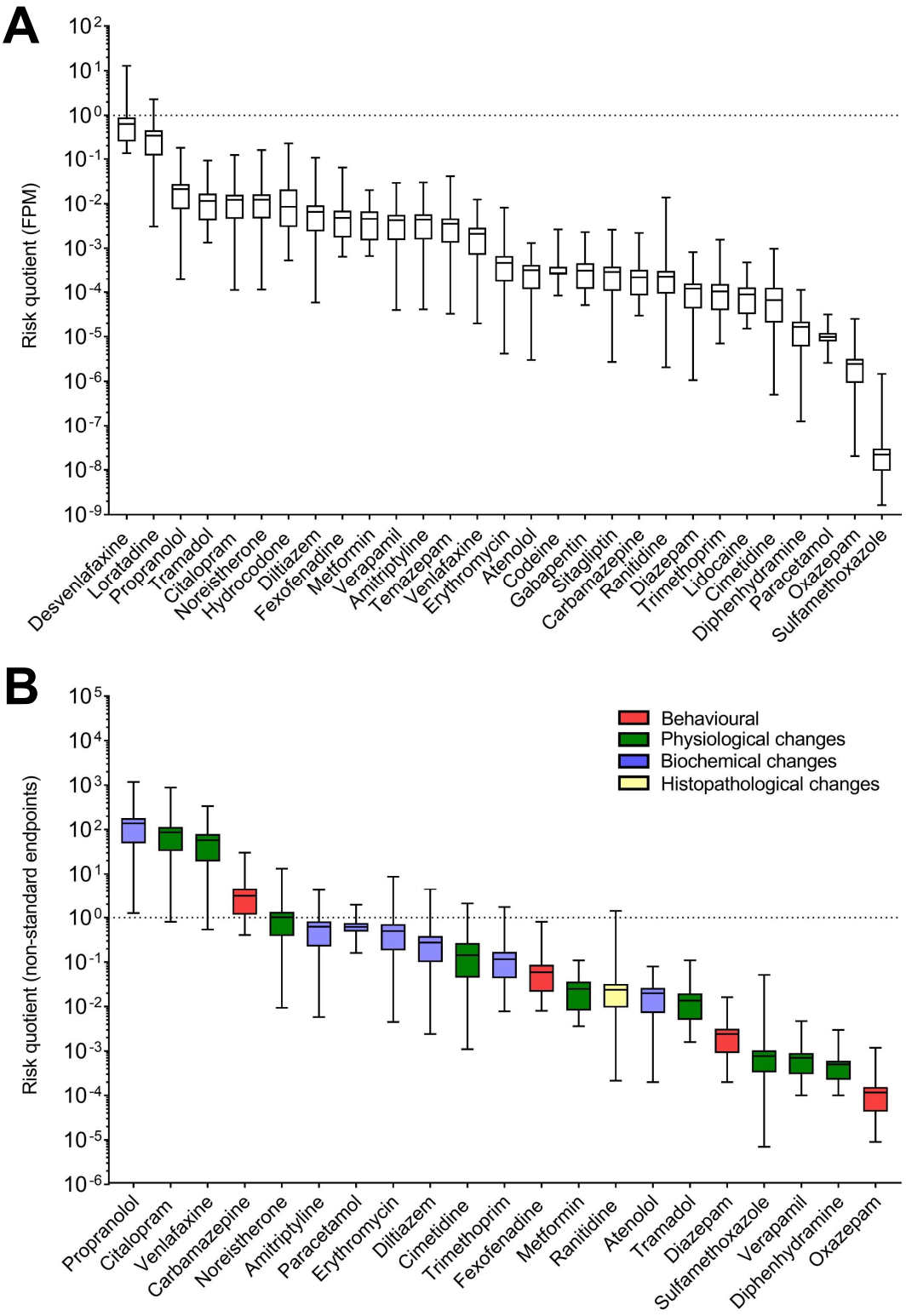


Figure 43. Risk quotients (RQs) based on all output from all river reaches modelled area. A) RQs calculated using the fish plasma model and B) based on the most sensitive non-standard endpoint found in the literature. Box and whisker plots indicate the median, lower and upper quartile and minimum and maximum RQ.

6.3 Conclusion

We have compared the performance of the simple PEC model proposed by the EMA[112] and the higher-tier spatial model, LF2000-WQX. This was achieved by evaluating PECs from these two models, for 22 pharmaceuticals, against annually averaged MECs in the larger more urban River Ouse and smaller more rural River Foss. Overall, the simple PEC underestimated eight compounds by a factor of 10, while the LF2000-WQX model performed much better with a mean predictive factor of 0.82 across all nine study sites. The LF2000-WQX predictions were superior to predictions based on simple PECs in both rivers. The goodness-of-fit of the LF2000-WQX model was better for study sites in the larger River Ouse, which is characterised by multiple pharmaceutical inputs, than the smaller River Foss with a single pharmaceutical input. Conversely, the simple PEC performed much better in the River Foss than the River Ouse. Local usage data was used in the modelling exercise therefore underestimated predictions, particularly in the River Foss are hypothesised to result from overestimated WWTP removal. In-stream losses were estimated in the River Foss and found to be affecting model performance in this river. A catchment risk assessment using the FPM identified desvenlafaxine and loratadine as highest risk pharmaceuticals with 4% and 18% of river reaches at risk, respectively. RQs based on non-standard endpoints were orders of magnitude higher with risks posed to 100% of river reaches by propranolol and 98% by citalopram and venlafaxine, while carbamazepine posed a risk to 88% of river reaches.

Chapter 7

Discussion and recommendations

7.0 General discussion of research findings

The study of freshwater environments is of great importance due to their critical role in delivering a range of ecosystem services essential for sustaining life. The contamination of these systems is an expansive area of research, which drives the creation of regulations and policies aimed to maintain healthy ecosystem functioning with respect to anthropogenic contamination. A good example of this is the priority substance list (Decision 2455/2001/EC) within the Water Framework Directive (WFD), which is the major regulatory instrument for achieving and maintaining clean and healthy waterbodies in the European Union. For these substances, environmental quality standards (EQS) have been derived, which member-states are required to monitor and develop action plans to achieve compliance. In recent decades, the detection of contaminants outside current regulations has spurred significant investigation to determine whether these emerging contaminants, which enter the environment through human use or manufacture, could accumulate to levels of concern for human and/or environmental health. As the knowledge surrounding these emerging contaminants has grown, a WFD watch list, which includes six pharmaceuticals (17-β-estradiol, 17-α-estradiol, diclofenac, erythromycin, clarithromycin and azithromycin) has been produced. The goal of the watch list is to gauge Europe wide contamination by these compounds in order to assess risks. The results will inform whether legally binding EQS values or other mitigation measures are required to protect waterbodies. The inclusion of pharmaceuticals on the WFD watch list highlights the fact that the presence of pharmaceuticals in the environment is of significant concern. Therefore, further work to characterise the risks of less studied pharmaceuticals is of great importance to ensure that further possible risks to the environment are identified and can be mitigated if necessary.

With over 1500 pharmaceuticals currently in use, the task to quantify environmental exposure and assess all ecotoxicological endpoints to estimate risk of these compounds is a daunting and lengthy task. Pharmaceutical prioritisation, which can be used to direct research efforts to those pharmaceuticals suspected to pose the greatest risk to the environment, may be part of the solution. To address the problem of limited environmental or ecotoxicity data availability for many pharmaceuticals, prioritisation is

based on modelling approaches to predict exposure and effects. Exposure models generally used for prioritisation are simplistic and the suitability of these models and the implications for risk assessment and prioritisation is not known. Therefore, the overall aim of this thesis was to evaluate the performance of simpler and higher tier exposure models used for prioritisation and risk assessment and to develop recommendations on how best to assess exposure in the future.

A literature review of pharmaceutical prioritisation approaches revealed that over 320 priority pharmaceuticals have been identified as a potential concern throughout the world. For only 29% of these are environmental data available in the form of a publicly accessible environmental risk assessment (ERA) held by the European Medicines Agency (EMA) (Chapter 2). The models underpinning previous prioritisation exercises were identified, with the most promising pulled together in a new holistic prioritisation approach. The developed prioritisation approach can identify which pharmaceuticals are risky as well as in which environmental compartment these risks are likely to occur. An evaluation of the confidence in models required to underpin the framework revealed that exposure models commonly used for both prioritisation and risk assessment have been subject to limited experimental validation. The most widespread and significant pharmaceutical pathway to the environment was identified as municipal WWTP discharges to the receiving aquatic environment,[15,363] indicating aquatic exposure models based on this emission pathway are most pertinent to assess. Monitoring studies were therefore initiated to develop robust datasets in order to evaluate the performance of commonly used exposure models and the implications of model performance (e.g. under or over estimations) for pharmaceutical prioritisation and risk assessment exercises.

In a scoping study (Chapter 3), predicted environmental concentrations (PECs), based on a commonly used EMA model,[112] were calculated and compared with monitoring data from a single grab sampling-based campaign in the Rivers Ouse and Foss in York, UK (Chapter 3). A pre-existing analytical method was used to measure 95 target pharmaceuticals in water samples collected across the river network, 25 of which were quantified. Measured environmental concentrations (MECs) were greater than the PECs for 38% and 78% of pharmaceuticals in the River Foss and Ouse, respectively. The major finding was that this discrepancy did affect prioritisation results, as risk quotient

rank order, based on model predictions, deviated from that calculated based on MECs. This was especially apparent for the River Ouse data, where risk quotient (RQ) ranks based on PECs and MECs overlapped for only 22% of pharmaceuticals. The outcomes were better for the River Foss data, with 36% of priority rankings overlapping. A previous study quantified how differing effect models influence priority rankings[6] and the results presented in Chapter 3 highlight that exposure models can also affect prioritisation rankings. The common use of these simple exposure models for prioritisation and risk assessment may therefore not be appropriate as they could lead users to the wrong conclusions over which pharmaceuticals are risky, potentially undermining the assessments. There are however, limitations with the evaluation approach used, namely the monitoring data used to compare with PECs was limited, and the PECs are meant to represent the average exposure conditions experienced in the river system, which may not have been captured by monitoring single grab samples. A more thorough monitoring campaign was therefore performed.

To support the detailed monitoring campaign an HPLC-MS/MS method for the quantification of 33 pharmaceuticals in aqueous matrices was developed and validated (Chapter 4). The method is rapid in that no sample pre-concentration/clean-up is needed. The selection of compounds was based on those detected in the scoping study as well as including an additional compound (gabapentin) thought to be present in the study area. The new method was developed from the pre-existing method used in Chapter 3. The chromatographic separation time was halved and lower limits of detection achieved. Limits of detection ranged from 0.9 ng/L (carbamazepine) to 12.4 ng/L (gabapentin). The method was validated for surface water, and WWTP influent and effluent. The larger injection volume enabled analytes to be detected without sample pre-concentration and matrix recovery was comparable with or even better for certain analytes, than that of methods employing sample clean-up (e.g. solid phase extraction); all 33 analytes had recoveries between 70-120% in surface water from an 80 ng/L matrix spike.

The rapid HPLC-MS/MS method was then applied to a year-long monitoring campaign of 11 sites along the Rivers Ouse and Foss (Chapter 5). The goal was to build up a robust monitoring dataset suitable for validating the exposure models and also characterising spatial and temporal drivers of exposure reflective of the river system. To

do this, a sampling design reflective of spatial (11 sites) and temporal (12 months) variability was employed. Significant spatial and temporal differences in the concentrations of pharmaceuticals were observed.

In the River Foss, temporal differences in concentrations could be explained by changes in river flow and pharmaceutical usage over the year, which is consistent with results of similar monitoring studies reported elsewhere.[39,324] Spatial analysis indicated that in-stream attenuation may be occurring as significant differences arose between sampling sites moving downstream of the WWTP for most pharmaceuticals, while others known to be recalcitrant to environmental degradation (e.g. carbamazepine) did not exhibit significant differences.

In the River Ouse, few pharmaceutical concentrations significantly differed temporally, and pharmaceutical usage and flow were not well correlated with monthly measured concentrations for many pharmaceuticals. When concentrations were converted to loads, more pharmaceuticals exhibited seasonally significant fluctuations, which was hypothesised to be the result of seasonal variability in WWTP removal efficiency. Spatially, concentrations differed significantly between all sites for recalcitrant compounds (e.g. carbamazepine) indicating that changes in river volume or further sources of pharmaceuticals (e.g. tributaries) are significant drivers of concentrations of these substances in this system. Previous research has identified that flow, pharmaceutical usage, WWTP removal, and in-stream attenuation all influence observed concentrations and the results presented in Chapter 5 are consistent with this. The key finding is that these factors influence concentrations differently, even in neighbouring rivers within the same catchment.

The monitoring campaign also revealed that pharmaceuticals were transported from upstream to the city, and significant spatial differences between sampling sites downstream of WWTPs were observed in both rivers. The simple PEC (Chapter 3) is therefore not suitable for predicting concentrations in these rivers, as it models the riverine concentration immediately downstream of WWTP inputs, does not incorporate upstream contributions and overlooks in-stream fate. Therefore, a higher-tier model capable of incorporating spatial variability and modelling all inputs from the catchment source would be more appropriate for to predict concentration in the study system.

Studies were therefore performed comparing a spatial exposure modelling system developed for use on down-the-drain chemicals with the simple PEC.

The model chosen was LF2000-WQX which includes the entire river system from England within a GIS platform and all WWTP discharges are spatially incorporated (Chapter 6). The model can also incorporate in-stream decay, but this was not included as previous river modelling has suggested the impact of in-stream decay will be minimal[158] and experimental decay rates have not been characterised for many of the pharmaceuticals studied. The performance of the model, as well as the simple PEC was evaluated against annually averaged MECs for each sampling location. The Nash-Sutcliffe model efficiency (E_1), was used to evaluate the model, which gives an indication of how closely modelled versus predicted concentrations fall around a 1 to 1 line. An E_1=1.0 indicates a perfect model fit, while E_1=0 indicates that the measured mean is just as good a predictor as the model.[334] The E_1 ranged from 0.83 to 0.89 in the River Ouse (at well-mixed sites) and 0.64 to 0.75 in the River Foss for LF2000-WQX. Model efficiency was much poorer for the simple PEC, ranging from -0.27 to 0.28 in the River Ouse and from 0.57 to 0.70 in the River Foss. The better performance of the simple PEC in the smaller River Foss is likely due to the limited upstream inputs, single WWTP discharge and limited changes in river volume along the sampling sites in this river. In both rivers, PECs were more commonly underestimated, with an overall predictive factor (i.e. PEC/MEC) for all pharmaceuticals of 0.83, ranging from 0.29 (ranitidine) to 3.18 (propranolol) for LF200-WQX and a mean of 0.22 ranging from 0.01 (paracetamol) to 1.79 (propranolol) for the simple PEC. Consistent model performance (E_1) moving downstream in the River Ouse was observed. Conversely, in the River Foss, model performance was affected moving downstream from the WWTP, which was determined to be the result of in-stream decay affecting model performance in this river. Overall the predictive power of LF2000-WQX was comparable with that of previous validation exercises,[167,304] as well as for similar spatial models applied in Europe (e.g. GREATER-ER).[158,168] These results indicate that spatial exposure models are better suited to predicting pharmaceutical concentrations in river systems than simple PECs as they incorporate pharmaceuticals transported from upstream, WWTP locations, factors which affect flow (e. g. tributaries), and can include in-stream decay.

The spatial exposure model produces output for all river reaches within the modelled catchment and can be simulated from the source waters, making it highly suited to assessing catchment risks. For example, a pharmaceutical may be a risk immediately downstream of the WWTP as indicated by the simple PEC; however, flow dynamics or in-stream decay could indicate that spatially, exposure is limited. Conversely, risks could be higher than expected from simple PECs due to upstream pharmaceutical inputs. A catchment risk assessment based on LF2000-WQX output was conducted using experimental, non-standard ecotoxicity data available currently in the peer-reviewed literature (Chapter 6). Risks were identified for all river reaches in the study area for propranolol and citalopram, while venlafaxine and carbamazepine were estimated to pose a risk in over 75% of the river reaches modelled. Another eight pharmaceuticals could be of risk in a small proportion of reaches. This catchment-based assessment is additionally advantageous as it puts risks in a spatial context, helping to assess the severity of a risk (i.e. localised or widespread). Furthermore, spatial exposure modelling approaches have been shown to influence prioritisation results by identifying risky compounds and locations which would have been missed by the simpler approach.[203]

To evaluate the impact on prioritisation of using LF2000-WQX rather than long-term monitoring data to derive exposure, two prioritisations based on annual average MECs or LF2000-WQX PECs for both rivers were conducted, similarly to the approach used in Chapter 3. Modelled (LF2000-WQX) and monitoring data from sites O2-O6 and F2-F5 were used to calculate a range of possible ranks, Figure 44 and 45. For the River Ouse data, the agreement between PEC and MEC rankings is much improved over the simple PEC approach (Chapter 3). The three exceptions for which rankings did not overlap are hydrocodone, ranitidine and atenolol where risk quotients (RQ) were underestimated compared to MECs by LF2000-WQX by an average factor of 0.61, 0.58 and 0.22, respectively (Figure 44). Ranitidine is available over-the-counter (OTC) and hydrocodone is a metabolite of codeine, which is also available in many OTC preparations. Usage of OTCs is not included in prescription data used as the input to the model, which could explain the underestimation of PECs resulting in lower RQ rankings than MECs for these pharmaceuticals. If the OTC pharmaceuticals are excluded, only a single compound did not have an overlapping risk rank, indicating that the higher-tier spatial model is more suited for prioritisation than the simpler approach (Chapter 3), for the River Ouse.

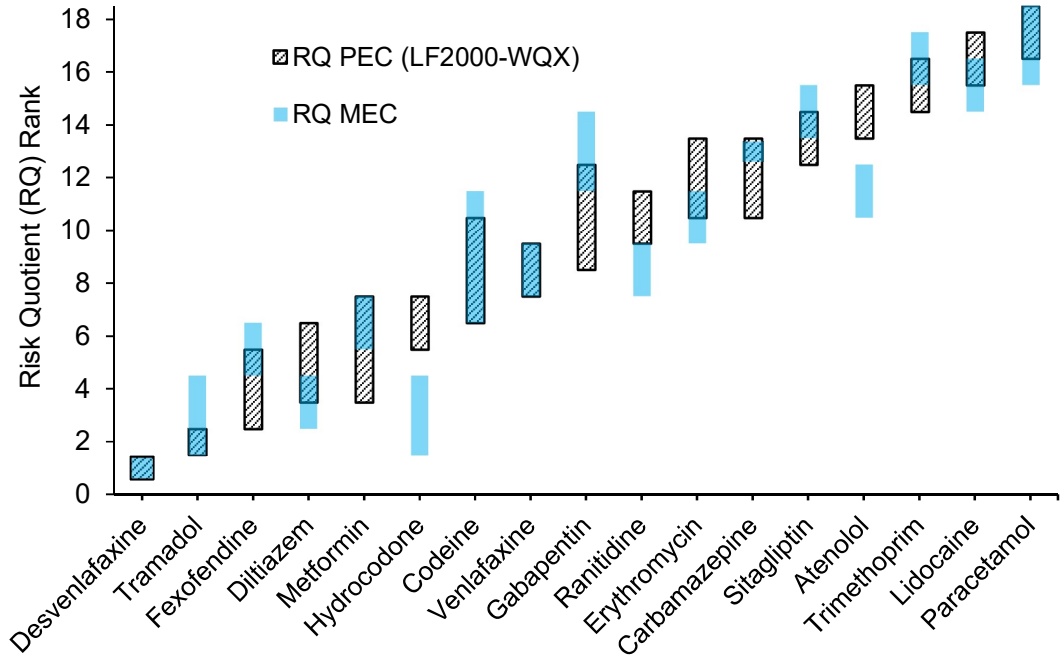


Figure 44. The range of possible ranks resulting from risk quotients calculated using MECs (annual averages) or PECs (LF2000-WQX) for sites O2 - O6 in the River Ouse. Ranks are presented by decreasing risk, where rank 1 corresponds to highest risk.

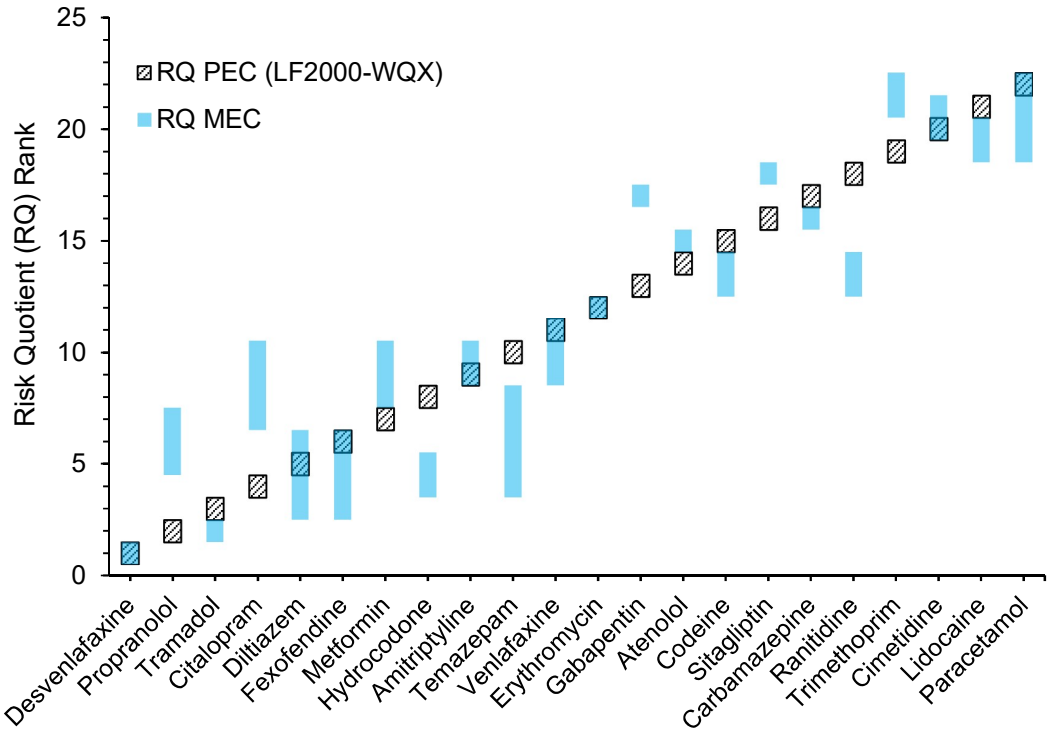


Figure 45. The range of possible ranks resulting from risk quotients calculated using MECs (annual averages) or PECs (LF2000-WQX) for sites F2 - F5 in the River Foss. Ranks are presented by decreasing risk, where rank 1 corresponds to highest risk.

Conversely to the conclusions of Chapter 3, the prioritisation rankings were less similar for the River Foss data than those for the River Ouse, Figure 45. This is consistent with poorer overall LF2000-WQX model performance for the River Foss. Six pharmaceuticals (hydrocodone, temazepam, codeine, carbamazepine, ranitidine and lidocaine) had MEC-based RQ ranks that did not overlap (lower) with PEC RQ ranks, indicating based on the monitoring data, that these compounds pose greater risks than predicted. Four are available *via* OTC formulations (hydrocodone, codeine, ranitidine and lidocaine), again highlighting that this is an important consumption pathway which needs to be captured in calculated emissions. On the other hand, seven pharmaceuticals had higher MEC-based RQ ranks (less risky) than corresponding PEC ranks, indicating that the model predicted higher risks than the monitoring data. This is a preferable scenario than underestimated risks as it helps ensure risks are not missed; however, refinement of the spatial exposure model could further improve PEC performance and potentially the consistency of PEC- and MEC-based RQ rankings.

The exposure drivers identified (Chapter 5) that were not considered in the model, could be influencing performance. Firstly, a method to incorporate OTC pharmaceutical usage needs to be included as several OTCs in both rivers were underestimated. Secondly, under/over estimates could occur due to the way the model predicts WWTP removal, as a constant removal rate. The rate used in this study was based on experimental data from a single sampling, but this may not have been representative of removal efficiency throughout the year. Recent WWTP monitoring studies have identified that constant removal rates temporally (both short and longer term) are unlikely.[51,62] One option could be to consider WWTP removal as a distribution instead of a constant rate, similarly to how flow is estimated within the LF2000-WQX model. In-stream losses were also observed to impact model performance in the River Foss, highlighting that this could also be an important driver to consider. This consideration is more complex than some of the others, as in-stream loss was not observed to affect model performance in the River Ouse. This could be because these processes are masked in the larger River Ouse by changes in flow and confluence with tributaries carrying pharmaceutical residues. Conversely, it could be possible that in-stream decay in the River Ouse is not operating to the same extent as it does in the River Foss due to river characteristics: for example, residence time, flow, depth and turbidity. The relationship between in-stream losses and

these parameters is not well defined and may need to be assessed to determine spatially where in-stream losses are important.

The work presented in the thesis advances the knowledge of the applicability of simple and higher-tier spatial pharmaceutical exposure models for use in prioritisation and risk assessment exercised for aquatic systems. It was determined that simple exposure models are limited in their usefulness as they are only capable of estimating exposure to a single scenario, immediately downstream of a WWTP with no upstream pharmaceutical sources. Such a scenario is highly uncommon, especially in urbanised regions where pharmaceutical usage is greatest. The unsuitability of simple exposure models to complex scenarios may seem obvious; however the impact these simplistic approaches have on prioritisation and risk assessment had not been previously assessed. Several authors have highlighted that these simple PEC approaches are inaccurate;[150,160,166] however they continue to be used for risk assessment and prioritisation.[6,143,147,153–155] The accuracy of these simple exposure models was demonstrated to be poor and as a result impact pharmaceuticals prioritisation (Chapter 3). This is a particularly important finding considering the prolific use of such exposure estimates in risk-based prioritisation. The extended monitoring campaign, enabled by the development and validation of a rapid HPLC-MS quantification method (Chapter 4), significantly contributed to the understanding of spatiotemporal pharmaceutical exposure and the driving factors in two contrasting river systems (Chapter 5). This robust set of monitoring data was then used to validate a higher-tier spatial model, LF2000-WQX (Chapter 6). It was determined that concentrations were better predicted than with the simple exposure estimates, and so were more suitable for prioritisation and risk assessment for both rivers. Therefore, efforts should be made to include these spatial models in prioritisation and risk assessment approaches. The processes which affect pharmaceutical exposure operate to varying degrees, even in neighbouring rivers, which in future could be incorporated into spatial models to improve performance further. Finally, risks based on experimental subtle effect data for several of the pharmaceuticals in this study were demonstrated across the catchment, highlighting the need to better understand the implications of these non-standard effects on individual fitness, populations and the ecosystem.

7.0.1 Limitations of current approach

It is important to consider the limitations of the presented work in conjunction with the findings. The sampling grab strategy used to determine environmental concentrations is limited as it provides only a snapshot in time of the pharmaceutical concentrations in each of the rivers. These concentrations may fluctuate throughout the day,[364] therefore grab sampling at a single point in time may not be representative. A composite sampling experiment was undertaken to validate the grab sampling approach. Limited significant differences were found between the two approaches, however, the composite samplers used were not assessed for potential compound degradation as no cooling of the composite sample was used. In future, the composite samplers need to be validated. The sampling protocol also required samples to be filtered in the field, which is important for maintaining sample integrity but limits analysis to the dissolved phase.[264] This is important as significant concentrations of pharmaceuticals have been found to be associated with particulates removed by the filter.[263] These particulates could still be available for uptake by biota, thus only sampling the dissolved phase could be underestimating pharmaceutical exposure in the water column. In future, analysis of the suspended particulate matter should be incorporated.

The analytical analysis, while cost and time effective is also limited by the number of compounds in the method which do not possess their own isotopically labelled internal standard (ILIS). The ILIS is required to compensate for matrix effects, which are common in the matrices studied.[275]Matrix effects can significantly impact quantification accuracy, thus results from any compound without an ILIS needs to be considered semi-quantitative. Furthermore, analytical results from Chapter 3 are limited and need to be considered with caution as these results were based on an external calibration. To compensate, matrix effects were evaluated for every sample, however this is not a matrix effect compensation strategy, but rather a check to determine the extent of possible matrix effects. The monitoring data is affected by both the analytical and sampling limitations, which could lead poor representation of pharmaceutical exposure in the studied rivers. This could be particularly important when evaluating the model as conclusions of model performance could be impacted. In future evaluations, these limitations need to be considered and addressed.

Similarly to limitations pertaining to the monitoring data, there are also limitations associated with how the PEC and simple PEC models were parameterised. Metabolism values were collected from the literature and a range of metabolism values created. Metabolism will be variable amongst patients, therefore a more robust approach would be to derive a weighted average and error estimate based on the quality and size of the pharmacokinetic study. This weighted averaged and error associated could be used in the model instead of a single value representing the top and bottom of the metabolism range found in the literature. The WWTP removal in the model is also handled as a single value without an error estimate. The variability and error associated with deriving WWTP removal suggests that using a single value is not suitable and that similarly to metabolism should be an average with error associated. This parameterisation could affect how the model preformed and should be investigated.

7.0.2 Wider Implications of research findings

The work presented in this thesis contributes to advancing the confidence in and highlighting the weaknesses of pharmaceutical prioritisation approaches. Prioritisation is often limited in scope in terms of both the number of compounds included and the environmental exposure routes considered. Simultaneously, assumptions made during the environmental risk assessment process could also be limiting evaluations to the aquatic compartment.[139] To overcome this, the holistic prioritisation framework was proposed (Chapter 2). Currently, prioritisations are generally used to inform monitoring campaigns or chose compounds to undertake effects research. The number of pharmaceuticals currently missing an ERA and the number for which there are data could be limited in terms of endpoints/environmental compartments considered, indicate that risks may be missed. This presents another opportunity for pharmaceutical prioritisation, to inform the risk assessment process. To achieve this, the prioritisation framework intentionally resembles a risk assessment; however it differs in that assessment trigger values are not required to assess exposure/effects in all relevant environmental compartments. This is because modelling approaches are used instead. By identifying which pharmaceuticals are of greatest risk and in which environmental compartments

these risks are most likely to emerge, it provides considerable direction for further experimental studies which can inform an ERA, Figure 46.

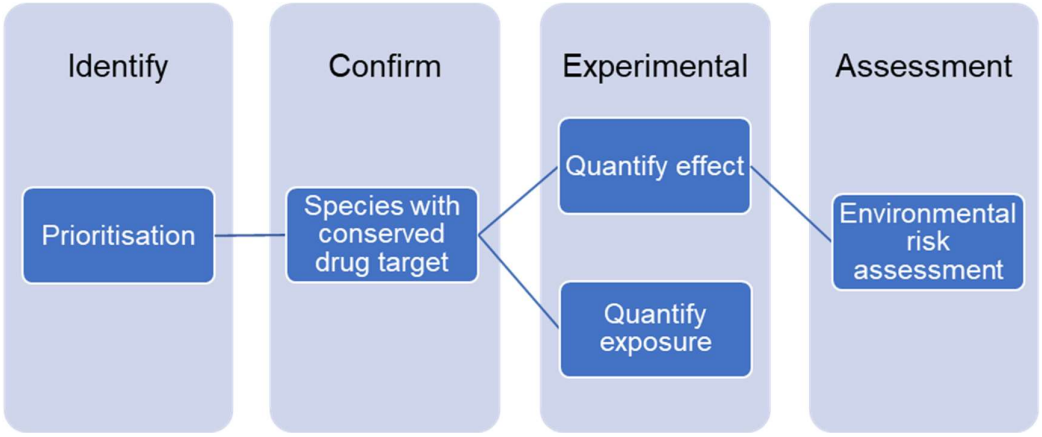


Figure 46. An overview of how the pharmaceutical prioritisation framework can be used to inform the risk assessment process.

The precise methods used to bridge the gap between prioritisation and risk assessment may not necessarily follow the schematic in Figure 46, but the principles are consistent. The prioritisation will identify which pharmaceuticals are of greatest risk and as it is holistic, in which environmental compartments/food chains these risks are most likely to emerge. In the confirmation phase, species in the relevant environmental compartment or food chain can be identified, for example using bioinformatic approaches where the percentage of evolutionary conservation of drug targets within the target species can be a precursor to testing effects.[117] In this way, effect studies could be directed towards the most sensitive species and the most pertinent endpoints to study, resulting in a reduction in the number of test animals required. If evolutionary conservation of a drug target is identified in a species, the evaluation can enter the experimental stage. Targeted chronic effects testing is undertaken and environmental exposure can be demonstrated through monitoring. As the effect endpoints are most likely non-standard or molecular, approaches such as the adverse outcome pathway (AOP) framework[365] could be used to help put this mechanistic toxicological data in context and then fed into the risk assessment process. In this way, pharmaceutical prioritisation would serve as a basis to inform further risk assessment; therefore confidence in prioritisation outcomes are important. The models identified for the holistic prioritisation framework need validation, refinement or development. Aquatic exposure from WWTP discharge was the core topic of the work presented in this thesis as it is one

of the major exposure pathways; however further work is needed to assess models for partitioning to sludge, soils and sediment, as well as pharmaceutical uptake models for plants and invertebrates (benthic, terrestrial and in the water column). Much research is still required to confidently administer the prioritisation framework; nevertheless it could form an important part of the risk assessment process to ensure risks to the environment are not missed.

7.1 Conclusions

In conclusion, this thesis demonstrates that currently used simple exposure models used for the prioritisation and risk assessment of pharmaceuticals are not suitable for predicting concentrations in river systems. Rivers are complex hydrological systems with multiple pharmaceutical inputs, which the simple exposure model overlooks. This oversimplification was shown to have ramifications for pharmaceutical prioritisation in a scoping study, thus a more representative set of monitoring data was collected.

The monitoring data revealed that significant spatial and temporal variability of pharmaceutical concentrations was observed in both rivers. Concentrations were roughly inversely related to flows, and upstream pharmaceutical inputs were observed in both rivers. Therefore, further refined spatial exposure models were deemed more appropriate to evaluate exposure as they have the capacity to incorporate both factors. The predictive power of the spatial exposure model was much improved over the simplistic approach and thus used to conduct a catchment risk assessment based on non-standard experimental endpoints. A risk ratio greater than 1 was observed for every river reach within the modelled area for propranolol and 98% for citalopram and venlafaxine, highlighting that risks from pharmaceuticals are present in both rivers. Finally, a re-prioritisation with the spatial model indicated that the improved predictive power translated into better agreement of prioritisation outcomes with MECs.

While the experimental characterisation of pharmaceuticals in the environment is important, modelling approaches that underpin prioritisation are also vital to meaningfully direct these efforts and/or effect based studies. Guidance through pharmaceutical prioritisation for the assessment of the large proportion of

pharmaceuticals for which there is no or limited knowledge, is vital to ensure that environmental risks can better be fully characterised and not overlooked.

7.2 Recommendations for further research specific to this thesis

The research presented in this thesis has provided novel insight into how simple and higher-tier exposure models impact prioritisation and risk assessment. There are however many open questions that need addressing before we can prioritise and risk assess pharmaceuticals in a robust way. Future work could consider the following aspects:

1. In-stream losses estimated using LF2000-WQX dilution factors in the River Foss indicated that for several pharmaceuticals these losses are significant along this stretch of river. A similar analysis could not be undertaken in the River Ouse due to other pharmaceutical sources downstream. To determine whether these in-stream losses could improve model performance in the River Ouse, LF2000-WQX could be re-simulated using the in-stream losses estimated from the River Foss. This analysis may identify that modelling in-stream losses at the same rate in both rivers may not be appropriate as river characteristics such as turbidity, depth and temperature could impact in-stream attenuation. Laboratory-derived degradation data is difficult to link to environmental losses, therefore it may be more beneficial, in terms of modelling, to derive those river characteristics that are likely to limit in-steam losses. This could be achieved through further monitoring of both similar and dissimilar river systems and characterising estimated in-stream losses when possible and relating them to parameters such as sunlight hours, temperature, depth, turbidity, residence time and flow. Mesocosm studies, simulating differing environmental conditions, could also be used to try to derive general degradation trends and drivers of these.
2. The physico-chemical properties of studied pharmaceuticals will affect their behaviour and fate in the environment. This includes their structure, logKow and pKa. These parameters will influence the distribution between aquatic compartments (suspended particulate matter, sediment, dissolved in water), and also the potential for degradation by processes such as photodegradation,

hydrolysis and microbial degradation, which could be important for evaluating and also simulating the model. An investigation of how these parameters influence the observed concentrations spatially and temporally could be important to gaining a better understanding of environmental fate. This evaluation could also include the impact of environmental conditions such as temperature, pH and solar radiation on environmental fate. This knowledge will be important for improving spatial exposure modelling and help identify why particular pharmaceuticals may not have been accurately predicted by the model.

3. The robust pharmaceutical monitoring data generated in this study were used to evaluate the performance of a single spatial exposure model (LF2000-WQX) (Chapter 5,6). The dataset could also be used to validate other existing spatial exposure models or serve as a training set for exposure models in development. Their performance with LF2000-WQX can be compared, furthering our knowledge surrounding the usefulness and capability of these higher-tier spatial exposure models.
4. The monitoring campaign identified that seasonally significant variations in pharmaceutical loads were present in the River Ouse, but not the River Foss (Chapter 5). These variations are not thought to be related to trends in pharmaceutical usage. It is possible that seasonal variations in WWTP removal efficiency are occurring. A seasonal monitoring campaign in the WWTPs in this study could help determine whether a) there are seasonal trends in WWTP removal efficiency in the York system and b) whether this trend is the same for the trickling filter and activated sludge treatment plants.
5. It was suggested that a single WWTP removal estimate may not be suitable for use in the model, potentially due to seasonal changes in removal efficiency (Chapter 6). With the present monitoring data, an inverse modelling exercise, where removal rates are predicted based on monitoring data and human emissions, similar to that previously undertaken with the LF2000-WQX model could be completed.[304] After the predicted removal rates are derived, the LF2000-WQX model can be simulated again and the outcomes compared with monitoring data. This could help determine whether the underestimated

emissions from the WWTPs in the present study are the result of the WWTP removal rate used or the pharmaceutical usage estimate.

6. The consideration of pharmaceuticals only dissolved in the aquatic environment could be an underestimate of the true aquatic exposure for many of the compounds in the analytical method. A significant fraction could be bound to the suspended particulate matter. Without consideration of pharmaceuticals bound to particulates, environmental risks could be missed. Therefore inclusion of particulate analysis will be important for ensuring pharmaceutical risks in the Rivers Foss and Ouse are not missed.

7.3 General recommendation for further research

The optimum prioritisation framework identified current knowledge surrounding the models and their assumptions which underpin the prioritisation framework (Chapter 1). The work in this thesis focused on the assessment of aquatic exposure models. Development and validation of exposure models for sediment and soils are also required. There is a real need to expand the consideration of environmental pathways past WWTP effluent discharge to also include manufacturing discharge and landfill leachate. Additionally, we should expand the currently assessed exposure routes to include food chains (aquatic and terrestrial), and humans in terms of agriculture (livestock and plants). Much work is needed to develop ways to estimate and incorporate these pathways and exposures; however, their inclusion will greatly enhance the confidence in outcomes provided by the framework. As knowledge develops, other emerging contaminants such as veterinary medicines, metabolites, transformation products, illicit drugs and mixtures of these can begin to be incorporated into the framework.

1. In the current research, a rapid HPLC-MS/MS quantification method for 33 pharmaceuticals was developed and validated (Chapter 4). The application of this method to the long-term monitoring campaign was highly successful. Further development of this rapid quantification method would be very useful for further monitoring efforts. A focus could be placed on incorporating a wider range of antibiotics which are of increasing environmental concern throughout the world.[34]

2. Several pharmaceutical predictions that performed poorly against monitoring data were likely due to the availability of OTC medicines. Consideration of the prescription pathway alone is therefore not suitable for predictions of those pharmaceuticals that are also available OTC. Development of an approach to help estimate the OTC usage of pharmaceuticals is needed.
3. Several of the highest risk pharmaceuticals, identified using the fish plasma model,[118] have no experimental effects data available, for example desvenlafaxine and hydrocodone. Further research should include identifying the drug targets for these pharmaceuticals and determining the extent of evolutionary conservation in aquatic species relevant to the ecosystem.[117] This information can then be used to design effects studies that quantify endpoints relevant to the mode of action of the pharmaceutical.

Effects of pharmaceuticals in the aquatic environment have been demonstrated *in situ,* such as the feminisation of male fish from synthetic estrogen exposure[109] and the decline in the vulture populations in Pakistan resulting from food chain exposure to diclofenac.[107] Moreover, chronic subtle effects related to the mode of action of pharmaceuticals are increasingly being reported in the laboratory.[121,239,346,366] The results from this work suggest that concentrations that can illicit these subtle effects are the levels that occur in the environment. The current EMA risk framework focuses on standard ecotoxicity endpoints (reproduction, growth, survival), which are ecologically important, but could overlook these subtle effects. Therefore, an approach to translate these MoA-related endpoints into a regulatory risk assessment framework would be highly beneficial. The AOP framework may be a potential approach to do this.[365,367] The incorporation of mixture toxicity, metabolites and also veterinary pharmaceutical usage will also need to be addressed to ensure risks are accurately characterised. Furthermore, as an increasing breadth of data is presented that indicates environmental risks from pharmaceuticals are possible, more work should focus on the effectiveness and cost-benefit of various risk mitigation options. This point is particularly pertinent as pharmaceutical usage in the UK and throughout the world is expected to rise, potentially increasing risks.[38]

Appendix

Appendix 1. Assessment of the availability of EPARs (see main text) for the top 350 prescribed pharmaceuticals by mass in England from the year 2012. Usage data was collected from the National Health Service Prescription Cost Analysis 2012.

Pharmaceutical	Mass prescribed (2012)	EPAR available	Pharmaceutical	Mass prescribed (2012)	EPAR available	Pharmaceutical	Mass prescribed (2012)	EPAR available
Acamprosate Calcium	4633.995	N	Amiodarone HCl	3129.719	N	Baclofen	848.1401	N
Acrivastine	40.1273	N	Amisulpride	2504.559	N	Balsalazide Sodium	8578.065	N
Acyclovir	15503.25	N	Amitriptyline HCl	10431.37	N	Bendroflumethiazide	1504.737	N
Adapalene	5.75915	N	Amlodipine	5623.418	Y	benzerazide	1711.785	N
Alendronic Acid	2485.896	Y	Amoxicillin	123080.7	N	Benzoyl Peroxide	2.777593	N
Alfuzosin HCl	198.6387	N	Anastrozole	19.01404	N	Benzydamine HCl	8.119974	N
Alimemazine Tartrate	42.47056	N	Aripiprazole	168.2959	Y	Betahistine HCl	1640.354	N
Allopurinol	35355.61	N	Acetylsalicylic acid	76766.37	Y	Betamethasone Valerate	0.103622	N
Alverine Citrate	2008.694	N	Atenolol	19849.26	N	Bezafibrate	1.102586	N
Amantadine HCl	577.1469	N	Atorvastatin	18301.26	N	Bicalutamide	7808.065	N
Amiloride	202.2389	N	Azathioprine	2824.994	N	Bisacodyl	662.9771	N
Aminophylline Hydrate	5359.066		Azithromycin	15020.11	N	Bisoprolol Fumarate	126.8647	N
Brinzolamide	1987.094	N	Cetomacrogol	65.58092	N	Centrimide	0.492776	N
Bumetanide	63.38782	N	Chloramphenicol	4.057731	N	Cetirizine HCl	1726.327	N

Appendix 1. (continued) Assessment of the availability of EPARs (see main text) for the top 350 prescribed pharmaceuticals by mass in England from the year 2012. Usage data was collected from the National Health Service Prescription Cost Analysis 2012.

Pharmaceutical	Mass prescribed (2012)	EPAR available	Pharmaceutical	Mass prescribed (2012)	EPAR available	Pharmaceutical	Mass prescribed (2012)	EPAR available
Budesonide	73.6161	Y	Chlohexidine Gluconate	0.047155	N	Clonazepam	24.876	N
Digoxin	12.81834	N	Erythromycin	17586.66	N	Flecainide Acetate	2029.77	N
Buprenorphine	65.85415	Y	Chlorphenamine Maleate	215.1903	N	Clonidine HCl	1086.108	N
Bupropion HCl	51.79441	Y	Chlorpromazine HCl	843.0926	N	Clopidogrel	13584.8	Y
Buspirone HCl	3.122872	N	Chlortalidone	297.7189	N	Clotrimazole	204.5456	N
Calcipotriol	3.116136	N	Ciclosporin	641.8038	Y	Codeine	47949.39	N
Calcium Acetate	1711.371	N	Cimetidine	2734.228	N	Colchicine	7.831715	N
Candesartan Cilexetil	2282.735	N	Cinnarizine	496.6023	N	Crotamiton	1.484619	N
Captopril	365.6421	N	Ciprofloxacin	6233.46	N	Cyclizine HCl	1750.654	N
Carbamazepine	37897.98	N	Citalopram Hydrobromide	8734.843	N	Cyprote Acetate	38.63865	N
Carbidopa	2875.356	Y	Clarithromycin	14320.11	N	Dabigatran Etexilate	1222.095	Y
Carbimazole	204.5619	N	Clavulanate	818.0046	N	Dantrolene	140.8969	N
Carbocisteine	62872.02	N	Clindamycin	-	N	Desmopressin Acetate	1.849407	N
Carvedilol	327.1891	N	Clobazam	107.8139	N	Desogestrel	16.96663	N
Cefalexin	9965.047	N	Clobetasol Propionate	2.279332	N	Desoloratidine	58.47969	N
Celecoxib	2534.791	Y	Clomipramine HCl	730.8683	N	Dexamethasone	17.56267	Y

Appendix 1. (continued) Assessment of the availability of EPARs (see main text) for the top 350 prescribed pharmaceuticals by mass in England from the year 2012. Usage data was collected from the National Health Service Prescription Cost Analysis 2012.

Pharmaceutical	Mass prescribed (2012)	EPAR available	Pharmaceutical	Mass prescribed (2012)	EPAR available	Pharmaceutical	Mass prescribed (2012)	EPAR available
Dexamfetamine Sulfate	24.88827	N	Doxazosin Mesilate	1027.151	N	Famotidine	-	N
Dexketoprofen	4.034275	N	Doxycycline Hyclate	3430.624	N	Felodipine	815.3964	N
Dextromethorphan Hydrobromide	OTC	Y	Duloxetine HCl	1991.763	Y	Fenofibrate	3771.716	Y
Dextroprop HCl	571.3053	N	Dutasteride	8.334655	N	Fentanyl	-	Y
Diazepam	619.8726	N	Enalapril Maleate	1346.481	N	Fesoterodine Fumarate	39.92008	Y
Diclofenac Sodium	8240.328	N	Entacapone	4430.08	Y	Fexofenadine HCl	9935.872	N
Dicycloverine HCl	184.5628	N	Eplerenone	230.6903	N	Finasteride	376.9499	N
Gabapentin	124353.5	N	Ibuprofen	99212.55	Y	Lercanidipine HCl	846.4954	N
Dihydrocodeine Tartrate	9609.232	N	Escitalopram	368.8627	N	Flucloxacillin Sodium	53702.76	N
Diltiazem HCl	21015.41	N	Estriol	5.740766	N	Fluconazole	-	N
Dimeticone	21.90098	N	Ethinylestradiol	14.66202	N	Fludrocortisone acetate	1.274797	N
Dipyridamole	2527.264	N	Ethosuximide	779.641	N	Fluorouracil	1.001543	N
Docusate Sodium	9354.674	N	Etodolac	3335.156	N	Fluoxetine HCl	5236.459	N
Domperidone	1320.033	N	Etoricoxib	892.8282	N	Flupentixol HCl	8.633705	N
Donepezil HCl	305.721	Y	Etynodiol Diacetate	2.65181	N	Fluticasone Propionate	5.465809	Y
Dorzolamide	0.183812	N	Exemestane	99.7522	N	Folic Acid	644.0475	N
Dosulepin HCl	3032.475	N	Ezetimibe	685.6289	N	Frusemide	155.3189	N

Appendix 1. (continued) Assessment of the availability of EPARs (see main text) for the top 350 prescribed pharmaceuticals by mass in England from the year 2012. Usage data was collected from the National Health Service Prescription Cost Analysis 2012.

Pharmaceutical	Mass prescribed (2012)	EPAR available	Pharmaceutical	Mass prescribed (2012)	EPAR available	Pharmaceutical	Mass prescribed (2012)	EPAR available
Mebendazole	112.712	N	Metoclopramide HCl	637.5506	N	Neomycin Sulfate	62.92856	N
Gemfibrozil	-	N	Imipramine HCl	299.5535	N	Letrozole	38.31931	N
Gilbenclamide	27.25068	N	Indapamide	220.0683	N	Levetiracetam	44519.27	Y
Gliclazide	36347.48	N	Indometacin	640.33	N	Levocetirizine	27.01289	N
Glimepiride	74.04899	Y	Indoramin	119.0657	N	Levodopa	19906.56	Y
Glipizide	57.49691	N	Ipratropium Bromide	3.282406	N	Levofloxacin	-	Y
Glyceryl Trinitrate	50.02094	N	Irbesartan	16480.61	Y	Levonorgestrel	0.437523	N
Haloperidol	24.58707	N	Isosorbide Mononitrate	6874.212	N	Levothyroxine Sodium	65.59779	N
Hydralazine HCl	257.4103	N	Itraconazole	475.082	N	Lidocaine HCl	0.565125	Y
Hydrochlorothiazide	-	Y	Ivabradine	71.02108	Y	Lisdexamfetamine Dimesylate	-	N
Hydrocortisone	--	Y	Ketoconazole	23.99151	Y	Lisinopril	4759.135	N
Hydroxycarbamide	2954.171	Y	Ketoprofen	235.9665	N	Lofepramine HCl	1129.279	N
Hydroxychloroquine Sulfate	533.517	N	Labetalol HCl	1644.713	N	Loperamide HCl	236.7046	N
Hydroxyzine HCl	536.2263	N	Lacidipine	62.06591	N	Loratadine	819.2082	N
Hyoscine Butylbromide	865.77	N	Lamotrigine	8726.156	N	Lorazepam	36.56924	N
Hypromellose	0.387105	N	Lansoprazole	16175.15	N	Losartan Potassium	16690.98	N
Ibandronate Sodium	-	N	Lantanoprost	0.531841	N	Lymecycline	20293.65	N

Appendix 1. (continued) Assessment of the availability of EPARs (see main text) for the top 350 prescribed pharmaceuticals by mass in England from the year 2012. Usage data was collected from the National Health Service Prescription Cost Analysis 2012.

Pharmaceutical	Mass prescribed (2012)	EPAR availabl e	Pharmaceutical	Mass prescribed (2012)	EPAR available	Pharmaceutical	Mass prescribed (2012)	EPAR available
Oxazepam	76.3154	N	Pramipexole	103.1683	Y	Quinine Bisulfate	6206.635	N
Mebeverine HCl	23210.73	N	Metoprolol Tartrate	2294.196	N	Nicorandil	1646.397	N
Medroxyprogesterone acetate	10027.43	N	Metronidazole	11153.06	N	Nicotine	384.8292	N
Mefenamic Acid	55.98768	N	Miconazole Nitrate	185.0328	N	Nifedipine	2999.857	N
Melatonin	289.0645	Y	Mirtazapine	3693.804	N	Nitrazepam	118.9559	N
Meloxicam	172.5621	N	Mometasone Furoate	0.155947	N	Nitrofurantoin	3179.265	N
Memantine HCl	10027.43	Y	Montelukast	502.6383	N	Norethisteron e	189.848	N
Meptazinol HCl	1194.181	N	Morphine Sulfate	4215.292	N	Nortriptyline	482.7553	N
Mesalazine	77618.76	N	Moxifloxacin HCl		N	Nystatin	-	N
Metformin	937082.8	Y	Moxonidine	5.73321	N	Oestrogens Conjugated	34.36688	Y
Methadone	1557.91	N	Mycophenolate Mofetil	8471.95	Y	Olanzapine	423.4855	Y
Methocarbamol	9850.463	N	Nabumetone	1900.28	N	Olmesartan Medoxomil	480.2685	N
Methotrexate	159.9306	Y	Naftidrofuryl Oxalate	1315.567	N	Olopatadine HCl		Y
Methycellulose	2211.906	N	Naloxone HCl		N	Omeprazole	20213.56	N
Methyldopa	2485.886	N	Naproxen	144631.8	N	Ondansetron HCl		N
Methylphenidate HCl	770.8237	N	Nebivolol	82.73712	N	Orlistat	6022.41	Y
Methylpredisolone	-	N	Nefopam HCl	863.1576	N	Oseltamivir Phosphate	-	Y

Appendix 1. (continued) Assessment of the availability of EPARs (see main text) for the top 350 prescribed pharmaceuticals by mass in England from the year 2012. Usage data was collected from the National Health Service Prescription Cost Analysis 2012.

Pharmaceutical	Mass prescribed (2012)	EPAR available	Pharmaceutical	Mass prescribed (2012)	EPAR available	Pharmaceutical	Mass prescribed (2012)	EPAR available
Simeticone	649.7117	N	Tamsulosin HCl	198.2635	N	Tranexamic Acid	13377.8	N
Oxybutynin	357.7791	Y	Pravastatin Sodium	2858.58	Y	Quinine Sulfate	23334.29	N
Oxycodone HCl	1252.609	N	Prazosin HCl	5.749455	N	Raberprazole sodium	254.6749	N
Oxytetracycline	17705.12	N	Prednisolone	1447.857	N	Raloxifene	-	Y
Pantoprazole	1031.968	Y	Pregabalin	21033.26	Y	Ramipril	5454.358	N
Paracetamol	2222361	N	Primidone	1738.296	N	Ranitidine HCl	34853.71	N
Paroxetine HCl	1168.383	N	Prochlorperazine Maleate	398.1138	N	Rasagiline Mesilate	4.18359	Y
Perindopril Arginine	26.24768	N	Procyclidine HCl	163.6549	N	Repaglinide	10.79511	Y
Perindopril Erbumine	927.4732	N	Promazine HCl	183.0343	N	Risedronate Sodium	-	N
Permethrin	0.645626	N	Promethazine HCl	372.5662	N	Risperidone	82.93323	N
Phenobarbital	566.2101	N	Propranolol HCl	9604.497	Y	Ropinirole HCL	73.94691	N
Phenoxymethylpenicillin	30213.85	N	Propylthiouracil	193.4775	N	Rosuvastatin Calcium	785.6995	N
Phenytoin	12046.42	N	Pseudoephedrine HCl	329.8656	Y	Salbutamol	78.13675	N
Pholcodine	103.1683	N	Pyridostigmine bromide	717.8097	N	Saxagliptin	40.0278	Y
Pioglitazone HCl	1285.504	Y	Pyridoxine HCl	242.4529	N	Sertraline HCl	14646.35	N
Piroxicam	24.32061	N	Quetiapine	9937.301	N	Sevelamer	7370.68	Y

Appendix 1. (continued) Assessment of the availability of EPARs (see main text) for the top 350 prescribed pharmaceuticals by mass in England from the year 2012. Usage data was collected from the National Health Service Prescription Cost Analysis 2012.

Pharmaceutical	Mass prescribed (2012)	EPAR available	Pharmaceutical	Mass prescribed (2012)	EPAR available	Pharmaceutical	Mass prescribed (2012)	EPAR available
Pizotifen Malate	24.88417	N	Quinapril HCl	-	N	Sildenafil Citrate	-	Y
Simvastatin	43228.17	Y	Telmisartan	942.8226	Y	Trazodone HCl	3261.608	N
Sitagliptin	6084.335	Y	Temazepam	660.796	N	Triamcinolone Acetonide	-	N
Sodium Cromoglicate	165.2364	N	Terazosin Hydrochloride	-	N	Triamterene	-	Y
Sodium Feredate	2654.143	N	Terbinafine HCl	6419.333	N	Trifluoperazine	14.17442	N
Sodium Fluoride	3.177142	N	Testosterone	158.8291	Y	Trihexyphenidyl HCl	19.41714	N
Sodium Picosulfate	301.9395	N	Tetracycline	945.4462	N	Trimethoprim	9618.376	N
Solifenacin	452.7087	N	Theophylline	7152.281	N	Trosoium chloride	350.868	N
Sotalol HCl	2722.237	N	Thiamine HCl	8037.643	N	Ursodeoxycholic Acid	5716.05	N
Spironolactone	2345.547	N	Tibolone	31.1085	N	Valaciclovir	467.6434	N
Sulfamethoxazole	-	N	Ticagrelor	777.7494	Y	Valproic Acid	10533.69	N
Sulfasalazine	53559.59	N	Tiotropium	2.634269	N	Valsartan	6512.622	Y
Sulpiride	1961.786	N	Tizanidine HCl	29.67087	N	Verapamil HCl	5771.087	N
Sumatriptam Succinate	749.3011	N	Tolbutamide	2603.915	N	Varenicline Tartrate	18.53741	Y
Tacrolimus	23.43627	Y	Tolterodine	118.3774	N	Venlafaxine	11206.86	N
Tadalafil	92.56042	Y	Topiramate	2315.892	Y	Warfarin Sodium	1192.922	N
Tamoxifen Citrate	456.7937	N	Tramadol	43206.84	N	Zolpidem Tartrate	141.9548	N
Zonisamide	509.5191	Y	Zopiclone	721.6331	N	Zuclopenthixol hydrochloride	74.3017	N

Appendix 2

Reviewed prioritisations and risk assessments

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Appendix 3. Parameters needed to estimate environmental pharmaceutical exposure in multiple compartment. Summarised in Table 3 of main text. Numbers refer to the framework presented in Chapter 2.

Number	Variable	Experimental source	Model/ predictive tool/ default	Applicability domain	Experimental validation	Method limitations/ Suggestions
(1) API usage in specific region	Consumption (mg/yr)	Prescription analysis	N/A	N/A	1. Found good agreement between PECs/MECs in WwTP effluent (11 APIs).[166]	• No over-the-counter API usage. • Hospital usage should be included if possible.[390] • Method can be paired with the Fpen approach to cover all APIs.
		Sales data	N/A	N/A	PECs derived from sales data are greater than local maximum MECs in study of 56 APIs.[369]	• Not publically available.
		Market penetration (Fpen) estimate	Fpen 1% (default)[112]	• All APIs.	1.) Derived 1% Fpen default based on 95th percentile of 800 APIs.[112] 2.) Evaluated 10 MECs with PECs derived using default Fpen, PECs were conservative.[143]	• Generalised, consumption over/under estimations likely.
(2) API emission to sewage	% excreted unchanged ($F_{excreta}$)	In vivo metabolism studies in man	N/A	• All administered internally (including metabolites).	Variation in reported $F_{excreta}$ identified as source of PEC error (0-200% change in PEC) [7].	• Topical and ophthalmic preparations generally no metabolism, assume 100% excretion.[153] • Sulfato-and glucuronide metabolites (cleaving) possible in WwTP, could increase wastewater parent API loads, include this fraction in PEC.[248] • Suggested that largest reported $F_{excreta}$ value in literature generates the most relevant PECs.[262]
(3) Wastewater dilution	Wastewater (L/person·day)	Wastewater entering WWTP averaged per capita	Default: 200 L/day[398,399]	• Europe	1.)Validated 200 L/day per capita wastewater generation for Germany.[400] 2.) Wastewater dilution will vary based on water usage practices throughout world, 50-400 L/day.[181]	• Consider regional water usage patterns to not overestimate environmental dilution.

Appendix 3. (continued) Parameters needed to estimate environmental pharmaceutical exposure in multiple compartment. Summarised in Table 3 of main text. Numbers refer to the framework presented in Chapter 2.

Number	Variable	Experimental source	Model/ predictive tool/	Applicability domain	Experimental validation	Method limitations/ Suggestions
(4) WwTP removal	% removal efficiency (%RE)	Estimated removals based on difference between influent and effluent concentrations	SimpleTreat 4.0[401]	• Monovalent organic acids, bases, neutrals • pH 3-7, -1 < logKow >3). • Koc may be underestimated for organic acids, more so for bases. • Not suitable for ionic surfactants.	1.) Neutral organics, predicted within +/- 5% removal.[402] 2.) 10 compounds to challenge applicability domain, found K_{OC} regressions good for acids, but not for bases (K_{OC} better to be experimentally determined.[56]	• Improvements in mechanistic understanding and modelling of sorption for ionsables still needed.[403] • Experimental values vary substantially, if used, use lowest % RE reported in literature. • Organic bases preform more poorly than acids because when ionised, cations could have electrostatic interactions with negatively charged particles (ie. sediment, colloids, sludge), so use experimental K_{oc} when possible. • SimpleTreat 3.1 (and newer) suggested for first tier risk assessment (considers ionic state of API).[112]
(5) Environmental Dilution	Dilution factor	Monitor river flow and WWTP discharge rate	Default:10 [112]	Rivers (up to dilution factor of 1000).	1.) Site specific dilution is prefered to calculate PEC.[163] 2.)Dilution factor can lead to an uncertainty of up to 695% in calculation of PECs.[7]	• In general, a 10 default dilution factor will provide the worst-case assumption (Europe). • Caution should be taken using this value to estimate the dilution factor of small rivers with seasonal fluctuations.
(6) Equilibrium partitioning	Soil-water partition coefficient (Kd)	OECD 106	QSAR [404]	• Includes ionisables and neutral organics • Bases: pKa>2, -1.66< logKow >7.03 • Acids: pKa 0-12, -2.19 < logKow > 8.50	1.) QSARs applied to realistic exposure scenario of 3 compounds, results compared with monitoring data and output from conventional fugacity modelling.[405] 2.) QSARs applied to 415 acids and 496 bases in a multimedia fate and effect model (USES-LCA), indicated partitioning to solid-phase underestimated when ionisables not considered, (e.g. TGD method).[406].	• $PEC_{porewater}$ is also calculated with this Kd estimation approach. • Further model refinement required for APIs specifically • The multimedia fate model SimpleBox 4.0 has also been updated with this approach.[407]
	Sediment-water partition coefficient (Kd)	OECD 106 (modified) OPPTS 835.110	QSAR [404]	N/A	N/A	• The water-soil Kd QSARs[404] has been suggested to use, nothing specifically developed for water-sediment partitioning.[405]

Appendix 3. (continued) Parameters needed to estimate environmental pharmaceutical exposure in multiple compartment. Summarised in Table 2 of main text. Numbers refer to the framework presented in Chapter 2.

Number	Variable	Experimental source	Model/ predictive tool/	Applicability domain	Experimental validation	Method limitations/ Suggestions
(7) Degradation	DT50 (soil)	OECD 307 (degradation test)	BIOWIN	• Able to predict not-readily biodegradable substances with high accuracy in contrast to ready biodegradability.[236]	1.) Compared API experimental anaerobic biodegradation with BIOWIN estimates, found a similar order in anaerobic biodegradability (n=4).[408] 2.)Validated the BIOWIN model using experimental data from 110 compounds.[236]	• Poor model predictions for chemicals that contain moieties or combinations of moieties that are not adequately represented in database to build models (e.g. pharmaceuticals). • Does not account for stereochemistry in predictions which is important for chiral molecules (e.g. pharmaceuticals).[409]
	DT50 (sediment)	OECD 308 (309) (degradation test)	BIOWIN	• Can be applied for sediments, but not validated.	N/A	• Degradation in sediment will be subject variety of environmental conditions, for example experimental differences in degradation rates between moving and flat bed sediments observed.[410] • More work is needed to determine the appropriateness of BIOWIN for API degradation in sediment.
(8) Application rate	Application (kg/hectare (dry weight) per year)	Localised application rate	Default: • 5000 kg/ha·yr agricultural • 1000 kg/ha·yr grassland (dry weight)	• Europe, however the Danish EPA suggest 6000 kg/ha·yr (dry weight) application for risk assessment.	N/A	• The suitability of the defaults is dependent on the country-specific biosolid practices and legislation, for example the US applied $4.0x10^6$ tons of dry weight biosolids, while Europe applied $2.39x10^6$ tons in 2006.[138] • The magnitude and impact of the application rate of biosolids throughout the world in terms of APIs is largely unexplored.

Appendix 4 Parameters needed to estimate pharmaceutical effects for prioritisation. Numbers refer to the framework presented in Chapter 2

Number	Variable	Experimental source	Model/ predictive tool	Applicability domain	Experimental validation	Method limitations
(10) $EC(LC)_{50}$	Fish LC_{50}	OECD 203	1.API specific ecotoxicity QSARs[212] 2. ECOSAR[411]	1. Applied QSAR to 1267 APIs and the percent of APIs that fell in the applicability domain (AD) was ≥ 74% 2. ECOSAR -3 < LogKow < 5. 8 Molecular weight <1000	1. Relatively new QSARs, no external validation yet published. 2. Limitations of ECOSAR demonstrated by many, notably Hulzebos and Posthumus.[236]	• ECOSAR was developed with a small set of industrial chemicals with simple structures.[412] • APIs have complex structures with multiple functional groups, which could have a specific mode of action.[212,237] • Experimental ecotoxicity data is limited.
	Invertebrate EC_{50}	OECD 202				
	Algae EC_{50}	OECD 201				
	Benthic Invertebrate EC_{50}	OECD 218	Not yet developed	N/A	N/A	• Unique exposure scenario where organisms could be exposed to water column, sediments and pore water.
	Earthworm LC_{50}	OECD 207	1. Equilibrium partitioning concept applied to aquatic data for screening. 2. Earthworm QSAR reported in Guo (2016).[147]	1. QSARs developed based on 11 compounds is valid for short-term toxicity of several chlorophenols, chlorobenzenes and chloroanilines.[413] 2. None reported.	1. API specific validation of this approach has not been attempted. 2. None reported.	• Equilibrium partitioning method may not be suitable for lipophilic compounds or substances with a specific mode of action (e.g. APIs).[413] • Does not consider the effects on soil organisms for chemicals that are adsorbed to soil particles and taken up by ingestion or contact with soil or sediment adsorbing chemicals (log Kow > than 3).
	Soil Invertebrate EC_{50}	OECD 218	Not yet developed	N/A	N/A	• Despite experimental field evidence of exposure, modelling consideration of this exposure pathway is largely unexplored for APIs.
	Terrestrial Plant EC_{50}	OECD 208	Not yet developed	N/A	N/A	

Appendix 4. (continued) Parameters needed to estimate pharmaceutical effects for prioritisation. Numbers refer to framework in Chapter 2

Number	Variable	Experimental source	Model/ predictive tool	Applicability domain	Experimental validation	Method limitations
(11) Chronic	Fish	OECD 210	1. ECOSAR: ChV (geometric mean of LOEC and NOEC) 2. Claey:[414] QSARs for substances acting via nonpolar and polar narcosis.	1. LogKow < 5.8, neutral organics. When chronic data is lacking acute to chronic ratios are used. 2. Nonpolar narcosis: 0.92 < logKow < 6.8 Polar narcosis: 6.83 < pKa <10.7 1.46 < logKow < 5.76	1. Validated for 23 neutral organics (not APIs). Concluded when functional groups could have a specific mode of action (e.g. pharmaceuticals), ECOSAR not suitable and only when a compound is within the AD the QSAR is suitable.[237] 2. Method has not been externally validated for APIs by others.	• Chronic experimental data is rare putting a reliance on acute to chronic ratios for many structural classes in ECOSAR. • ECOSAR was creating using a limited number of compounds whose relevance to APIs is questioned.[412] • De Haas[237] and Claeys,[414] suggest chronic ECOSAR is not robust.
	Invertebrate	OECD 211	ECOSAR Chv	1. LogKow < 5.8, neutral organics. When chronic data is lacking acute to chronic ratios are used.	Not validated for APIs	• Chronic experimental data for invertebrates and aquatic plants, validation and development has focused on fish.
	Algae	OECD 221				
	Benthic Invertebrate	OECD 219	No QSAR for APIs developed	N/A	N/A	• Despite experimental field evidence of exposure, modelling consideration of this exposure pathway is largely unexplored for APIs.
(12) Assessment Factors	Acute assessment factor (AF)		Defaults suggested: EPA (1995)[415] EMEA 2006[112] OECD 1992[416]	AF: No greater than 1000, regardless of whether species is a standard test organism. AF: No less than 100 even when acute LC(EC)50 is from most sensitive species.	• Fish are most sensitive species and assessment factors applied to acute data may be acceptable when chronic data is missing (unless a mode of action concern is present)[235].	• Account for inter- and intra-species variability and extrapolate from lab to field or in silico prediction to field. • Derived from policy, assessment factors are arbitrary values which may have little scientific relevance, but reduce the likelihood of underestimating risk.[417]
	Chronic AF		ECHA (2008)[418]	AF: 10 if ecotoxicity is available for 3 trophic levels. AF: 50 if ecotoxicity available for 2 trophic levels.		

Appendix 4. (continued) Parameters needed to estimate pharmaceutical effects for prioritisation. Numbers refer to framework in Chapter 2

Number	Variable	Experimental source	Model/ predictive tool	Applicability domain	Experimental validation	Method limitations
(13) Fish plasma concentration	FPC		1. $logP_{blood:water}$[118] 2. BCF estimation (ionisables)[192]	1. 0 < LogKow < 8 2. Three equations covering acids, bases and neutral compounds: 1< logKow <7 Acid: -0.36 < pKa <10.6 Base: 2 < pKa < 11.4	1. Tested the read across hypothesis (using pH corrected logKow) for Fluoxetine, concluded powerful tool for risk assessment.[240] 1b. Tested read across hypothesis for ibuprofen, provided evidence to support it.[241] 2. Investigation of parameters used to model FPC, suggested approach (2) with logDow most robust.[250]	• BCF method that considers ionisables preferred.[192,250] • C_{max} values are more readily available.[250] • The read-across approach for risk assessment has limited validation.[240,241]
(14) Internal concentration invertebrate	IPC	Method not yet suggested	Not yet developed	N/A	N/A	• An invertebrate internal concentration estimation method needs to be developed for invertebrates associated with the benthos and the water column. • A similar approach like the FPC could be possibility.[119]
	$IPC_{benthic}$	Method not yet suggested	Not yet developed	N/A	N/A	
(15) Therapeutic plasma concentration	H_tPC	C_{max} (peak plasma concentration) AUC_{conc} (area under the time-concentration curve)	N/A	N/A	The area under the curve (AUC) compared to C_{max} does not have a large impact of FPC results [250].	• Highly dependent on the administered therapeutic dose/brand. • Lowest reported values taken to represent worst case. • Available in peer-reviewed pharmacokinetic literature or drug approval reports (EMA, FDA).

Appendix 4. (continued) Parameters needed to estimate pharmaceutical effects for prioritisation. Numbers refer to framework in Chapter 2

Number	Variable	Experimental source	Model/ predictive tool	Applicability domain	Experimental validation	Method limitations
(16) Bioconcentration factors (BCF)	Fish BCF	OECD 305	1. See FPC 2. QSAR [419]	1. See FPC 2. 1< LogKow <10	1. Not validated specifically for pharmaceuticals. 2. Meylan evaluated 694 logBCF/logKow data values 610 non-ionic and 84 ionic covering a logKow range of 3.98-13.98 to derive QSARs.	• Linear and parabolic approaches to cover logKow 2- 10 suggested,[419] LogKow >10 BCFs should be treated as qualitative. • These models are not applicable to ionic compounds.[420] • Neither approach validated explicitly for pharmaceuticals.
	Invertebrate BCF	BCF minimised design[421]	Not yet developed	N/A	N/A	• Uptake of APIs in invertebrate has been shown [422,423], but a suitable predictive model for neutrals and ionisables has yet to be developed. • OECD 305 methods may be inappropriate for invertebrates.
	Benthic Invertebrate BCF	Test method not yet suggested	Not yet developed	N/A	N/A	• QSARs are only available to predict fish and algal BCFs. • Field studies demonstrated pharmaceutical accumulation in benthic invertebrates.[218,424]
	Earthworm BCF	OECD 317	QSAR [425,426]	0 < logKow < 8	BCF/BAF estimation approach has been validated, but not for pharmaceuticals specifically.[427]	• Current predictive method not suitable for ionisable organic chemicals and poor performance for chemicals of moderate to high hydrophobicity.[399,425]
	Plant BCF	Test method not yet suggested	Not yet developed	N/A	N/A	• Pharmaceutical uptake has been demonstrated in the lab and in the field.[234,428] • Developing a predictive tool for this uptake pathway will be especially important as human intake stemming from biosolid use and reclaimed wastewater on cropland has been demonstrated.[429]
(17) NOEC	Mammal	Clinical and pre-clinical data Toxicity studies –repeated-dose toxicity (NOAEL 28, 90 day) or chronic study	Assessment factor : $NOEC_{mammal}$, 28 days=300 90 days =90 Chronic=30	N/A	N/A	• Available as pre-clinical data. • Convert NOAEL to NOEC but not appropriate to extrapolate LC50 tests to derive NOEC unlike birds.[399]

Appendix 5

A 1L composite sample composed of three sampling points across the river channel was collected at each of the sites in Table S.3 on February 11, 2015 for the Chapter 3 scoping study.

Appendix 5. The national grid referenced locations of sampling sites in Chapter 3.

Site Number	Site name	National grid reference	River
1	Earswick	54.007484, -1.060723	Foss
2	Heworth	53.965412, -1.073496	Foss
3	Tower Bridge	53.95421, -1.077873	Foss
4	A1237	53.983455, -1.129493	Ouse
5	Rawcliffe Outfall	53.977546, -1.118611	Ouse
6	Water's End	53.967854, -1.103354	Ouse
7	Skeldergate	53.954222, -1.081372	Ouse
8	Millennium	53.944812, -1.082022	Ouse

Appendix 6. Liquid chromatography gradient and flow rate based on Furlong[77] used in the Chapter 3 scoping study.

Time (minutes)	% Mobile phase	Flow (µL/min)
0	10	0.45
1	10	0.45
5	40	0.45
10	60	0.45
15	100	0.45
23	100	0.6
23.01	10	0.45
33	10	0.45

Appendix 7. Target compound quantifier and qualifier transitions with MS/MS parameters used for Chapter 3 scoping study. All samples were analysed at the USGS NQWL.

Compound	MS/MS transitions	Fragmentor voltage	Collision energy voltage	Retention time (minutes)
10-Hydroxy-amitriptyline	**294 → 276.1**	110	8	10.29
	294 → 215.1	110	48	
Abacavir	**287.1 → 191.2**	100	15	8.28
	287.1 → 150.1	100	30	
Acetaminophen	**152.1 → 110.1**	105	12	4.00
	152.1 → 93.1	105	32	
Aciclovir	**226.1 → 152.0**	90	8	1.98
	226.1 → 135.0	90	28	
Albuterol	**240.2 → 222.1**	100	4	4.78
	240.2 → 166.2	100	10	
	240.2 → 148.0	100	16	
Alprazolam	**309.1 → 281.0**	140	24	14.11
	309.1 → 205.0	140	44	
Amitriptyline	**278.2 → 233.1**	120	15	13.76
	278.2 → 191.3	120	24	
Amphetamine	**136.1 → 119.1**	80	4	6.87
	136.1 → 91.0	80	16	
Antipyrene	**189.1 → 147.1**	120	20	8.50
	189.1 → 104.1	120	25	
Atenolol	**267.1 → 190.1**	120	16	5.00
	267.1 → 145	120	24	
Benztropine	**308.0 → 167.2**	135	28	13.60
	308.0 → 152.2	135	56	
Bupropion	**240 → 184.0**	100	8	10.20
	240.0 → 166.0	100	16	
Carbamazepine	**237.2 → 194.2**	115	16	12.94
	237.2 → 193.2	115	36	
Carisoprodol	**261.1 → 176.1**	85	4	13.96
	261.1 → 158.1	85	4	
Chlorpheniramine	**275.1 → 230.0**	105	12	11.04
	275.1 → 167.0	105	44	
Cimetidine	**253.1 → 159.0**	60	10	4.93
	253.1 → 95.0	60	30	
Citalopram	**325.3 → 262.2**	135	15	11.36
	325.3 → 109.1	135	25	
Clonidine	**230.0 → 213.0**	125	24	6.27
	230.0 → 44.1	125	28	
Dehydronifedipine	**345.1 → 284.1**	145	28	13.27
	345.1 → 268.1	145	28	
Desmethyl-diltizem	**401.1 → 178.1**	120	15	12.60
	401.1 → 150.1	120	40	

Appendix 7. (continued) Target compound quantifier and qualifier transitions with MS/MS parameters used for Chapter 3 scoping study.

Compound	MS/MS transitions	Fragmentor voltage	Collision energy voltage	Retention time (minutes)
Desvenlafaxine	**264.4 → 58.1**	110	20	8.61
	264.4 → 107.0	110	5	
Dextromethorphan	**272.1 → 215.1**	135	24	11.54
	272.1 → 171.0	135	40	
Diazepam	**285.0 → 193.1**	135	32	15.19
	285.0 → 154.1	135	28	
Diltiazem	**415.1 → 178.1**	130	24	12.52
	415.1 → 370.1	130	15	
	415.1 → 150.1	130	48	
Diphenhydramine	**256.1 → 167.1**	60	10	11.66
	256.1 → 152.0	60	45	
Erythromycin	**734.5 → 576.4**	110	15	13.86
	734.5 → 158.1	110	30	
Ezetimibe	**392.4 → 201.1**	115	48	15.38
	392.4 → 133.2	115	28	
Fadrozole	**224.1 → 116.0**	120	30	7.39
	224.1 → 82.1	120	30	
Fenofibrate	**361.1 → 233.0**	110	12	17.20
	361.1 → 139.0	110	28	
Fexofenadine	**502.4 → 484.2**	145	20	13.61
	502.4 → 466.2	145	28	
Fluconazole	**307.1 → 238.0**	110	12	9.20
	307.1 → 220.0	110	16	
Fluoxetine	**310.3 → 148.1**	95	4	14.16
	310.3 → 44.1	95	8	
Fluticasone	**501.2 → 313.1**	110	8	16.00
	501.2 → 293.1	110	12	
Glipizide	**446.0 → 321.1**	105	8	14.27
	446.0 → 304.0	105	20	
Glyburide	**494.0 → 369.0**	105	8	15.75
	494.0 → 169.0	105	36	
Hydrocodone	**300.1 → 199.0**	140	28	5.87
	300.1 → 171.1	140	40	
Hydrocortisone	**363.2 →309.1**	115	10	13.32
	363.2 → 121.0	115	24	
Hydroxyzine	**375.0 → 201.0**	90	15	13.90
	375.0 → 166.0	90	45	
Iminostilbene	**194.1 → 193.1**	100	35	15.47
	194.1 → 179.1	100	35	

Appendix 7. (continued) Target compound quantifier and qualifier transitions with MS/MS parameters used for Chapter 3 scoping study.

Compound	MS/MS transitions	Fragmentor voltage	Collision energy voltage	Retention time (minutes)
Ketoconazole	**531.1 → 489.1**	160	32	14.93
	531.1 → 82.1	160	48	
Lamivudine	**230.1 → 112.1**	60	5	2.60
	230.1 → 95.1	60	45	
Lidocaine	**235.3 → 86.2**	105	16	7.90
	235.3 → 58.2	105	40	
Loperamide	**477.1 → 266.2**	60	25	14.46
	477.1 → 210.1	60	60	
Loratadine	**383.2 → 337.1**	130	20	16.75
	383.2 → 266.1	130	48	
Lorazepam	**321.1 → 303.0**	110	5	14.05
	321.1 → 275.0	110	15	
Meprobamate	**219.0 → 158.1**	80	4	10.59
	219.0 → 55.1	80	20	
Metaxalone	**222.1 → 161.1**	90	4	13.10
	222.1 → 105.1	90	24	
Metformin	**130.2 → 71.1**	90	20	1.39
	130.2 → 60.1	90	10	
Methadone	**310.2 → 265.1**	110	12	13.60
	310.2 → 105.0	110	28	
Methocarbamol	**242.1 → 199.1**	80	4	9.35
	242.1 → 118.0	80	4	
Methotrexate	**455.2 → 308.1**	120	16	7.38
	455.2 → 175.0	120	40	
Metoprolol	**268.2 → 116.1**	115	16	9.00
	268.2 → 74.1	115	20	
Morphine	**286.1 → 201.1**	140	24	2.30
	286.1 → 165.0	140	44	
	286.1 → 152.1	140	60	
Nadolol	**310.0 → 254.3**	110	12	7.73
	310.0 → 201.2	110	20	
Nevirapine	**267.1 → 226.1**	120	25	10.51
	267.1 → 184.1	120	35	
Nizatidine	**332.1 → 155.0**	110	16	3.58
	332.1 → 131.0	110	24	
Norethindrone	**299.5 → 213.0**	125	16	8.28
	299.5 → 91.1	125	16	
Nordiazepam	**271.1 → 208.1**	155	28	14.84
	271.1 → 140.1	155	28	
Norverapamil	**441.3 → 165.2**	150	25	12.73
	441.3 → 150.1	150	45	
Omeprazole	**346.1 → 198.1**	70	10	13.10
	346.1 → 180.0	70	30	

Appendix 7. (continued) Target compound quantifier and qualifier transitions with MS/MS parameters used for Chapter 3 scoping study.

Compound	MS/MS transitions	Fragmentor voltage	Collision energy voltage	Retention time (minutes)
Oseltamivir	**313.2 → 225.1**	100	4	12.00
	313.2 → 166.0	100	16	
Oxazepam	**287.1 → 269.1**	70	10	14.04
	287.1 → 241.1	70	15	
Oxycodone	**316.1 → 298.1**	110	16	6.27
	316.1 → 241.1	110	28	
Paroxetine	**330.1 → 192.1**	120	20	13.20
	330.1 → 70.1	120	30	
Penciclovir	**254.1 → 152.1**	115	16	2.40
	254.1 → 135.1	115	36	
Pentoxyfylline	**279.1 → 181.0**	115	12	9.76
	279.1 → 99.1	115	16	
Phenazopyridine	**214.0 → 122.0**	120	16	12.74
	214.0 → 80.1	120	28	
Phendimetrazine	**192.1 → 147.1**	115	16	6.73
	192.1 → 117.1	115	24	
Phenytoin	**253.1 → 182.1**	105	12	12.37
	253.1 → 104.0	105	36	
Piperonyl butoxide	**177.1 → 119.1**	95	12	17.30
	177.1 → 91.1	95	24	
Prednisolone	**361.2 → 343.2**	100	4	13.29
	361.2 → 325.2	100	4	
Prednisone	**359.1 → 341.2**	110	4	12.57
	359.1 → 147.1	110	24	
Promethazine	**285.1 → 198.0**	100	28	12.84
	285.1 → 86.1	100	12	
Propoxyphene	**340.0 → 266.2**	85	4	13.45
	340.0 → 58.1	85	12	
Propranolol	**260.1 → 183.1**	120	16	11.34
	260.1 → 116.1	120	16	
Pseudoephedrine	**166.1 → 148.1**	85	4	6.00
	166.1 → 133.1	85	20	
Quinine	**325.0 → 172.0**	90	40	9.93
	325.0 → 160.0	90	25	
Raloxifene	**474.3 → 112.1**	145	32	11.54
	474.3 → 84.1	145	56	
Sertraline	**306.0 → 275.0**	100	5	14.40
	306.0 → 129.0	100	25	
Sitagliptin	**408.1 → 235.0**	120	16	9.32
	408.1 → 174.0	120	28	
Sulfadimethoxine	**311.1 → 156.0**	125	16	9.82
	311.1 → 92.1	125	32	

Appendix 7. (continued) Target compound quantifier and qualifier transitions with MS/MS parameters used for Chapter 3 scoping study.

Compound	MS/MS transitions	Fragmentor voltage	Collision energy voltage	Retention time (minutes)
Sulfamethizole	**271.0 → 156.1**	105	8	7.21
	271.0 → 92.1	105	28	
Sulfamethoxazole	**254.1 → 156.1**	110	10	8.03
	254.1 → 108.0	110	24	
	254.1 → 92.0	110	24	
Tamoxifen	**372.2 → 72.1**	135	24	16.04
	372.2 → 44.0	135	60	
Temazepam	**301.1 → 283.0**	120	8	14.32
	301.1 → 255.0	120	20	
Theophylline	**181.1 → 124.0**	110	16	5.96
	181.1 → 96.1	110	20	
Thiabendazole	**202.1 → 175.0**	120	24	9.49
	202.1 → 131.0	120	36	
	202.1 → 65.0	120	48	
Tiotropium	**392.1 → 170.1**	140	32	8.80
	392.1 → 152.1	140	28	
Tramadol	**264.3 → 58.1**	105	16	8.60
	264.3 → 42.1	105	80	
Triamterene	**254.1 → 237.0**	100	30	8.40
	254.1 → 104.0	100	40	
Trimethoprim	**291.2 → 261.1**	135	24	7.37
	291.2 → 230.1	135	20	
	291.2 → 123.0	135	24	
Venlafaxine	**278.1 → 260.2**	110	8	10.82
	278.1 → 58.1	110	16	
Verapamil	**455.3 → 165.2**	140	25	12.73
	455.3 → 150.1	140	45	
Warfarin	**309.0 → 251.0**	100	16	15.10
	309.0 → 163.0	100	8	

Appendix 8. Accuracy (reported as % recovery from HPLC-grade water), precision (indicated by %RSD), dynamic range and linearity as reported by Furlong et al.[80]

Pharmaceutical	10 ng/L		80 ng/L		200 ng/L		R^2	Range
	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)		
10-Hydroxy-amitriptyline	92.2	16.3	92.4	10.1	99.6	6.6	>0.98	2–8000
Abacavir	91.0	9.4	95.9	9.4	103.1	5.4	>0.98	1–8000
Acyclovir	73.0	39.9	80.4	23.2	91.6	14.4	>0.98	2–8000
Albuterol	97.7	13.2	96.5	7.6	103.9	4.2	>0.98	1–8000
Alprazolam	98.7	7.8	94.8	10.1	101.5	2.1	>0.98	1–8000
Amitriptyline	145.9	60.5	90.5	23.1	91.4	13.9	>0.98	4–8000
Amphetamine	90.7	50.9	88.1	7.9	104.1	5.8	>0.98	1–8000
Antipyrine	123	48.9	100.2	16.6	110.6	8.8	>0.98	10–8000
Atenolol	89.3	67.2	81.6	23.3	104.1	7.8	>0.98	4–8000
Benztropine	215	69.8	88.3	16.7	92.2	17.2	>0.98	1–8000
Bupropion	129	34.2	96.4	10.4	103.5	3.9	>0.98	1–8000
Carbamazepine	91.8	9.8	99.3	7.0	107.3	3.8	>0.98	1–8000
Carisoprodol	104	10.9	99.8	8.8	105.1	5.7	>0.98	2–8000
Chlorpheniramine	114	51.4	88.7	5.1	102.9	9.9	>0.98	1–8000
Cimetidine	180	37.1	90.2	13.7	98.0	12.3	>0.98	4–8000
Citalopram	90.6	6.6	92.5	8.9	102.6	4.7	>0.98	2–8000
Clonidine	79.2	29.8	88.5	19.1	108.5	8.8	>0.98	10–8000
Dehydronifedipine	109	10.3	100.3	4.6	104.7	4.8	>0.98	1–8000
Desmethyl-diltiazem	119	25.0	84.3	24.5	89.9	20.0	>0.98	1–8000
Desvenlafaxine	114	21.7	95.2	10.8	101.9	5.0	>0.98	2–8000
Dextromethor-phan	115	34.1	89.2	15.7	99.5	7.4	>0.98	1–8000
Diazepam	105	7.7	92.6	10.0	104.2	7.4	>0.98	1–8000
Diltiazem	98.5	8.9	90.4	14.4	98.8	6.6	>0.98	1–8000
Diphenhydramine	99.5	9.4	92.8	8.3	103.2	7.3	>0.98	1–8000
Erythromycin	80.0	30.6	92.6	7.2	103.7	7.6	>0.98	1–8000
Ezetimibe	103	58.4	74.6	50.1	84.9	39.1	>0.98	20–8000
Fadrozole	114	25.0	94.7	5.0	102.3	6.0	>0.98	4–8000
Fenofibrate	99.0	10.3	93.6	13.6	105.4	8.4	>0.98	1–8000
Fexofenadine	127	23.9	98.0	11.1	106.1	5.2	>0.98	1–8000
Fluconazole	93.7	18.7	101.9	20.5	110.6	5.0	>0.98	4–8000
Fluoxetine	547	83.0	121.9	25.0	92.6	6.2	>0.98	2–8000
Fluticasone propionate	102	15.9	93.2	16.1	104.2	9.1	>0.98	1–8000
Glipizide	97.3	36.7	101.2	16.4	103.4	10.8	>0.98	4–8000
Glyburide	102	6.7	93.9	11.8	101.8	7.4	>0.98	1–8000
Hydrocodone	176	62.7	94.2	11.0	98.2	7.2	>0.98	4–8000

Appendix 8. Accuracy (reported as % recovery from HPLC-grade water), precision (indicated by %RSD), dynamic range and linearity as reported by Furlong et al.[80]

Pharmaceutical	10 ng/L		80 ng/L		200 ng/L		R^2	Range
	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)		
Hydrocortisone	86.0	69.8	87.3	23.3	101.6	8.6	>0.98	10–8000
Hydrocortisone	86.0	69.8	87.3	23.3	101.6	8.6	>0.98	10–8000
Hydroxyzine	96.2	6.1	95.0	8.0	103.7	9.4	>0.98	1–8000
Iminostilbene	93.9	64.0	86.4	19.2	100.2	9.6	>0.98	4–8000
Ketoconazole	114	53.0	85.8	7.2	97.3	17.6	>0.98	4–8000
Lamivudine	120	30.8	96.3	13.5	104.4	5.1	>0.98	2–8000
Lidocaine	134	42.1	97.3	4.9	104.1	3.7	>0.98	2–8000
Loperamide	159	47.5	86.5	14.9	98.8	10.7	>0.98	1–8000
Loratadine	97.2	15.7	94.3	12.7	104.9	7.0	>0.98	1–8000
Lorazepam	222	72.2	104.1	43.4	95.7	12.4	>0.98	20–8000
Meprobamate	143	43.3	96.6	19.5	105.8	8.9	>0.98	4–8000
Metaxalone	117	29.3	98.4	6.1	104.3	6.1	>0.98	4–8000
Metformin	99.5	30.1	94.1	9.5	105.8	4.1	>0.98	1–8000
Methadone	125	46.4	87.7	16.1	100.0	9.5	>0.98	1–8000
Methocarbamol	102	54.4	94.6	9.3	106.9	6.2	>0.98	1–8000
Methotrexate	158	58.9	113.0	19.8	108.2	6.7	>0.98	10–8000
Metoprolol	159	51.4	97.9	12.6	98.9	9.8	>0.98	4–8000
Morphine	123	67.2	101.8	17.1	106.3	5.9	>0.98	2–8000
Nadalol	141	43.0	97.1	5.7	102.3	6.1	>0.98	10–8000
Nevirapine	108	25.2	89.0	20.7	102.3	6.0	>0.98	1–8000
Nizatidine	141	40.4	92.8	14.0	97.1	12.5	>0.98	4–8000
Nordiazepam	110	35.5	94.7	19.1	104.2	13.8	>0.98	4–8000
Norethindrone	95.0	43.8	91.2	11.5	103.7	7.3	>0.98	4–8000
Norverapamil	157	55.9	87.0	21.5	88.7	16.4	>0.98	1–8000
Omeprazole + esomeprazole[1]	98.9	15.6	102.2	8.2	103.1	6.1	>0.98	1–8000
Oseltamivir	121	20.6	99.1	7.1	104.7	4.4	>0.98	4–8000
Oxazepam	71.0	45.3	57.0	56.8	104.5	12.1	>0.98	10–8000
Oxycodone	76.5	76.3	90.3	12.7	98.8	8.9	>0.98	2–8000
Paracetamol	88.7	15.5	96.4	11.8	107.3	6.7	>0.98	1–8000
Paroxetine	165	79.1	82.6	24.8	93.1	15.1	>0.98	1–8000
Penciclovir	84.5	62.7	77.5	48.1	100.3	13.1	>0.98	10–8000
Pentoxifylline	109	28.0	97.4	9.2	106.3	5.4	>0.98	1–8000
Phenazopyridine	110	28.8	96.3	9.1	101.5	3.1	>0.98	1–8000
Phendimetrazine	139	75.6	99.0	16.4	99.9	3.4	>0.98	4–8000
Phenytoin	297	64.8	56.4	70.2	98.0	19.8	>0.98	40–8000

Appendix 8. Accuracy (reported as % recovery from HPLC-grade water), precision (indicated by %RSD), dynamic range and linearity as reported by Furlong et al.[80]

Pharmaceutical	10 ng/L		80 ng/L		200 ng/L		R^2	Range
	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)		
Piperonyl butoxide	97.3	6.2	92.1	13.1	104.7	7.7	>0.98	1–8000
Prednisolone	85.4	133	89.4	19.2	106.9	5.3	>0.98	10–8000
Prednisone	149	80.6	101.0	38.4	106.1	8.2	>0.98	10–8000
Promethazine	152	74.7	80.9	32.1	89.3	17.3	>0.98	10-8000
Propoxyphene	103	15.2	93.8	11.9	102.8	5.9	>0.98	2–8000
Propranolol	131	34.3	96.7	9.1	99.3	7.2	>0.98	4–8000
Pseudoephedrine + ephedrine[1]	86.1	44.7	89.7	11.1	103.7	5.5	>0.98	1–8000
Quinine	145	28.5	100.3	8.4	100.2	7.1	>0.98	10–8000
Raloxifene	118	104.4	95.1	33.7	115.5	13.1	>0.98	1–8000
Ranitidine	401	83.9	103.8	19.3	119.5	89.5	>0.98	20–8000
Sertraline	301	59.8	98.3	10.3	94.2	9.4	>0.98	2–8000
Sitagliptin	158	67.9	91.3	23.8	110.9	12.5	>0.98	10–8000
Sulfadimethoxine	126	37.5	98.8	9.2	107.8	5.6	>0.98	4–8000
Sulfamethizole	94.6	NA	88.0	20.2	102.1	10.0	>0.98	10–8000
Sulfamethoxazole	247	43.2	101.8	23.4	103.3	22.0	>0.98	10–8000
Tamoxifen	195	117	97.8	24.9	95.1	25.0	>0.98	1–8000
Temazepam	102	12.1	97.3	9.8	108.6	7.1	>0.98	4–8000
Theophylline	128	82.0	99.6	10.3	97.1	6.9	>0.98	20–8000
Thiabendazole	136	55.8	97.5	8.9	106.0	4.6	>0.98	2–8000
Tiotropium	354	79.4	96.6	11.5	82.9	29.6	>0.98	1–8000
Tramadol	99.5	10.7	96.1	9.2	104.7	4.4	>0.98	2–8000
Triamterene	119	44.0	94.1	7.4	103.1	7.9	>0.98	1–8000
Trimethoprim	116	24.8	95.9	9.7	106.2	4.2	>0.98	1–8000
Venlafaxine	96.5	8.7	96.2	7.4	103.4	6.1	>0.98	2–8000
Verapamil	155	50.5	89.9	13.0	97.0	17.0	>0.98	1–8000
Warfarin	93.5	6.4	92.2	10.3	103.3	8.7	>0.98	1–8000

Appendix 9. Pharmaceutical dispensaries used to indicate local usage of pharmaceuticals in York for January 2015 (Chapter 3) and each month in 2016 (Chapter 5).

Practice Name	Post Code	Practice Code
Beech grove medical practice	YO26 5LD	B82095
Clifton medical practice	YO30 6PS	B82006
Dalton terrace surgery	YO24 4DB	B82021
East & West York community service (1)	YO26 6EQ	Y03984
East & West York community service (2)	YO24 4HD.	Y03985
East & West York community service (4)	YO30 6PS	Y03987
East & West York community service (5)	YO31 0PR	Y03988
East & West York community service (8)	YO23 1AP	Y03991
East parade	YO31 7YD	B82103
Elvington medical practice	Yo41 4DY	B82081
Escrick surgery	YO19 6LE	B82018
Front street surgery	YO24 3BZ	B82100
Gale farm surgery	YO24 3BU	B82055
Haxby group practice	YO32 2LL	B82026
Health visitors-Acomb	YO26 5LD	Y03992
Health visitors-Clementhorpe	YO23 1AP	Y03993
Health visitors-Clifton	YO30 2JS	Y03994
Health visitors-Hob Moor	YO24 4PS	Y03995
Health visitors-new Earswick	New Earswick	Y03996
Health visitors-the avenue	YO31 0UT	Y03998
Jorvik Gillygate practice	YO1 7NP	B82098
Lifeline protect	YO24 1AU	Y03510
My health group	YO32 5UA	B82080
Ryedale/York community service (1)	YO32 2LL	Y03999
Ryedale/York community service (2)	YO32 2LL	Y04000
Ryedale/York community service (5)	YO10 4QE	Y04003
Palliative care-York	YO24 1GL	Y04007
Petergate surgery	YO30 4RZ	B82003
Priory medical group	YO24 3WX	B82005
South York heart failure nurses	YO23 1AP	Y04016
South York respiratory nurses	YO23 1AP	Y04400
The Old School medical practice	YO23 3UA	B82071
Unity health	YO10 4DU	B82047
York hospital	YO31 8HE	Y00030
York medical group	YO24 4HD	B82083
Yorkshire doctors	YO30 5PB	Y04950

Appendix 10. Parameters used for calculating predicted environmental concentrations (Chapter 3). The pharmaceutical usage is based on the pharmaceutical prescribed by the medical practices in Appendix 8 for the month of January 2015.

Compound	Usage (mg)	PEC (ng/L)	Unchanged excretion (%)		Fraction remaining (%) after WwTP removal	
			High	Low	High	Low
10-hydroxy-amitriptyline	1571614	11.15	10.6[430]	-	91[431]	-
Abacavir	0	0	38[432]	-	0.01[13]	-
Paracetamol	719426970	4225.	80[433]	2[433]	10[43]	0.01[43]
Acyclovir	2263240	37.8	91[434]	76[145]	25[145]	3[13]
Albuterol	820870	15.33	31.8[435]	-	100[184]	-
Amitriptyline	4490325	14.67	5[18]	-	45[91]	-
Amphetamine	6720	0.02	74[32]	-	5[43]	-
Alprazolam	0	0	20[436]	-	77[145]	-
Antipyrine	0	-	-	-	-	-
Atenolol	6379450	187.36	50[437]	40[437]	80[438]	3[438]
Benztropine	0	-	-	-	-	-
Bupropion	80100	1.20	34[145]	-	60[439]	-
Carbamazepine	12352300	45.35	5[440]	2[441]	100[20]	37.7[20]
Carisoprodol	0	-	-	-	-	-
Chlorpheniramine	45490	0.68	26[442]	3[443]	78[145]	-
Cimetidine	1178400	52.69	87[444]	-	70[438]	-
Citalopram	2962040	45.24	26[439]	12[445]	100[446]	82[446]
Clonidine	233.05	0.008	60[434]	-	78[145]	-
Dehydronifedipine	1055952	80.94	60[434]	-	76 [145]	-
Desmethyl-diltiazem	3292022	76.20	48.5[447]	-	80[448]	65[62]
Desvenlafaxine	1475172.88	77.44	55[434]	29[439]	130[446]	53[449]
Dextromethorphan	5458	0.07	32.5[450]	3[451]	55[431]	-
Diazepam	115632	0.21	3[145]	1[452]	84[453]	17[453]
Diltiazem	5486703.3	13.09	5[454]	2[434]	80[448]	65[62]
Diphenhydramine	13675	0.10	13[455]	1.9[455]	83[23]	40[456]
Erythromycin	9753360	143.22	20[457]	2[458]	100[20]	40[20]
Ezetimibe	112770	4.15	69[459]	-	73[145]	-
Fadrozole	0	-	-	-	-	-
Fenofibrate	699607	19.65	85[145]		0.45[295]	
Fexofenadine	3958680	206.94	80[434]	10[4]	89[62]	82[460]
Fluconazole	73250	4.30	80[145]	-	1[461]	-
Fluoxetine	1825380	12.38	11[32]	-	84[91]	-
Fluticasone	71793.1	4.86	95[145]	-	97[145]	-
Glipizide	27475	0.18	10[434]	-	91[431]	-
Glyburide	1960	0.06	50[145]	10[434]	78[438]	25[438]
Hydrocodone	1707472	13.51	11[462]	10[463]	98[313]	5[312]
Hydrocortisone	838502.2	50.54	1	-	82[431]	-
Hydroxyzine	95330	0.31	5[145]	-	90[145]	-
Ketoconazole	660600	0.80	4[464]	-	41[465]	-

Appendix 10. (continued) Parameters used for calculating predicted environmental concentrations (Chapter 3). The pharmaceutical usage is based on the pharmaceutical prescribed by the medical practices in Appendix 8 for the month of January 2015.

Compound	Usage (mg)	PEC (ng/L)	Unchanged excretion (%)		Fraction remaining (%) after WwTP removal	
			High	Low	High	Low
Lidocaine	232515	1.48	10[466]	3[467]	87[449]	50[449]
Loperamide	106044	0.03	2[466]	-	19[431]	-
Loratadine	240730	0.30	2[468]	1[469]	85[470]	67[51]
Lorazepam	7427	0.27	76[469]	-	65[61]	-
Meprobamate	44800	0.66	20[434]	-	100[471]	-
Metaxalone	0	-	-	-	-	-
Metformin	236329350	1873.9	90[434]	-	12[472]	2[472]
Methadone	565095	19.71	50[473]	-	95[474]	-
Methocarbamol	852000	22.36	65[437]	1[475]	55[145]	16.7[313]
Methotrexate	72453	3.74	90[434]	-	78[431]	-
Metoprolol	445850	9.82	30[32]	-	100[476]	-
Morphine	1111784	41.63	75[477]	3[478]	68[479]	1[479]
Nadolol	20160	0.81	72.9[480]	-	75[438]	-
Nevirapine	0	0	5[481]	-	100[13]	-
Nizatidine	44400	1.92	60[466]	-	98[431]	-
Norethindrone	42407	1.47	55[434]	5[4]	86[145]	86[145]
Nordiazepam	33534	0.72	65[482]	-	45[46]	-
Norverapamil	20663	0.02	2.2[483]	-	75[448]	-
Omeprazole	595439	0.38	1[484]	-	91.5[459]	-
Oseltamivir	4800	0.32	90[485]	24[486]	100[13]	37[487]
Oxazepam	11670	0.37	33[32]	-	130[46]	-
Oxycodone	427460	5.85	19[145]	-	98[145]	-
Paroxetine	221160	0.38	2.5[145]	-	93[91]	-
Penciclovir	20	0.0001	70[488]	-	13[13]	-
Pentoxyfylline	24000	0.08	6[489]	-	77[490]	-
Phenazopyridine	0	-	-	-	-	-
Phendimetrazine	0	-	-	-	-	-
Phenytoin	1435900	3.29	4[491]	-	78[145]	-
Prednisolone	510704	3.37	30[492]	-	0.3[493]	-
Piperonyl butoxide	0	-	-	-	-	-
Prednisone	0	0	5[145]	-	54[145]	-
Promethazine	34670	0.12	5[145]	-	98[145]	-
Propoxyphene	0	0	25[145]	-	25[145]	-
Propranolol	3831390	71.73	25.5[494]		99[459]	65[43]
Pseudoephedrine	51420	1.02	90[32]	40[32]	30[495]	5[496]
Quinine	8328600	120.13	20[434]	2[4]	98[431]	-
Raloxifene	52320	0.007	6.2[497]	-	3[145]	-
Ranitidine	11057850	471.7	70[498]	30[32]	83[499]	2[438]
Sertraline	7437550	52.53	13[145]	-	74[91]	-
Sitagliptin	1555499	89.7	80[500]	75[500]	98[431]	-

Appendix 10. (continued) Parameters used for calculating predicted environmental concentrations (Chapter 3). The pharmaceutical usage is based on the pharmaceutical prescribed by the medical practices in Appendix 8 for the month of January 2015.

Compound	Usage (mg)	PEC (ng/L)	Unchanged excretion (%)		Fraction remaining (%) after WwTP removal	
			High	Low	High	Low
Sitagliptin	1555499	89.7	80[500]	75[500]	98[431]	-
Sulfadimethoxine	0	-	-	-	-	-
Sulfamethizole	0	-	-	-	-	-
Sulfamethoxazole	260000	4.01	30[32]	-	70[438]	-
Tamoxifen	137980	2.13	30[32]	-	70[260]	-
Temazepam	140430	8.12	75[32]	-	1.05[46]	-
Theophylline	1434200	10.34	10[32]	-	98[431]	-
Thiabendazole	0	-	-	-	-	-
Tiotropium	110	0.005	74[145]	-	91[145]	-
Tramadol	10656400	190.2	32[501]	15[502]	76[449]	32[313]
Triamterene	0	0	22.6[503]	2[504]	110[446]	-
Trimethoprim	3087500	204.02	90[503]	40[466]	100[20]	13[313]
Venlafaxine	2682132.5	14.38	10[505]	5[439]	73[449]	48[449]
Verapamil	939193	2.71	5[506]	-	80[448]	-
Warfarin	382855	1.41	10[466]	-	50[145]	-

Appendix 11

Bioconcentration factor equations

Equations used to calculate the bioconcentration factors[192] required to estimated fish internal plasma concentrations using the FPM[118] to prioritise pharmaceuticals.

$$\log BCF = 0.85 * \log K_{ow} - 0.7 \quad \text{(Neutrals)}[192]$$

$$\log BCF = \log\left[f_n * 10^{(0.64\ \log k_{ow} - 0.12)} + f_d * 10^{(0.37\ \log k_{ow} + 0.06\ pK_a - 0.51)}\right] \quad \text{(Acids)}[192]$$

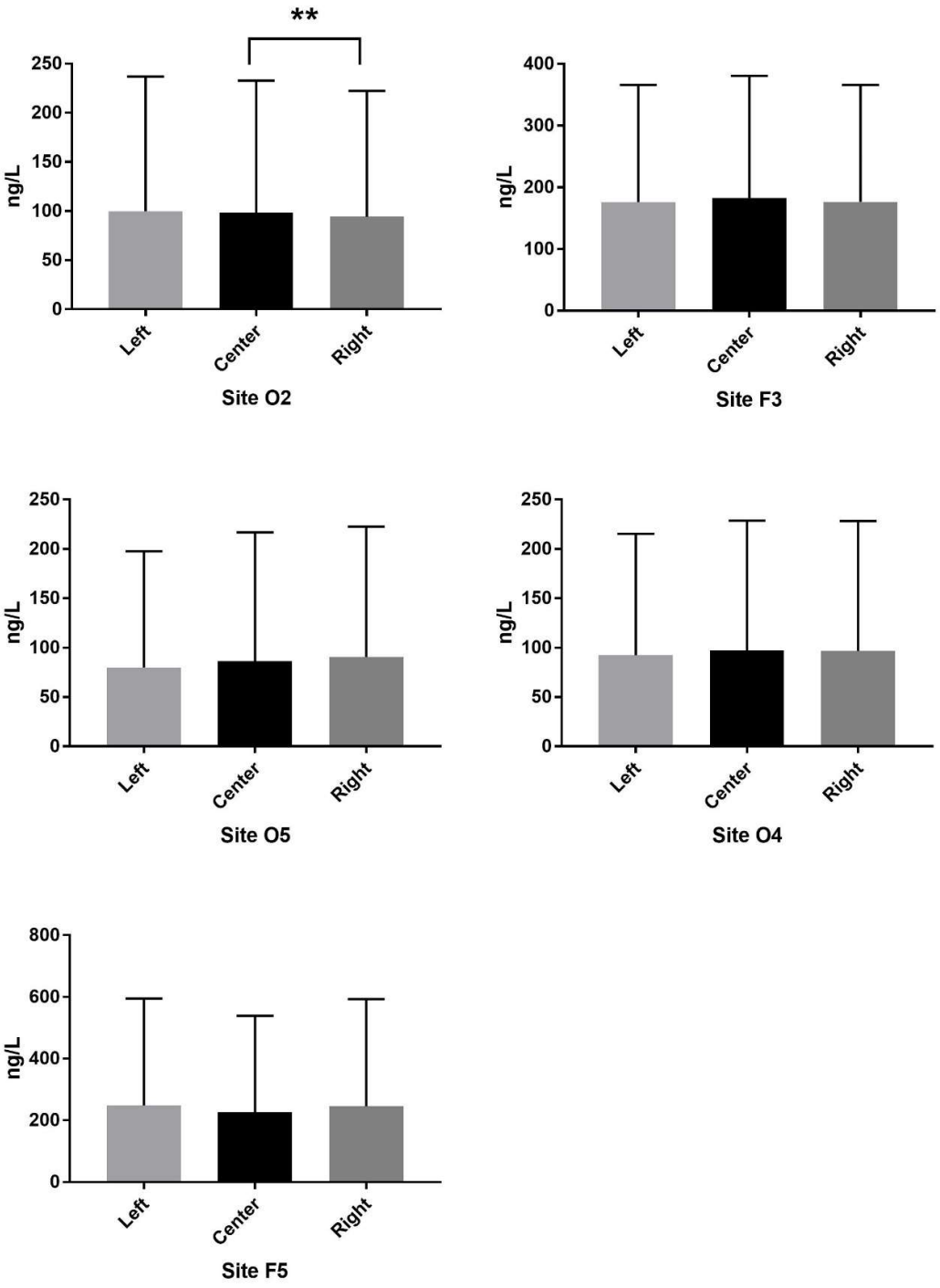
$$\log BCF = \log\left[f_n * 10^{(0.62\ \log k_{ow} - 0.15)} + f_d * 10^{(0.28\ \log k_{ow} - 0.07\ pK_a + 0.84)}\right] \quad \text{(Bases)}[192]$$

$$f_n = \frac{1}{1 + 10^{i(pka - pH)}}$$

$$f_d = 1 - f_n$$

Appendix 12 Channel sampling location

Prior to conducting the monitoring campaign described in Chapter 5, an assessment to determine whether the mixed the sampling sites was conducted. Samples were collected on three separate occasions at sampling sites where access across the entire width of the channel was possible. Metformin, gabapentin, carbamazepine and fexofenadine were selected to determine whether the pharmaceuticals were well mixed within the river channel at sampling sites. During each of the three sampling visits three replicates were taken at equidistant intervals (three in total) across the breadth of the channel. The results from the one-way ANOVA (Friedman's test) are presented in Figure S1. The majority of significance test results were not significant; therefore it was deemed suitable to take the monthly sample from anywhere along the width of the channel and to use it as representative of pharmaceutical concentrations across the channel at that point.



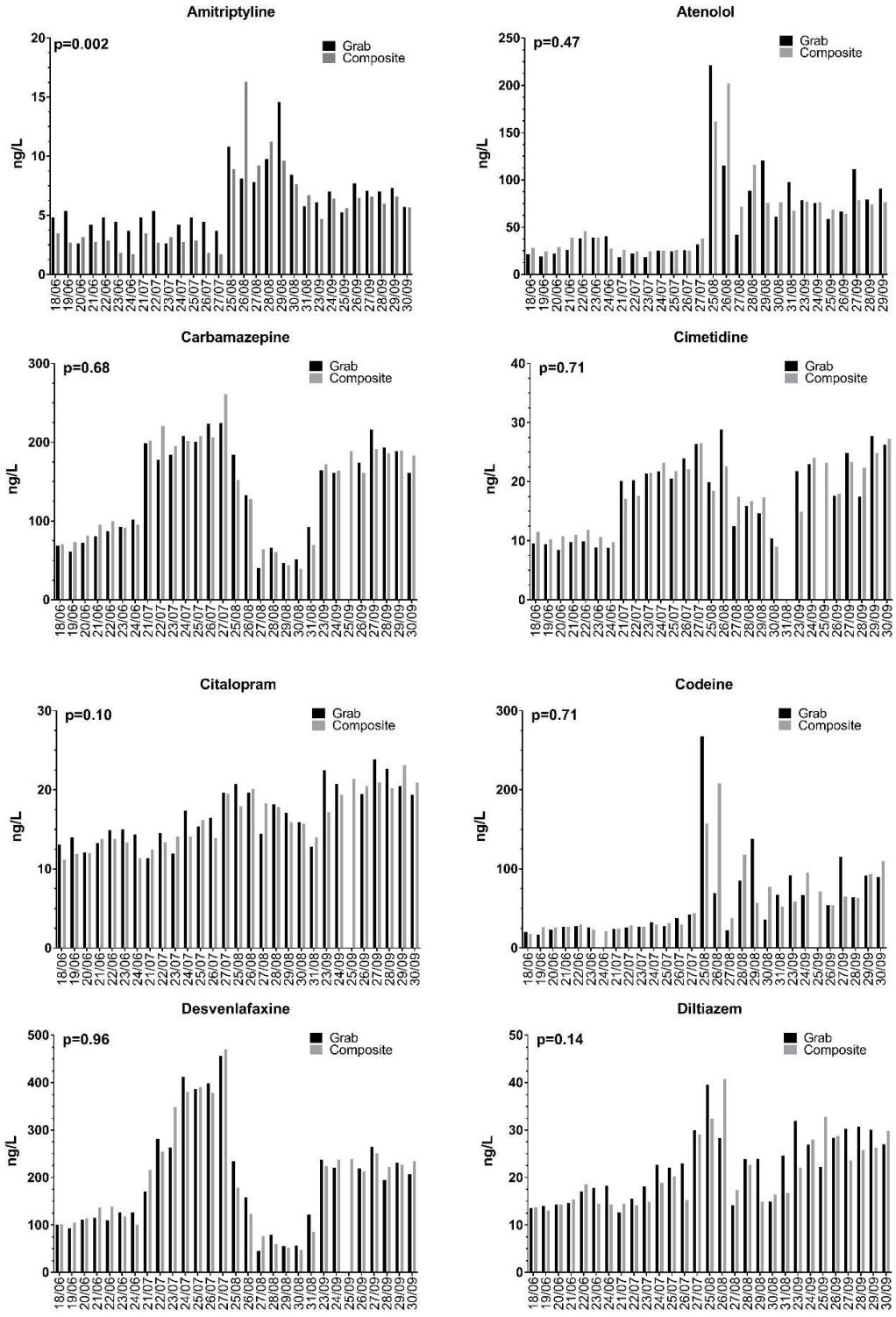
Appendix 11. Samples collected in triplicate at three points equidistant across the channel at each sampling location are presented as summed. This sampling approach was undertaken on three separate occasions. Friedman's test followed by post hoc testing using Dunn's multiple comparison test was calculated for each site to ensure representative sampling sites were well mixed. A significant result was obtained when $p<0.05$ (*) or $p<0.01$ (**).

Appendix 13. Local (York) pharmaceutical usage data for 2016 presented in kg/month.

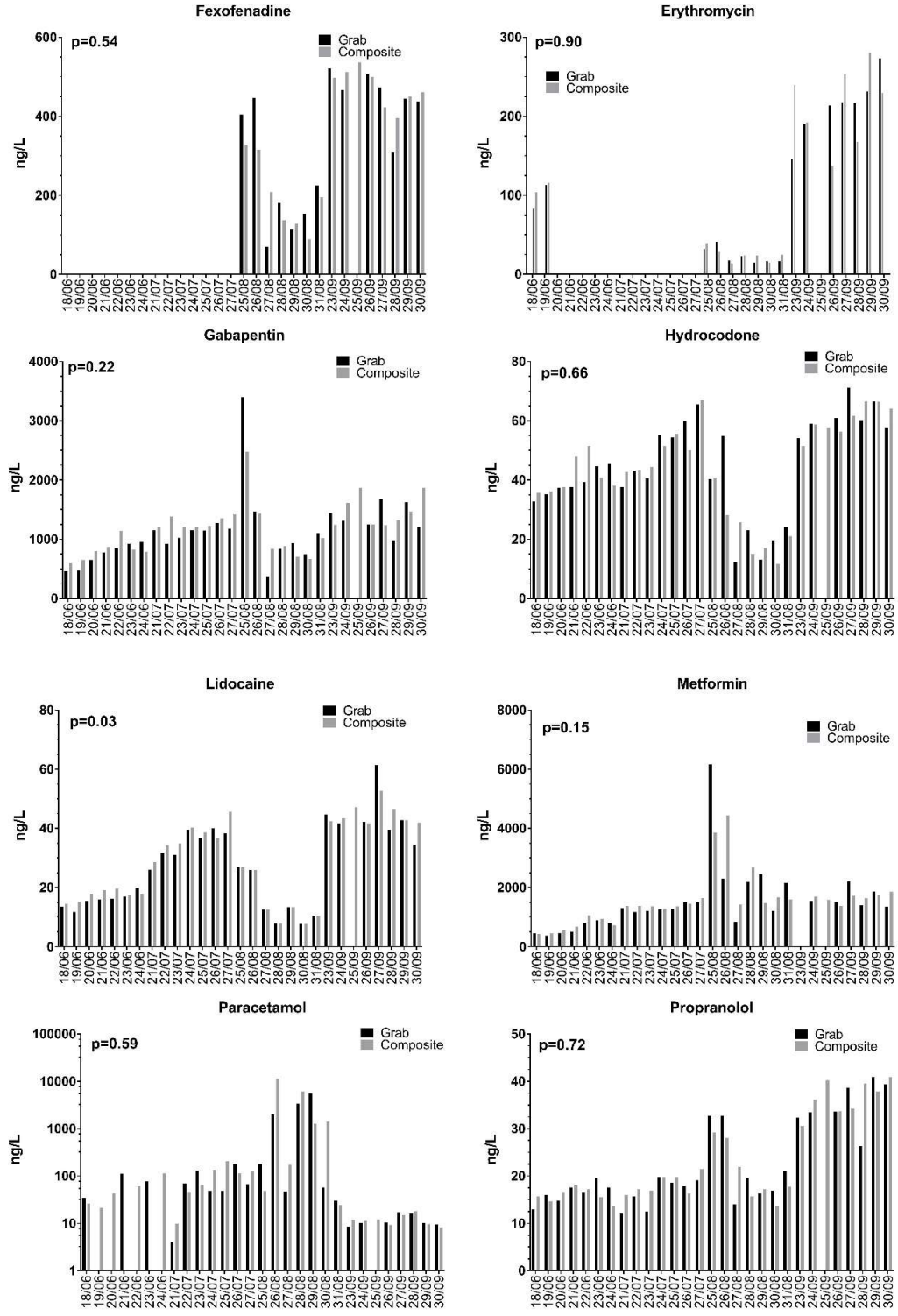
	kg/month											
Compound	January	February	March	April	May	June	July	August	September	October	November	December
Amitriptyline	3.87	3.86	4.21	4.21	3.95	3.68	4.16	4.15	4.02	4.00	3.94	4.22
Atenolol	5.20	5.25	5.57	5.39	5.12	5.01	5.25	5.30	5.25	5.04	5.00	5.29
Carbamazepine	9.85	11.45	10.46	11.42	10.92	9.87	11.14	10.95	10.07	11.08	10.98	11.94
Cimetidine	0.73	0.40	0.44	0.63	0.41	0.33	0.38	0.24	0.26	0.23	0.32	0.25
Citalopram	2.37	2.27	2.40	2.44	2.33	2.22	2.32	2.39	2.30	2.28	2.32	2.41
Codeine	11.40	11.50	12.19	12.24	11.97	11.44	12.12	12.21	12.25	11.87	12.01	12.56
Desvenlafaxine	1.39	1.41	1.43	1.56	1.41	1.32	1.44	1.54	1.46	1.52	1.48	1.58
Diazepam	0.12	0.11	0.12	0.12	0.12	0.10	0.12	0.12	0.12	0.11	0.12	0.12
Diltiazem	8.04	7.33	8.02	7.98	7.80	7.21	7.76	7.95	7.72	7.60	7.57	7.96
Diphenhydramine	0.01	0.00	0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.01	0.00	0.01
Erythromycin	6.94	5.95	6.55	6.62	6.30	6.25	5.72	4.86	4.87	0.66	5.58	5.33
Fexofenadine	3.49	2.73	4.20	5.11	6.23	7.06	5.22	5.48	4.72	4.27	3.71	4.12
Gabapentin	55.70	54.81	59.13	61.35	56.40	59.20	59.53	59.94	61.90	55.99	60.77	63.86
Hydrocodone	1.25	1.27	1.34	1.35	1.32	1.26	1.33	1.34	1.35	1.31	1.32	1.38
Lidocaine	1.33	1.01	0.62	1.11	1.16	0.95	1.12	1.17	1.20	1.40	1.10	1.13

Appendix 13. (continued) Local (York) pharmaceutical usage data for 2016 presented in kg/month.

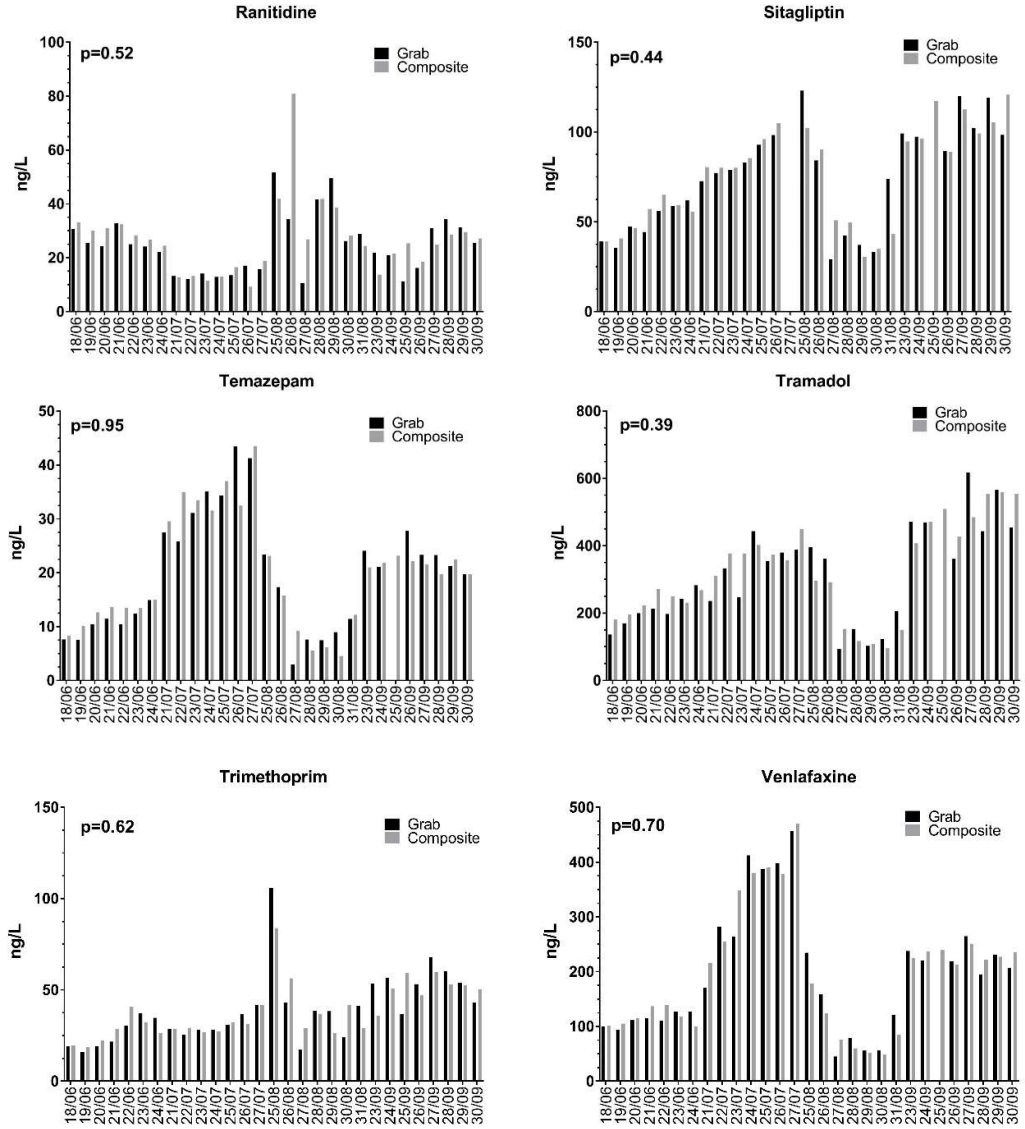
	kg/month											
Compound	January	February	March	April	May	June	July	August	September	October	November	December
Lidocaine	1.33	1.01	0.62	1.11	1.16	0.95	1.12	1.17	1.20	1.40	1.10	1.13
Loratadine	0.21	0.22	0.26	0.31	0.39	0.41	0.40	0.33	0.30	0.22	0.23	0.24
Metformin	174.67	177.47	194.0	190.1	184.94	175.81	189.1	193.76	188.82	181.19	189.67	194.50
Noreistherone	0.06	0.06	0.07	0.08	0.07	0.10	0.08	0.08	0.07	0.06	0.05	0.05
Oseltamivir	0	0	0	0	0	0	0	0	0	0	0	0
Oxazepam	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00
Paracetamol	670.57	413.89	709.8	714.0	684.27	673.19	695.4	706.22	696.65	664.28	682.03	704.23
Propranolol	3.26	3.29	3.52	3.42	3.42	3.14	3.37	3.42	3.29	3.41	3.47	3.43
Ranitidine	10.36	10.18	11.64	10.98	10.84	10.60	11.36	11.31	11.62	11.27	11.54	12.03
Raloxifene	0.03	0.04	0.03	0.03	0.04	0.03	0.03	0.04	0.03	0.03	0.03	0.02
Sertraline	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sitagliptin	1.10	1.17	1.28	1.27	1.20	1.21	1.25	1.33	1.34	1.19	1.32	1.39
Sulfamethoxazole	0.40	0.47	0.25	0.37	0.32	0.38	0.60	0.42	0.42	0.37	0.37	0.44
Temazepam	0.10	0.10	0.10	0.10	0.09	0.10	0.09	0.10	0.10	0.09	0.09	0.09
Tramadol	8.88	8.63	9.31	9.27	9.05	8.71	9.24	9.36	9.18	8.51	8.96	9.10
Triamterene	0	0	0	0	0	0	0	0	0	0	0	0
Trimethoprim	3.17	2.90	3.09	3.09	3.09	2.85	3.07	2.82	3.23	3.22	2.99	2.84
Venlafaxine	2.52	2.57	2.60	2.84	2.57	2.40	2.62	2.80	2.65	2.76	2.70	2.87
Verapamil	1.03	0.90	1.06	1.01	0.92	0.85	1.00	1.04	0.96	0.92	0.88	1.06



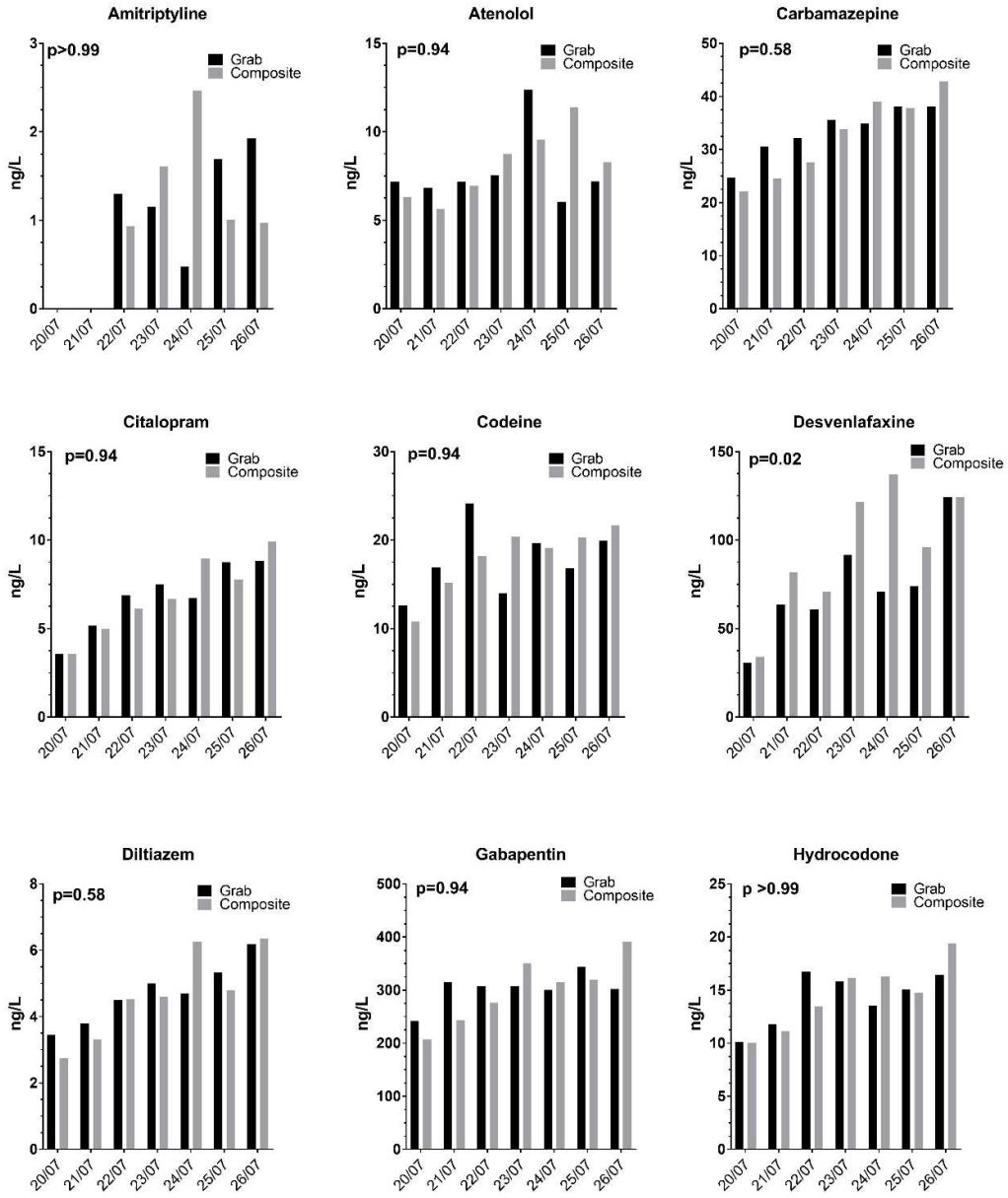
Appendix 14. Paired 24 h composite and grab samples collected at the F3 site (River Foss) during June, July, August and September 2016. The results of a paired t-test is also presented: $p > 0.05$, not significant.



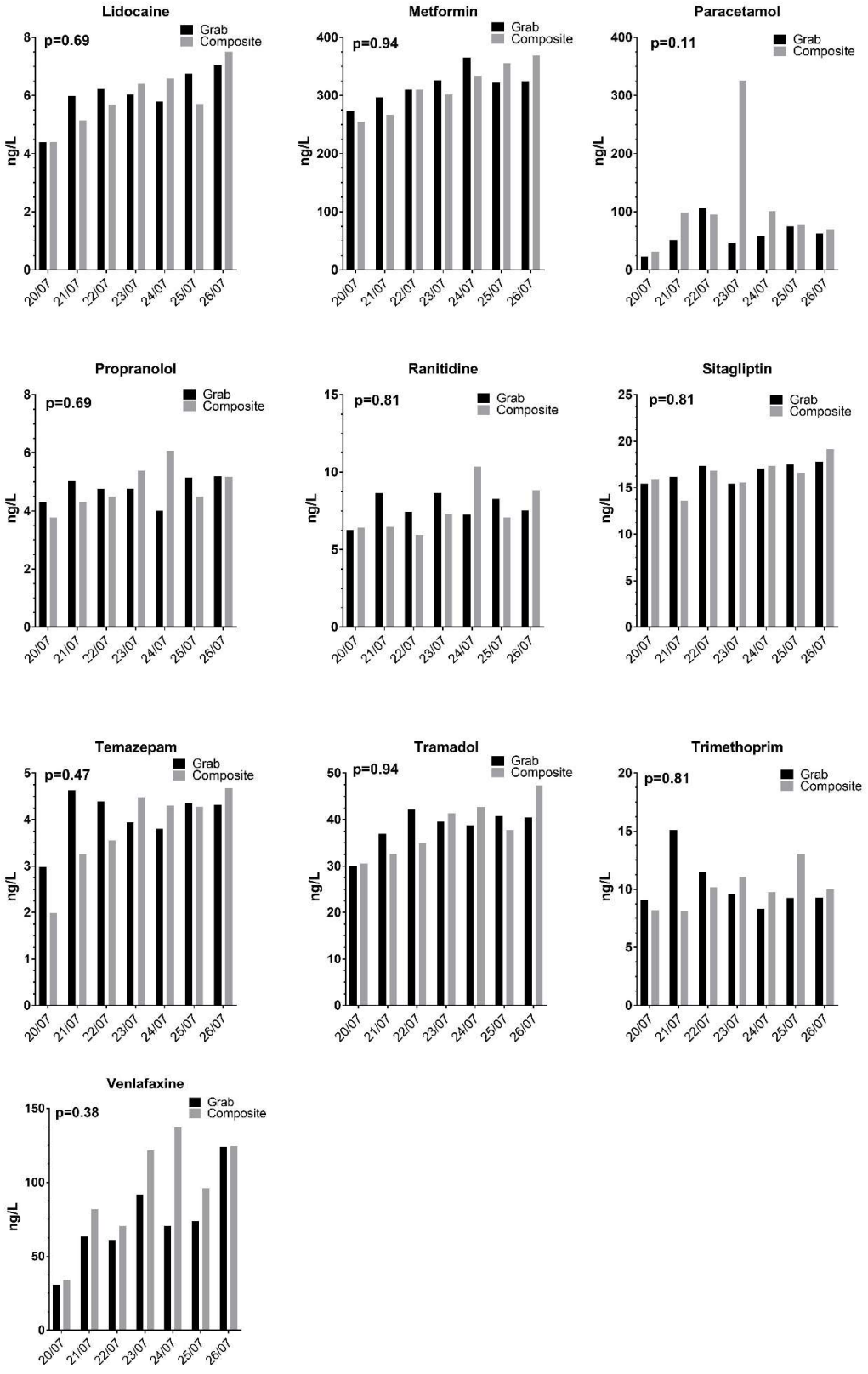
Appendix 14. (continued) Paired 24 h composite and grab samples collected at the F3 site (River Foss) during June, July, August and September 2016. The results of a paired t-test is also presented: $p>0.05$, not significant.



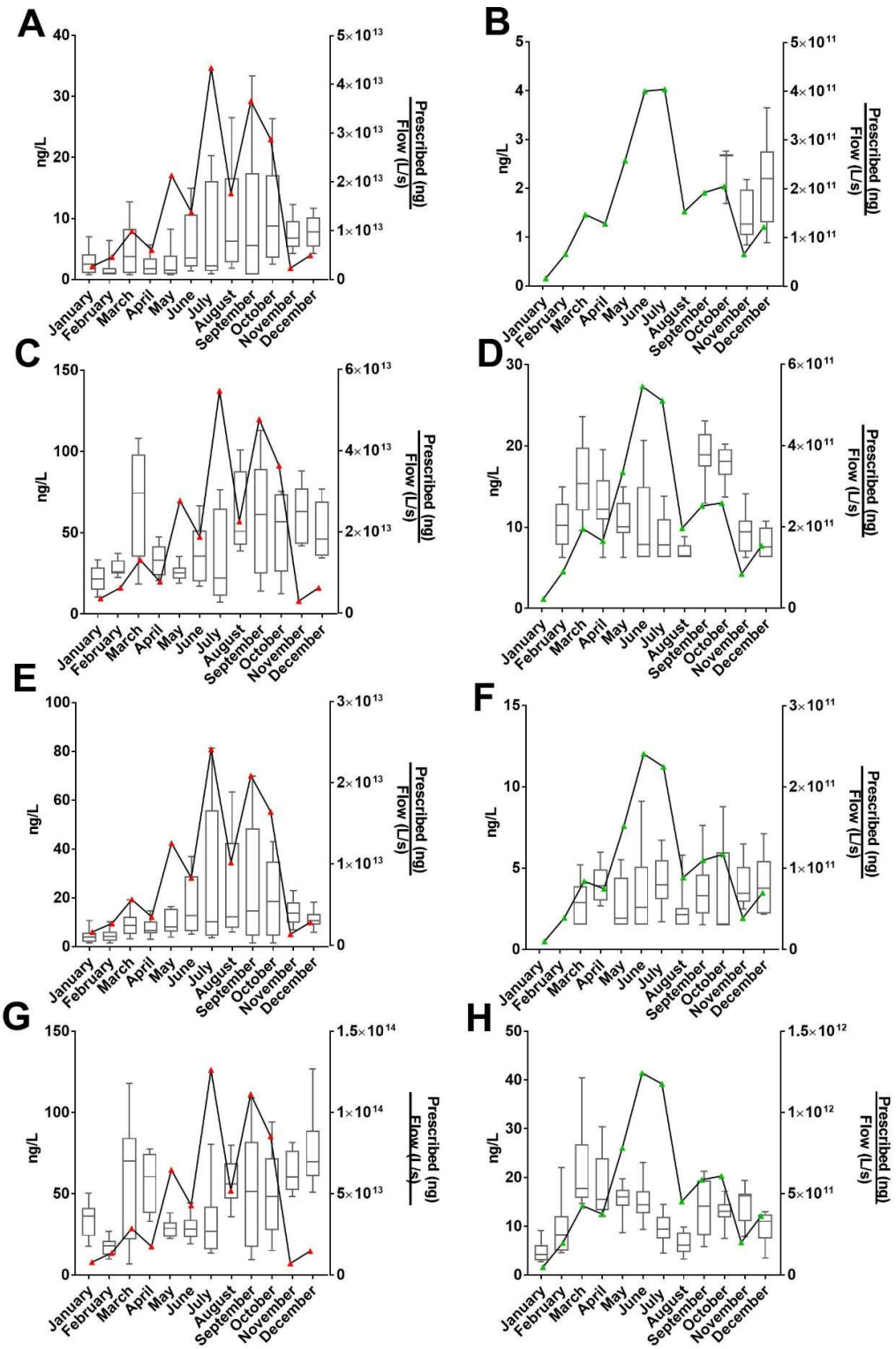
Appendix 14. (continued) Paired 24 h composite and grab samples collected at the F3 site (River Foss) during June, July, August and September 2016. The results of a paired t-test is also presented: $p>0.05$, not significant.



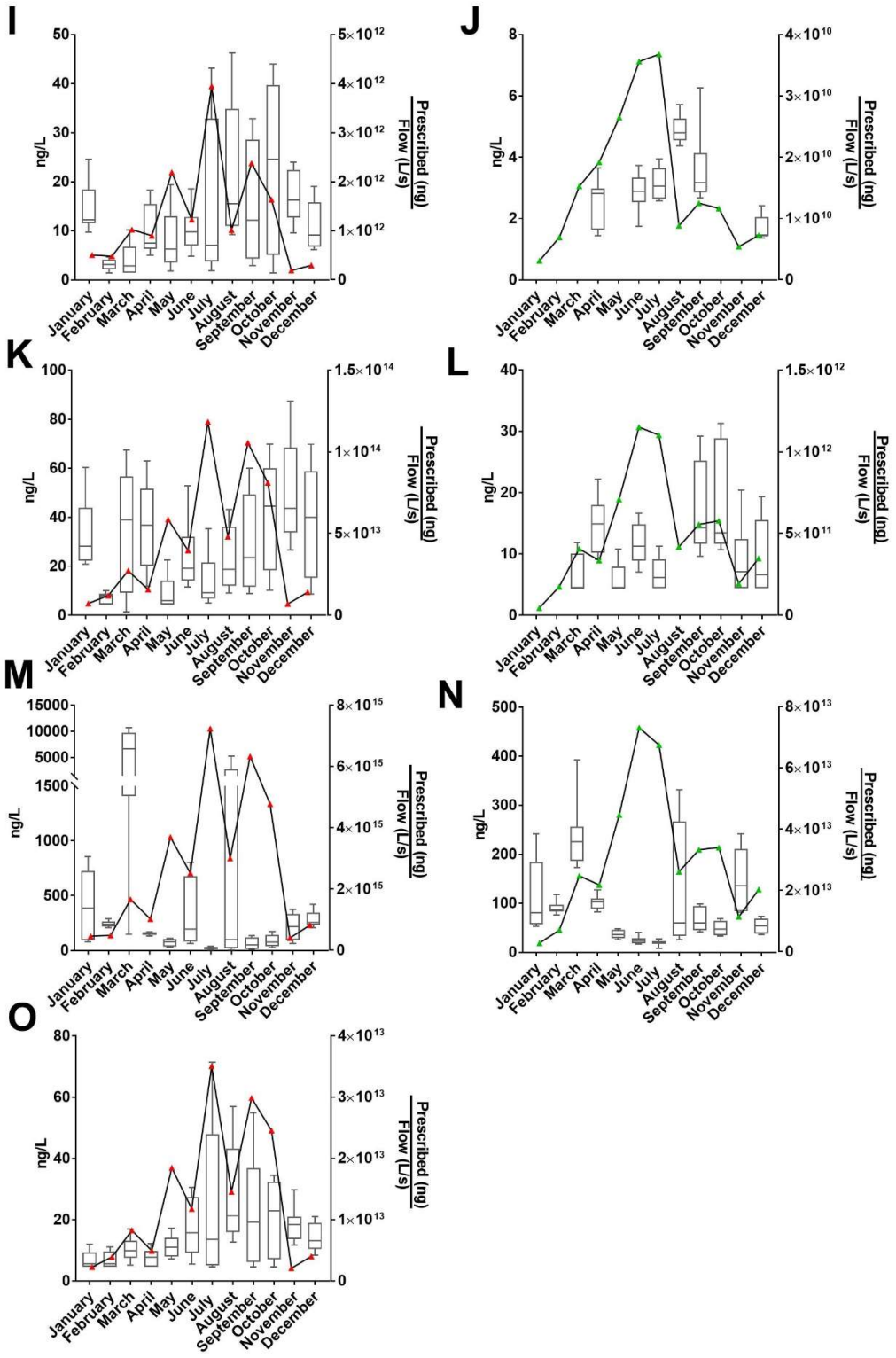
Appendix 14. (continued) Paired 24 h composite and grab samples collected downstream of the O5 site (River Ouse) during July 2016. The results of a paired t-test is also presented: $p > 0.05$, not significant.



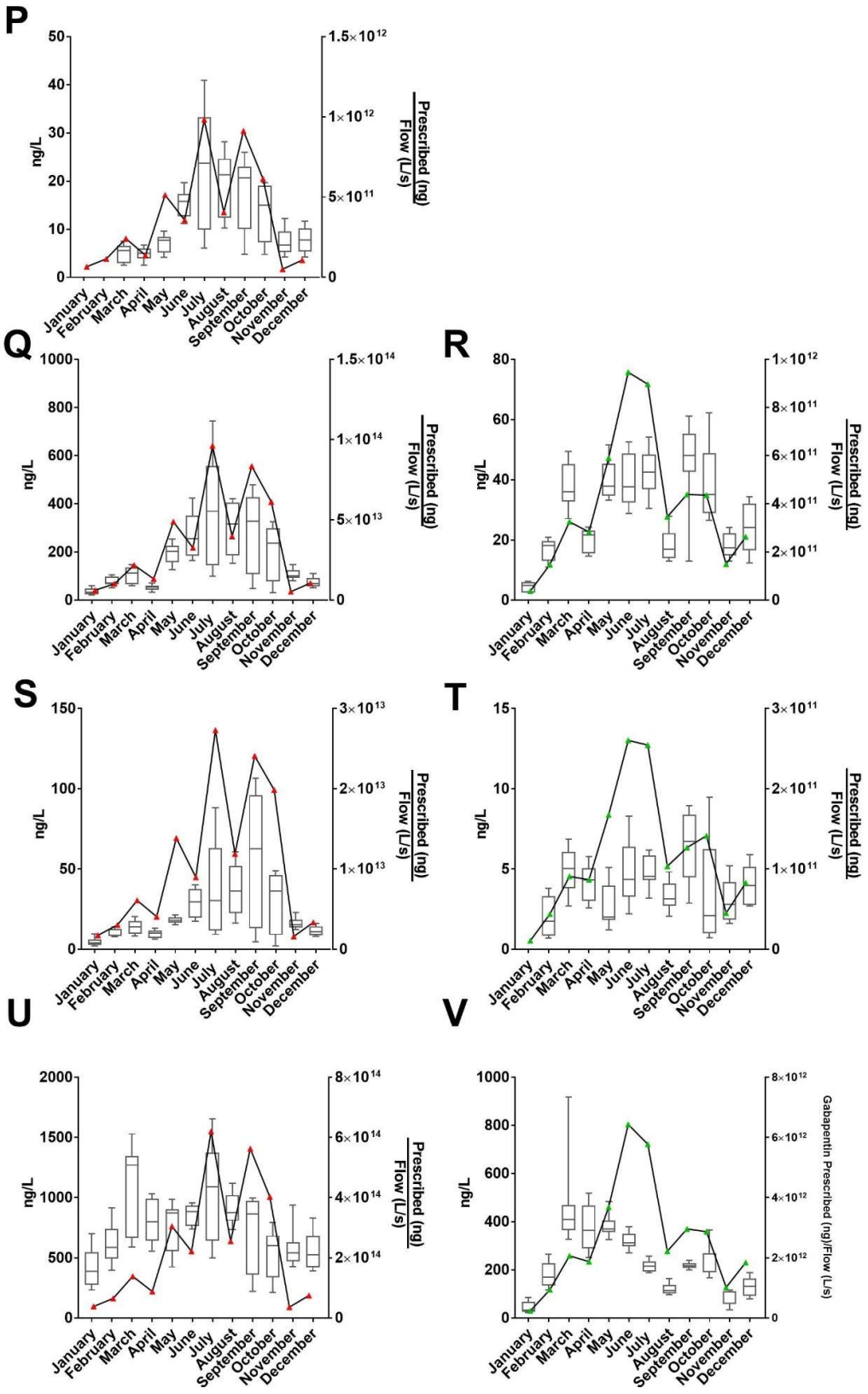
Appendix 14. (continued) Paired 24 h composite and grab samples collected downstream of the O5 site (River Ouse) during July 2016. The results of a paired t-test is also presented: $p>0.05$, not significant.



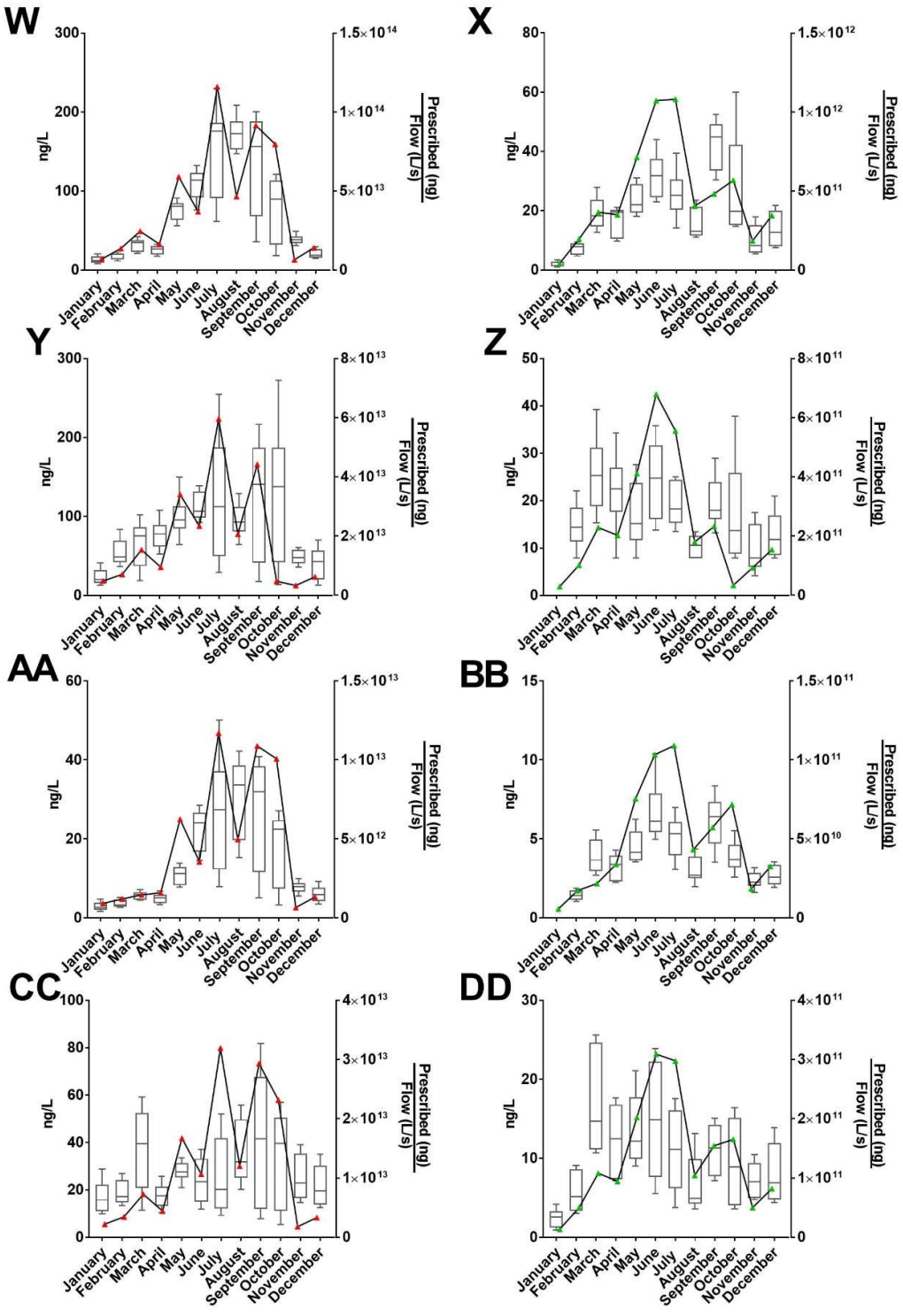
Appendix 15. Box plots of the monthly measured concentrations (median, 25th and 75th quartile and 10th to 90th percentile whiskers in the River Foss (left column) and River Ouse (right column). Plotted against monthly prescribed concentration divided by flow. Amitriptyline (A & B), atenolol (B & C), citalopram (E & F) and codeine (G & H).



Appendix 15. (continued) Box plots of the monthly measured concentrations (median, 25th and 75th quartile and 10th to 90th percentile whiskers in the River Foss (left column) and River Ouse (right column). Plotted against monthly prescribed concentration divided by flow. Cimetidine (I & J), ranitidine (K & L), paracetamol (M & N) and propranolol (O).



Appendix 15. (continued) Box plots of the monthly measured concentrations (median, 25th and 75th quartile and 10th to 90th percentile whiskers in the River Foss (left column) and River Ouse (right column). Plotted against monthly prescribed concentration divided by flow. Temazepam (P), tramadol (Q & R), venlafaxine (S & T) and gabapentin (U & V).



Appendix 15. (continued) Box plots of the monthly measured concentrations (median, 25th and 75th quartile and 10th to 90th percentile whiskers in the River Foss (left column) and River Ouse (right column). Plotted against monthly prescribed concentration divided by flow. Carbamazepine(W & X), erythromycin (Y & Z), lidocaine (AA & BB) and trimethoprim (CC & DD).

Appendix 16. Monitoring data from the River Foss. Annual average values were calculated from samples collected monthly during 2016.

Compound	Annual MEC (ng/L)				
	F1	F2	F3	F4	F5
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Amitriptyline	<LOD	11.9 (8.5)	5.5 (5.4)	3.2 (3.1)	2.3 (1.8)
Atenolol	<LOD	57.4 (28.3)	50.6 (25.9)	42.4 (24.0)	28.2 (16.0)
Carbamazepine	3.9 (3.9)	79.5 (68.6)	84.2 (67.4)	76.4 (61.9)	51.3 (52.9)
Cimetidine*	20.3 (15.8)	22.6 (13.5)	13.9 (10.3)	9.0* (4.5)	5.2* (3.9)
Citalopram	<LOD	28.8 (25.1)	14.9 (7.8)	7.3 (2.6)	5.6 (2.7)
Codeine*	5.4* (2.9)	56.1 (30.2)	52.3 (27.1)	46.1 (22.8)	33.5 (17.9)
Desvenlafaxine	19.7 (16.4)	111.3 (85.0)	98.5 (62.4)	86.9 (53.0)	49.0 (27.4)
Diazepam	<LOD	<LOD	<LOD	<LOD	<LOD
Diltiazem	2.8 (4.9)	21.5 (14.9)	17.2 (10.7)	11.3 (6.2)	5.8 (3.6)
Diphenhydramine*	<LOD	5.9* (5.0)	<LOD	<LOD	<LOD
Erythromycin	16.9 (9.8)	102.4 (64.3)	100.9 (63.0)	80.2 (35.7)	51.9 (34.8)
Fexofenadine	23.9 (29.2)	295.5 (330)	286.5 (259)	237.7 (200)	123.7 (104)
Gabapentin	87.1 (57.0)	786.1 (267)	826.2 (305)	783.8 (268)	595.3 (308)
Hydrocodone	1.9* (1.8)	36.3 (27.4)	31.0 (21.0)	22.5 (14.1)	11.6 (6.3)
Lidocaine*	2.1* (1.6)	17.0 (15.0)	16.5 (13.3)	14.8 (12.0)	7.1 (5.1)
Loratadine*	<LOD	<LOD	<LOD	<LOD	<LOD
Metformin	140.8 (80.1)	929.6 (518)	1094.9 (552)	1034.8 (570)	757.6 (491)
Noreistherone	<LOD	<LOD	<LOD	<LOD	<LOD
Oxazepam	<LOD	<LOD	<LOD	<LOD	<LOD
Paracetamol*	44.9 (39.7)	156.1 (210)	915.3 (2821)	1025.7 (2643)	990.2 (1856)
Propranolol	<LOD	26.3 (19.4)	17.9 (8.6)	11.0 (4.6)	7.9 (4.5)
Ranitidine*	<LOD	40.9 (23.4)	34.1 (22.7)	23.9 (16.9)	15.8 (9.5)
Sitagliptin	< LOD	56.0 (39.5)	50.4 (31.8)	42.1 (24.0)	23.1 (8.9)
Sulfamethoxazole	<LOD	<LOD	11.2 (8.7)	9.5 (6.2)	<LOD
Temazepam	<LOD	10.6 (11.5)	11.5 (9.1)	10.5 (8.8)	5.7 (3.5)
Tramadol	19.6 (17.5)	213.4 (191)	218.9 (161)	174.4 (113)	93.8 (57.1)
Trimethoprim	3.4 (2.9)	37.1 (17.6)	31.7 (16.2)	23.3 (9.0)	17.1 (10.8)
Venlafaxine	1.8* (1.1)	33.0 (31.3)	28.3 (22.1)	20.5 (12.8)	10.4 (5.6)
Verapamil	<LOD	<LOD	<LOD	<LOD	<LOD

Appendix 16. (continued) Monitoring data from the River Ouse. Annual average values were calculated from samples collected monthly during 2016.

Compound	Annual MEC (ng/L)					
	O1	O2	O3	O4	O5	O6
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Amitriptyline	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Atenolol	<LOD	9.7 (4.8)	10.7 (4.7)	9.7 (3.4)	10.4 (5.0)	13.0 (4.5)
Carbamazepine	6.9 (4.7)	12.8 (9.6)	23.7 (15.8)	13.8 (9.1)	15.2 (10.1)	24.2 (12.0)
Cimetidine*	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Citalopram	<LOD	<LOD	3.9* (2.1)	<LOD	2.3* (0.8)	4.2 (1.8)
Codeine*	8.9 (3.7)	10.5 (4.8)	13.2 (5.8)	11.6 (4.9)	12.8 (4.8)	9.0 (4.9)
Desvenlafaxine	5.3* (4.5)	10.9 (8.4)	21.8 (14.7)	13.0 (8.6)	11.8 (9.1)	23.2 (10.2)
Diazepam	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Diltiazem	<LOD	<LOD	3.6 (2.3)	1.7 (1.3)	1.8 (1.0)	3.0 (1.2)
Diphenhydramine*	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Erythromycin	<LOD	<LOD	19.8* (7.5)	12.6* (4.1)	12.2 *(5.4)	18.4* (9.1)
Fexofenadine	14.9 (12.4)	20.7 (16.3)	38.6 (24.2)	28.3 (24.1)	25.8 (17.2)	42.8 (30.1)
Gabapentin	131.0 (67)	189.8 (103)	240.1 (121)	202.3 (103)	212.6 (107)	227.3 (154)
Hydrocodone	1.3* (0.8)	3.2 (1.9)	8.1 (4.5)	4.1 (1.9)	4.0 (1.7)	5.9 (2.6)
Lidocaine*	1.4* (0.6)	2.7* (1.4)	4.0 (1.9)	2.8 (1.4)	3.1 (1.5)	4.5 (2.2)
Loratadine*	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Metformin	166.3 (80.3)	238.4 (116)	249.5 (119)	230.4 (100)	269.5 (114)	269.8 (106)
Noreistherone	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Oxazepam	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Paracetamol*	58.9 (43.9)	68.7 (53.8)	65.3 (47.9)	74.5 (57.6)	128.1 (119)	87.8 (58.2)
Propranolol	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Ranitidine*	<LOD	<LOD	14.0* (9.1)	6.9* (3.5)	<LOD	9.3* (5.3)
Sitagliptin	<LOD	8.7* (3.7)	15.6 (7.9)	9.7* (3.9)	9.8* (3.5)	15.5 (5.2)
Sulfamethoxazole	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Temazepam	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Tramadol	15.3 (9.1)	22.4 (12.3)	30.5 (14.8)	24.2 (13.1)	24.3 (13.0)	34.3 (13.4)
Trimethoprim	5.3 (5.6)	5.5 (2.1)	11.0 (5.2)	6.1 (3.0)	6.5 (2.8)	14.5 (4.9)
Venlafaxine	<LOD	2.5 (1.3)	3.9 (2.3)	2.5 (1.2)	2.8 (1.4)	4.3 (2.1)
Verapamil	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Abbreviations

%RSD	Percent relative standard deviation
ADI	Acceptable daily intake
ADME	Adsorption, distribution, metabolism, excretion
AF	Assessment factor
AOP	Adverse outcome pathway
API	Active pharmaceutical ingredients
BCF	Bioconcentration factor
CAS	Carbon activated sludge
CCC	Continuing calibration solution
$C_{effluent}$	Concentration in effluent
CF	Control filter (field blank)
$C_{influent}$	concentration in influent
C_{max}	Peak plasma concentration
CNF	Control no filter (field blank)
CRM	Charged residue model
CSO	Combined sewer overflow
dc	direct current
DWF	Dry weather flow
E_1	Nash-Sutcliffe model efficiency
EA	Environment Agency
EC	European Commission
EC50	Half maximal effective concentration
ECOSAR	Ecological structure activity relationships
EE2	Ethinylestradiol
EIC	Extracted ion chromatograms
eMC	Electronic medicines compendium
EMA	European Medicines Agency
EPAR	European Public assessment report
EQS	Environmental quality standard
ERA	Environmental Risk assessment
ESI	Electrospray ionisation
FPM	Fish Plasma Model
FWHM	Full width half maximum
GF/F	Glass-fibre filter
GIS	Geographical information system
GREAT-ER	Geo-referenced regional environmental exposure assessment tool for European rivers
HPLC	High-performance liquid chromatography
UHPLC	Ultra high-performance liquid chromatography
IS	Internal standard

ISS	Internal standard solution
iSTREEM	In -stream exposure model
Ka	Acid dissociation constant
K_{biol}	Pseudo-first order biological degradation rate constant
Kow	Octanol-water partition coefficient
IEM	Ion evaporation model
LB	Laboratory blank
LC	Liquid chromatography
LC50	Concentration required to kill 50% of the population
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LF2000-WQX	Low Flows 2000 Water Quality eXtension
LOD	Limit of detection
LOEC	Lowest observed effect concentration
LOQ	Limit of quantification
LOQC	Limit of quantification check
LRB	Laboratory reagent blank
P_{mass}	Mass of pharmaceutical consumed per capita (µg/person/day)
m/z	Mass-to-charge ratio
MEC	Measured environmental concentration
MoA	Mode of action
MRM	Multiple reaction monitoring
MRS	Matrix recovery spike
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
NHS	National Health Service
NOEC	No observable effect concentration
NWQL	National Water Quality Laboratory
OECD	Organisation for Economic Co-operation
OTC	Over-the-counter
PBT	Persistence, bioaccumulation, toxicity
PEC	Predicted environmental concentration
P*h*ATE	Pharmaceutical assessment and transport evaluation
pKa	Negative base-10 logarithm of the acid dissociation constant (Ka)
PNEC	Predicted no-effect concentration
Pop.	Population
Q1	The first quadrupole in a triple quadrupole mass spectrometer
q2	The second quadrupole in a triple quadrupole mass spectrometer
Q3	The third quadrupole in a triple quadrupole mass spectrometer
QA	Quality assurance
QC	Quality control
QqLIT	Quadrupole linear ion trap
QqQ	Triple quadrupole mass spectrometer
QSAR	Quantitative structual activity relationships

R	Global wastewater treatment removal rate
r	Pearson correlation coefficient
R^2	Coefficient of determination
RF	Radio frequency
RQ	Risk quotient
S/N	Signal-to-noise
SAS	Secondary activated sludge
SB	Secondary biological filter
SD	Standard deviation
SPE	Solid phase extraction
TA	Activated sludge with tertiary treatment
TB	Biological filter with tertiary treatment
tR	Retention time
UHPLC	Ultra high-performance liquid chromatography
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
WFD	Water Framework Directive
WWTP	Wastewater treatment plant

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