Pharmaceuticals in freshwater environments and their potential effects on freshwater invertebrates

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Saving the small things that run the planet

Correction notice

Corrections have been made to the original version of this report (dated September 26th 2021) to remove references to Ecological Quality Standards (EQS). EQS values are only applicable once a substance has been added to the priority substances list under the Water Framework Directive (WFD).

To provide appropriate clarification throughout the report, reference to “surface waters” has been changed to “waterbodies”, “freshwaters” or “up and downstream”.

Figures have been amended to remove up and downstream samples from Horsham New STW in the UKWIR data evaluation. This has been done to prevent misinterpretation of data from the sole STW that provides up and downstream concentrations in the UKWIR data.

1st November 2021

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Executive summary

i. The need for protection

a. Freshwater is a vital habitat that is inextricably linked to terrestrial ecosystems. Freshwater species are some of the most threatened and least studied organisms.

b. The interconnectedness of waterways makes them efficient spreaders of pollutants through the ecosystem and the position of invertebrates in the food chain make them ideal stepping-stones for chemicals into other animals.

c. Pharmaceuticals are a major emerging category of concern for chemical contamination in the environment. They are commonly detected in waterways globally and have poorly enforced, contradictory quality standards.

ii. Sources and sinks of Pharmaceuticals

a. Pharmaceuticals have both human and veterinary uses resulting in a variety of pathways into the environment during their manufacture, use and disposal.

b. Chemicals are not entirely eliminated through wastewater treatment and are released in effluent. Removal of a parent compound does not necessarily mean the removal of toxicity. Several pharmaceuticals have been shown to breakdown into substances that are more toxic than the parent compound.

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d. Sinks may be physical, or biological and include: Sediment, bioaccumulation, ground water, and the marine environment.

e. Wastewater Treatment Works are the best monitored and most manageable Source of Pharmaceutical contamination. Septic tanks represent a large diffuse source of pollutants that are largely unmonitored and difficult manage.

iii. How pharmaceuticals interact with invertebrates and the environment

a. Very few studies measure the effects of pharmaceuticals at environmentally relevant concentrations or conduct long term studies. Several substances have the potential to impact invertebrates in the environment, but data are sparse, especially in drug classes like antibiotics.

b. The most observed effect of pharmaceuticals in invertebrates are alterations in reproduction and growth, with some researchers suggesting peaks in effect at low concentrations for some substances.

c. Pharmaceuticals interact with each other and other chemicals resulting in toxic cocktails that are more harmful than single substances.

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e. Biomagnification is unlikely for pharmaceuticals, but this does not rule out potential for direct effects between single trophic levels.

iv. Conclusions from the second phase of the UKWIR Chemical Investigation Programme (CIP2) and CIP2 Scotland

a. Many pharmaceuticals are present in the UK freshwater environment that exceed Predicted No Effect Concentration (PNEC) values. Large spikes in concentrations recorded by CIP2 Scotland are regularly recorded in effluent and downstream samples, as well as in effluent samples recorded by UKWIR CIP2. At times these exceeding their PNEC and reflect a worst-case scenario for these waterbodies. Here PNECs are used as a reference point for target water quality in all samples, not at the WFD classification point.

b. In several cases spikes take concentrations to levels that have been observed to impact the growth, behaviour, and reproduction of freshwater invertebrates. It is currently unknown what effects such spikes in concentration have on freshwater communities.

c. Wastewater treatment works contribute to increases in downstream concentrations of most pharmaceuticals in this study. A significant increase was found for 50% of substances investigated.

d. In effluent, 11 of 14 substances exceeded recommended PNECs between 35% to 94% of the time.

e. Most breaches occurred for Ibuprofen which exceeded limits 62% of the time upstream and 84% of the time downstream of WWTWs, followed by 17-Alpha-ethinyloestradiol (EE2) (15% and 31%), diclofenac (7% and 34%) and 17-Beta-oestradiol (E2) (11% and 24%).

v. Pharmaceuticals of most concern

From the review of current literature and the second phase of CIP2 Scotland and CIP2 UKWIR data, the main chemicals of concern appear to be:

a. **Ibuprofen** – Non-steroidal anti-inflammatory medication.
Pervasive and found in all environmental samples exceeding the PNEC. High-risk quotients for 19 of 20 Scottish WWTWs in downstream waters. Recorded to occur at concentrations that impact invertebrates.

b. **Carbamazepine** – Anti-epileptic medication.
Appears to affect invertebrates below the current PNEC at levels that occur in the environment.

c. **Fluoxetine** – Anti-depressant medication
Occurs in the environment at concentrations that have been observed to alter invertebrate behaviour and reproduction. It is also known to bioaccumulate.
d. **Venlafaxine** - Anti-depressant medication
Not included in CIP2 sampling but causes stress responses in the freshwater snail, *Leptoxis carinata,* at concentrations well below the PNEC
e. **Diclofenac** – Non-steroidal anti-inflammatory medication.
Poorly eliminated through WWTWs, commonly occurs in waterbodies above the PNEC. Known to bioaccumulate in invertebrates and can impact some avian species.

vi. Recommendations

a. Further research and monitoring of the presence of pharmaceuticals in the natural environment.

b. Improved evaluation of the environmental risks posed by pharmaceutical products to include:

   i. Retrospective risk assessments carried out on approved products already in use.

   ii. Breakdown products(s) as well as parent pharmaceutical

   iii. Cocktail effect of products

c. Results of updated Environmental Risk Assessments considered in Water Framework Directive monitoring and in future chemical investigation programmes.

d. Wastewater treatment facilities must be improved to prevent novel pollutants such as pharmaceuticals entering the environment.

e. While CIP3 will include analysis of biosolids, and an evaluation of septic tanks is underway; we also encourage further examination of other sources of contamination beyond WWTWs – such as combined sewer overflows, river and pond sediments, landfill run off, and agricultural run off – as well as more in-depth, long-term examinations of effects on invertebrates in the environment.

f. Reduce the number of pharmaceuticals entering WWTWs through:

   i. Further education on correct usage and disposal of pharmaceuticals to supplement resources available for antibiotic disposal.

   ii. Increased regulation on availability of most prevalent/worst impacting pharmaceuticals.

   iii. Prescribing less damaging drugs or opting for alternative treatments such as blue-green social prescribing where appropriate as per the One Health Breakthrough Partnership (OHBP), a project in development that aims to address over prescription, and environmental release of pharmaceuticals.

1 – Introduction

In the last two decades, emerging chemicals of concern have been identified beyond the usual pesticides, heavy metals, and persistent organic pollutants[1–5]. Pharmaceuticals are a major emerging category of chemicals that pose real concern for the health of our terrestrial and freshwater ecosystems. Freshwater species are some of the most threatened and least studied organisms[6]. In Europe for example, around 44% of freshwater molluscs and 15% of dragonflies classed are threatened, and a quarter of dragonfly species are in decline[7,8]. As such, it is important to address what aspects of human use and abuse of the environment contribute to changes in biodiversity.

Aquatic and terrestrial ecosystems are inextricably linked. Rivers carry plant nutrients, foods, pollutants, native and invasive species further than they would be able to travel alone. Our waterways feed the land, and aquatic invertebrates feed birds, bats, otters, fish, and many other organisms. The interconnectedness of waterways makes them efficient spreaders of pollutants through the ecosystem and the position of invertebrates in the food chain make them ideal stepping-stones for chemicals into other animals[9–11].

Chemicals proven to be harmful often have contradictory or poorly enforced quality standards in freshwater. In the UK this has resulted in some substances like Diclofenac occurring in concentrations on average three times higher than its PNEC, without needing specific monitoring or removal[12,13].

The EU, as part of the Water Framework Directive (WFD), has identified a suite of pharmaceuticals to be included on Watch Lists as potentially harmful chemicals[14–16]. England, Wales, and Scotland have also recently completed the second phase of the Chemical Investigation Programme (CIP2) which highlights several pharmaceutical compounds of concern in UK freshwaters[17].

Here, we first review current literature for the potential ecotoxicity of pharmaceuticals with reference to their impacts on invertebrates, alongside a discussion of sources and sinks for these chemicals and current policy. This is followed by analysis of CIP2 data from across the UK exploring: the environmental concentrations of selected chemicals; the influence of Wastewater Treatment Works (WWTWs) on up and downstream concentrations; and a discussion of potential risks and impacts posed by pharmaceuticals.

2 – A review of pharmaceuticals in the environment

2.1 - Potential for ecotoxicity: lethal versus sub lethal effects

Most widely used measurements and standards for ecotoxicity of chemicals, like Predicted No Effect Concentration (PNEC), are based on EC50s and LC50s with an assumption that concentration below these levels for most pharmaceuticals in the environment will have negligible effects on most invertebrates[18].

However, while tests are based on establishing concentrations that cause mortality, in practice death is only one of many drivers of population changes and reductions in environmental health. Low concentrations may result in sub-lethal effects that can have substantial impacts on the ecology, behaviour, and evolution of an organism. Several pharmaceuticals have been shown to have a peak in sub-lethal effects at low, ecologically relevant concentrations[19,20].

General examples of sub-lethal effects include:

- Sterilisation – individuals cannot produce viable sperm and eggs. Usually, with a greater effect on males than females (no recorded example for pharmaceuticals in freshwater invertebrates).
- Alteration of sex ratios – one sex becomes more dominant through increased deaths for one sex or changes in development[21,22].
- Genotoxicity leading to increased rates of DNA damage in exposed populations[23,24].
- Changes in fecundity (egg production rates) – females increase or decrease egg production[21,25,26].
- Disruption to immune system function[27].
- Changes in behaviour (induced by underlying physiological changes) – a result of stress or changes in development. May lead to higher rates of predation or changes in reproductive success[20,28].
- Endocrine disruption, usually leading to changes in reproduction or growth[29,30], although its occurrence in molluscs - where most research has been directed - is disputed[31].
- Stunted or altered growth and development of individuals[29,30,32,33].
- Changes in population growth rates which could result in overpopulation or gradual decline[34,35].

In some cases, PNECs are based on laboratory studies under artificially high concentrations resulting in a skewed view of what is, or is not, potentially ecotoxic in the natural environment. Some pharmaceuticals may appear to have negligible effects on mortality and reproduction at low concentrations, but have specific, highly detrimental effects on certain life stages for some animals. For instance, carbamazepine causes non-biting midge larvae to be unable to emerge from pupation above measured concentrations of 0.14 and 0.234 mg/kg at 20 and 23°C[36] respectively.

The reliance on laboratory results is currently unavoidable due to a combination of a lack of substantial field studies, and the need for each substance to be assessed in isolation for inclusion in legislation and policy. Measures are taken to address potential unreliability and research is constantly updating these values. The real issue is that PNEC values appear to be addressed, or rather ignored, in a somewhat blasé manner in legislation and in practice. There are no laws that directly address the release of pharmaceuticals into the environment, only EU policy recommendations and advisory reports. This has resulted in the average concentration of pharmaceuticals like diclofenac and ibuprofen exceeding their PNEC by 4 and 40-fold respectively in some UK rivers[12,37].

2.2 - Potential for ecotoxicity: Breakdown products and metabolites

Chemicals are often not entirely eliminated during wastewater treatment and are released in effluent to waterbodies[12,17,38–40]. Some of these compounds persist for long periods in the environment where they can be degraded by sunlight (photodegradation) or broken down by organisms into metabolites (biodegradation) (see Figure 1). Breakdown products are also produced through wastewater treatment as complete elimination is not possible using current methods. Conversion into breakdown products can lead to the false appearance that the chemical has been removed and is no longer a threat as the parent compound (the original pharmaceutical) gradually disappears[3,41,42].

Removal of a parent compound does not necessarily mean the removal of toxicity. Some substances like carbamazepine, diclofenac and naproxen breakdown or are transformed into substances that are equally or more dangerous to human and environmental health[43–45]. Breakdown products of pharmaceuticals have been observed in the environment in high concentrations. For example, Ferrando-Climent et al. (2012) found ibuprofen breakdown products at concentrations two to tenfold higher compared to ibuprofen itself in Spanish WWTW influent, effluent, and waters 500m downstream of sewage effluent[46].

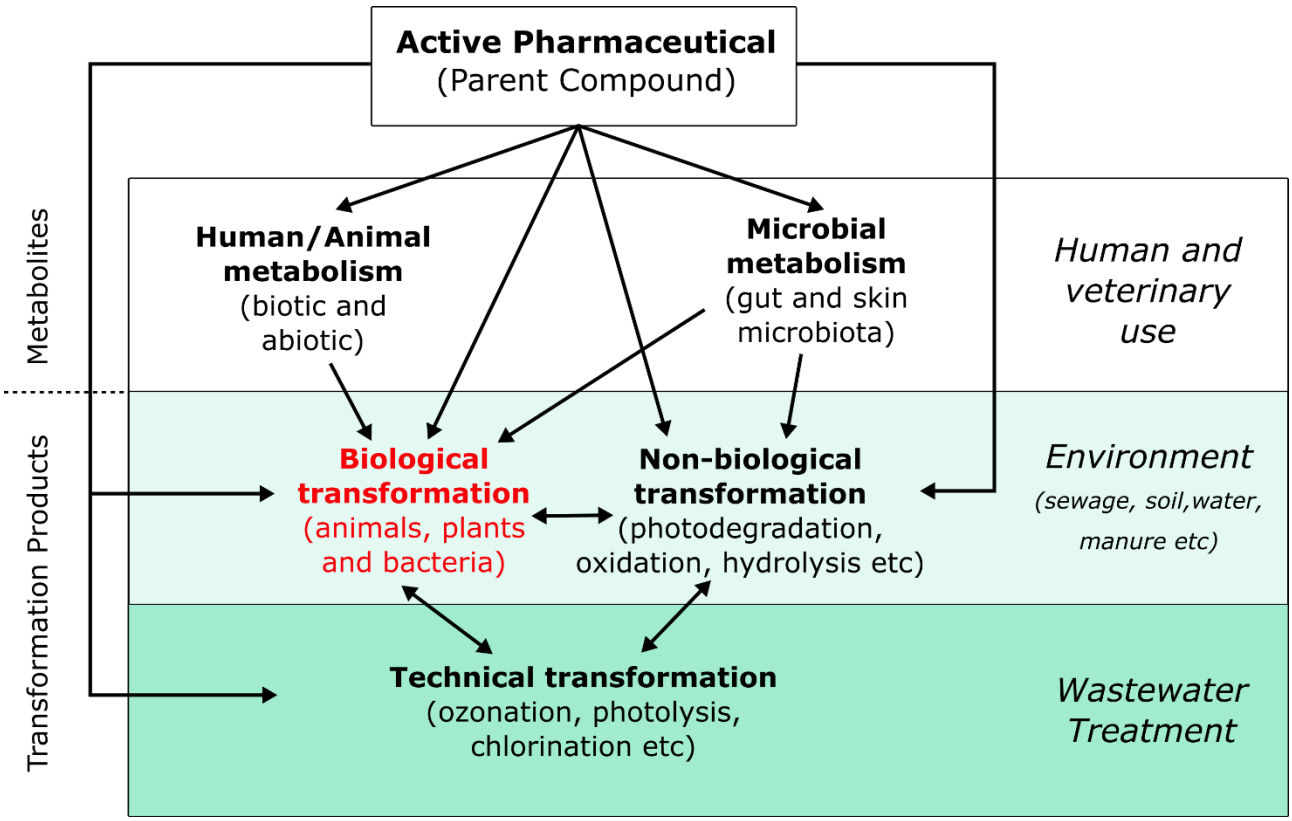

Figure 1. Pathways for pharmaceutical degradation and movement into wild organisms (highlighted red). Adapted from Diaz (2003) [47]

Several pharmaceuticals have been shown to breakdown into substances that are more toxic than the parent compound:

- Carbamazepine can breakdown into acridine in the laboratory under conditions found in estuarine waters[43]. Acridine is a known carcinogen, toxin and mutagen in mammals and has been shown to bioaccumulate in *Daphnia pulex* and fish as well as breaking down slowly in the natural environment[48].

- Naproxen itself is not considered particularly toxic, with the highest PNEC of all drugs addressed in this report[49]. However, under photodegradation (break down in UV light), naproxen forms compounds which are more toxic to planktonic organisms than the parent compound[44,50]. These breakdown products are on average: 4 to 14 times more acutely toxic to the rotifer (*Brachionus calyciflorus*); 4 to 16 times for crustacea (*Thamnocephalus platyurus* and *Ceriodaphnia dubia*)[44]; and 1 to 9 times for *Daphnia magna* [50]. As with many pharmaceuticals, these breakdown products also have considerably greater sub-lethal effects that occur at very low concentrations[44,51]. Reproduction was inhibited by naproxen's photo-transformation products at 50% effective concentrations (EC50s, affects 50% of the population at this concentration) of 0.026 to 1.06 to mg/L for *C. dubia* and 0.25 to 0.67 mg/L for *B. calyciflorus*. The EC50 of all but one breakdown product was below the EC50 measured for naproxen in each organism (0.33 to 0.68 mg/L and 0.56 to 0.79 mg/L respectively). Effects were more pronounced in the algae, *Pseudokirchneriella subcapitata*, where growth was inhibited at an EC50 between 1.9 to 6.86 mg/L for breakdown products, compared to 31.82 to 39.31 mg/L for naproxen itself[44].
- Diclofenac readily breaks down in sunlight leading to the impression that the chemical is removed. In photodegradation experiments, diclofenac increases in toxicity as it breaks down resulting in a sixfold reduction in algal growth after 53 hours[45]. Such photoproducts are not routinely measured in the environment, so it is not known what concentrations they exist at. Effects on algal communities could have knock-on effects for biofilm grazers like snails, mayflies, and some caddis larvae.

When assessing the toxicity of pharmaceuticals, it is important to examine the potential toxicity of their breakdown products and metabolites as well. Knowing the toxicity of a substance's breakdown products allows for predictions of current and future toxicity. Additionally, it is important know how long a substance is active in the environment. Pharmaceuticals like ibuprofen and paracetamol are also often pseudo-persistent thanks to near constant release into the environment. Length of exposure impacts toxic effects, with toxic effects increasing with time in the NSAIDs ibuprofen and diclofenac[90,115] and in some agricultural antibiotics[147].

2.3 - Potential for ecotoxicity: Toxic cocktails of multiple drugs

Mixing drugs is known to be dangerous in medical prescribing for humans, the same is true in aquatic environments. Pharmaceuticals act together and cause significantly greater negative effects in combination than on their own, resulting in lower growth and survival at lower concentrations, and greater effects on gene expression and reproduction[52–54]. The different combinations of drugs and other chemicals are associated with significantly different effects on communities of macroinvertebrates in freshwater[55].

Effects can be additive or synergistic (results either add up or their effects are multiplied when present together). Effects can occur at ecologically relevant levels[27], but most knowledge is from laboratory experiments, which often use concentrations several times higher than recorded concentrations in the environment[54]. They are also limited to specific artificial combinations of pharmaceuticals which may not reflect the real concoctions of pharmaceuticals and other chemicals found in our rivers. Nevertheless, it is a good starting point for understanding the potential impacts of mixtures of pharmaceuticals on invertebrates.

Gust *et al*. (2013) is one of the rare studies that compares the effects of concoctions of several different classes of pharmaceutical at environmentally relevant concentrations[27]. The authors

demonstrated that the immunocompetence of the freshwater snail, *Lymnaea stagnalis*, responds differently to different groups of pharmaceuticals. They found that the effects of a global mixture of all chemicals was most similar to sewage effluent mixtures. Out of the separate mixtures of each drug class, the antibiotic group reflected the global mixture the closest. Effects range from compromising immune system regulation to oxidative stress, which is indicative of toxic responses to the drugs.

Environmentally safe limits do not account for the combined effects of chemicals and are almost exclusively based on single chemical studies, often carried out in isolation in the laboratory. While extremely important groundwork, the use of single chemical measurements can lead to significant underestimation of chemical toxicity in the environment [56]. Combinations of stresses are nearly always more detrimental to animals and ecosystems than lone stresses. Some researchers have attempted to address mixture toxicity by developing standards that combine the toxicity of chemicals and their breakdown products, [57–59] as well as accounting for synergistic effects of some chemicals. For example, the relative hazard index (RHI) developed by Gutiérrez et al. (2008) addresses the combined toxicity of chemicals in a mixture, their individual toxicities, and their ability to bioaccumulate. These new standards are yet to be put in practice or adopted; individual PNEC values are currently the most common, widespread standard.

3 – Sources and sinks

Pharmaceuticals have both human and veterinary uses resulting in a variety of pathways into the environment (figure 2). This includes large input sources like WWTWs as well as small but numerous sources like septic tanks. Each source has their own set of challenges to overcome when addressing chemical contamination.

Sinks may be abiotic (river sediment, soil, groundwater, etc.) or biotic (plankton, invertebrates, amphibians, etc.). Sinks may interconnect and have knock on effects for organisms that interact with them. For example, small invertebrates disturb river sediment and may cause faster release of contaminants[60]. Alternatively, an invertebrate might store the contaminant in its tissues at a higher level than the surrounding water or sediment[61], increasing the risk that its predators will also be affected, possibly more than the invertebrate itself. Alternatively, the behaviour or reproduction of an invertebrate might be affected by the substance, with potential knock-on consequences for other species.

3.1 - Wastewater treatment plants (WWTWs)

WWTWs are the central hub through which most wastewater passes through so they play an important role in the removal of contamination before water is released into the environment. Biofilm reactors (including activated sludge and biofilters) in WWTWs are particularly important for the transformation and mineralisation of some drugs like ibuprofen which can be removed at rates to above 90%[62,63]. However other pharmaceuticals are not fully metabolised in biofilm reactors, so cannot be efficiently removed by traditional sewage treatment methods. For pharmaceuticals addressed in this report, a summary of recorded removal rates at WWTWs are as follows:

- Diclofenac removal is incredibly unreliable. It is usually removed at rates around 17% but rates of 0%, 50% and 69% have also been recorded[39,40,62].
- Ibuprofen is generally removed at rates of 88-93% and upwards but this is not always achieved. In addition, its breakdown products are not always removed[39,64].
- Carbamazepine and its products CBZ-ep and dh-hCBZ fall below 25% removal efficiency[40] down to 0%[13,64].

- Fluoxetine has up to ~50% removal efficiency in UK WWTWs[13], but has also been recorded to have ~0% removal efficiency in biofilm reactors[40].
- Azithromycin has been recorded to have ~40% removal efficiency[13]. Another study recorded a significant increase in effluent[40].
- Clarithromycin and Erythromycin are removed at rates between 50-60%[13,40].
- d-venlafaxine removal falls below 50% efficiency[40].
- Naproxen falls between 40 and 75%[40,62,64].
- Venlafaxine also falls between 40 and 75%[40,62,64].
- The natural hormones E1 and E2 are usually removed at relatively high efficiency around 90-100%, but the artificial hormone EE2 is around 60%[13]. EE2 is incredibly persistent in activated sludge and is the breakdown product of another hormone, mestranol [65]**.**
- Propranolol removal efficiency is around 25-30%[13].

All pharmaceuticals addressed in this report are however commonly found in UK waterways and some – like ibuprofen and diclofenac – are virtually ubiquitous[12,13].

Unsurprisingly WWTWs are a major contributing factor in the bioaccumulation of several pharmaceuticals in freshwater invertebrates[66]. Exposure to wastewater treatment effluent has been observed to increase rates of DNA damage in midge larvae[23] and has disruptive effects on reproduction, growth, and development in freshwater shrimps and mussels[29,30]. All WWTWs are different and the makeup of the waste they treat will vary with location. Addressing the issue of poor chemical processing would likely need to be bespoke to the treatment plant, though improvement of general treatment methods would be ideal. In particular, limiting the operation of storm combined sewer overflows which discharge raw or partially treated sewage to watercourses is essential.

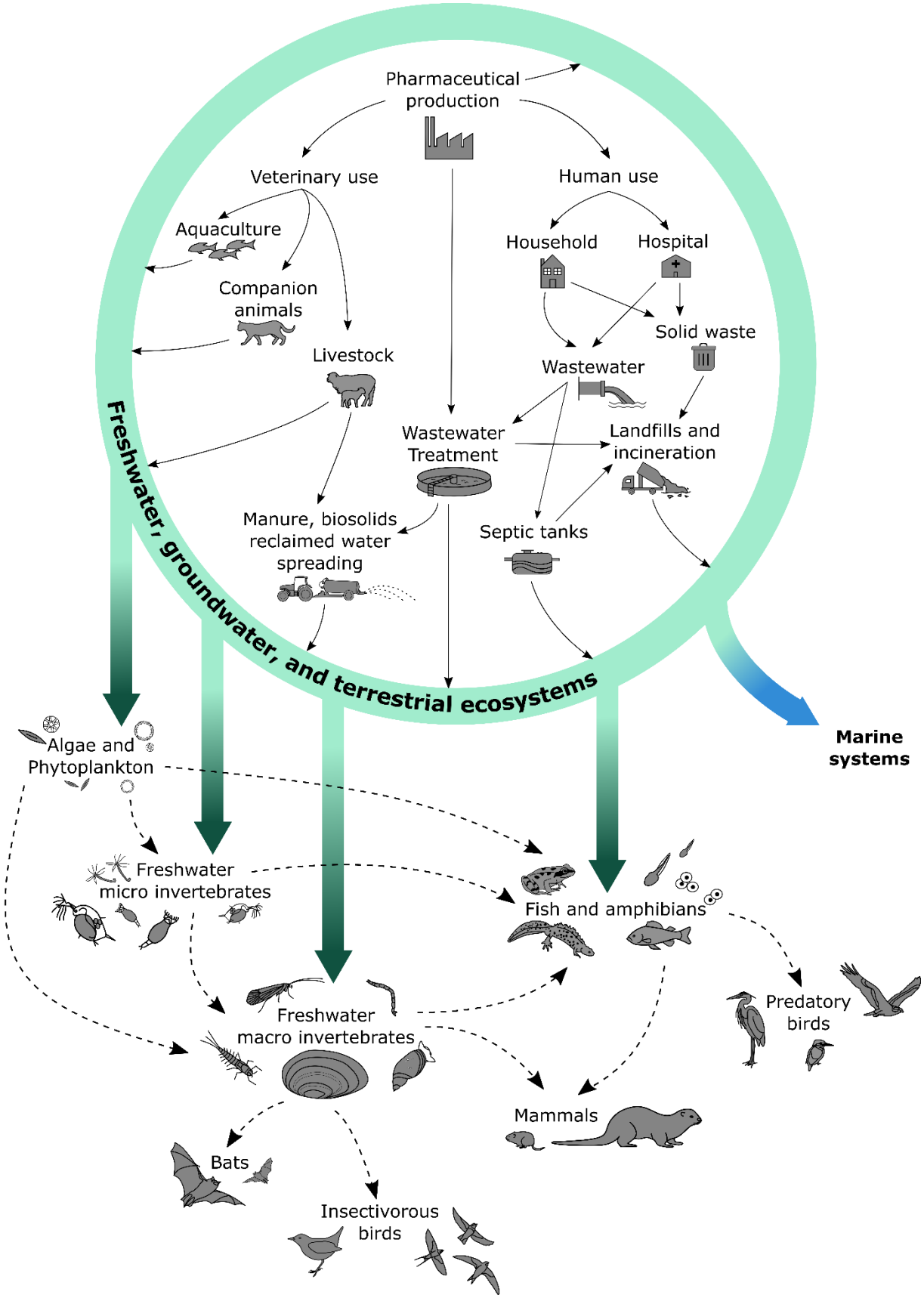

Figure 2. Sources and fates of pharmaceuticals in the environment. Solid arrows indicate direct pathways of pharmaceuticals, dotted arrows indicate possible routes for indirect effects and bioaccumulation. Adapted from OECD (2019)[67] and Taggart et al (2015)[1]. by Helen R. Davison

3.3 - Septic Tanks

Septic tanks are common in rural regions of the UK where sewage systems are not linked to a central wastewater treatment plant. The treatment method by septic tanks is a crude, small scale version of the biodigesters found in most WWTWs. They are relatively effective, but do not completely degrade contaminants and so contribute to leaching into the soil and groundwater systems[68–70].

Septic tank effluent contains similar, sometimes higher concentrations of contaminants to wastewater treatment plants[71]. Some septic tanks lead to drain field sites – where septic tank effluent is released into the soil through a filtering medium – which do help to reduce the concentrations of contaminants from septic tank effluent, but not completely[71]. Despite the potential importance of septic tanks as a huge, diffuse contamination source, there is a large knowledge gap in the literature. Only 2% of papers address septic tanks directly, compared to 37% addressing activated sludge - a method typically used in centralised sewage treatment in WWTWs[4]. In addition, many septic tanks are individually owned, so they are difficult to regulate and maintain to consistent standards that limit contamination. Scottish Water is currently preparing a report which should help fill the knowledge gap for contaminants in septic tanks and the variability of septic tank treatment.

3.4 - Agricultural runoff and spreading of reclaimed water and treated biosolids

Agricultural runoff from field irrigated with reclaimed water or treated biosolids can introduce contamination to receiving waterbodies and has the potential to impact soil and freshwater invertebrates[3,42,72,73]. Treatment for biosolids is not designed to remove chemical waste and they are known to retain pharmaceutical contamination throughout the treatment period for over six months[74]. Most investigated chemicals, including ibuprofen and diclofenac, have been recorded in activated sludge at very low concentrations (e.g., 122-588 ng/g for ibuprofen and 22-209.1 ng/g for diclofenac[5,74]). While others like ciprofloxacin are retained at levels up to 6500 ng/g (reviewed in Petrie et al., 2015)[5]. No legislation currently addresses chemical contamination of treated biosolids, resulting in terrestrial contamination as well as leaching into waterways.

Leaching of pharmaceuticals from fields treated with biosolids derived from sewage sludge can change dramatically over time. All chemicals act differently in the soil and demonstrate a variety of mobility that is dependent on their ability to adsorb to the solid medium[75]. Triclosan (a personal care product) for example is well retained by soil (a reason why it can be found in such high concentrations in biosolids), but over the course of a year it has been shown to dramatically decrease in concentration[42]. This decrease is a result of leaching out of the topsoil, changes in weather conditions (wetter weather leads to greater mobility), and degradation into methyl-triclosan[42]. Both triclosan and carbamazepine have been detected 266 days after initial contamination[73]; other pharmaceuticals like ibuprofen and paracetamol (acetaminophen) appear to be initially sequestered in the soil then gradually released over time[73]. Long term studies demonstrate the importance of monitoring the changes in chemical concentrations over time as levels of contamination are not static.

It should also be noted that the method of application can impact the levels of contamination in run off. Injection leads to far lower levels of pharmaceuticals in runoff compared to broadcast application[73]. However, care should be taken so that these different methods do not transfer the problem elsewhere, such as groundwater.

3.6 – Bioaccumulation in wildlife

Aquatic invertebrates are important routes for bioaccumulation, either through direct absorption from the water or through consumption of contaminated organisms[76]. Invertebrates known to bioaccumulate pharmaceuticals include snails, bivalves, water fleas, worms and insects[3,11,61,76–78]. Plants, fish, amphibians, and other mammals are also capable of retaining pharmaceuticals in their tissues, either through direct absorption from the environment or from their food (reviewed in Puckowski et al., 2016)[76].

Bioaccumulation can result in long term impacts that persist even after successful removal of the contamination, as well as secondary impacts on predators. Pharmaceuticals are unlikely to magnify up multiple levels of the food chain in the same way as other pollutants because they lack the properties to do so. To magnify, a substance must be strongly lipophilic and hard to degrade. Currently, only persistent organic pollutants and heavy metals are known to be capable of this. However, pharmaceuticals can be accumulated in tissues and tend to be active at low concentrations in both vertebrate and invertebrates. As a result, bioaccumulation of pharmaceuticals could cause unexpected effects in non-target organisms between single trophic levels.

Diclofenac for example is considered the main cause of the catastrophic collapse of vulture species across Eurasia and Africa[1]. Limited studies observe diclofenac outside of Gyps vultures, but toxic effects have been found in other bird species including Steppe eagles, chickens, Mynah birds, pigeon and Quail[79,80]. Diclofenac has been found to bioaccumulate in invertebrates downstream of wastewater treatment plants[66], which is a cause of potential concern to insectivorous bird and fish species if they are sensitive to it.

In the aquatic environment, absorption from the water column is the most important exposure route for invertebrates[81] and vertebrates[82]. Lagesson et al. (2016) suggests that the dietary route could be important for higher trophic level species like Common perch in the natural environment based on their finding of bioconcentration values 3 to 10 times higher than those estimated in the laboratory. To our knowledge, no study to date has directly addressed the uptake of pharmaceuticals between aquatic invertebrates and their vertebrate predators even though it is commonly quoted as a concern in the scientific literature.

Invertebrates can retain pharmaceuticals in their tissues, with bioaccumulation factors (BAFs) (also known as bioconcentration factors (BCFs)) ranging from 2.2 to 34,000[3,10,61,66,76]. Invertebrates are the food source for many other animals, including birds, bats, otters, water vole, frogs, and fish, and are responsible for the cycling of nutrients and energy up the food chain. As such there is potential for drugs to accumulate to biologically important levels in invertebrates that can potentially affect animals higher up the food chain[11,76,78].

3.7 - Sediment

Sediment contamination by pharmaceuticals and watch list substances is not currently universally addressed under the WFD or within freshwater monitoring schemes in the UK. The WFD gives the option for some EQS to be measured in sediment or from the tissue of appropriate biota, but this is specific to certain priority substances like mercury[14]. This lack of sediment monitoring is relevant to other forms of chemical contamination as well, such as insecticides and herbicides.

Chemicals in soil and sediment are known to affect invertebrates, but studies showing effects of pharmaceuticals in sediments on invertebrates are limited. Impacts are seen in soil and sediment dwelling (benthic) organisms, but it is difficult to isolate the purely sediment-induced effects. That

said, benthic species that live on or in the sediment are the most likely to bioaccumulate pharmaceuticals, implicating a relationship between pharmaceuticals and sediments[10]. Examples in soil and sediment include: beta-oestradiol accumulating in earthworms exposed to sewage effluent[78]; and benthic larvae of the midge *Chironomus riparius* displaying poorer growth rates, reduced fecundity and changes in gene expression when raised in sediments exposed to reclaimed water spiked with carbamazepine and triclosan[53].

Adsorption into sediment may limit immediate availability and toxicity; pharmaceuticals in sediment can be less toxic than the same substances in the water column[60]. The main issue is that they act as a sink and source that stores and then constantly releases chemicals into the water over long periods of time[3]. In addition, Gilroy et al. (2012) demonstrated that the actions of microfauna can cause chemicals to be released back into the water column faster than expected. Analgesics, NSAIDs, antibiotics and psychiatric drugs have all been shown to be particularly well retained in sediment in high quantities relative to other pharmaceuticals[38]. Sediments are also known to contain higher concentrations that can be equivalent to dangerous concentrations recorded for freshwater bodies[3,38].

4 – Water quality and current UK legislation

Despite great strides in tackling diffuse and point source pollution from traditional sources such as agriculture, mine drainage, and the sewerage network, there is still much to do to tackle the growing number of novel pollutants. Water quality in the UK is reported separately in each country with England having the poorest freshwater status with 0% of freshwater in good overall health[83]. Northern Ireland (31.3%)[84], Wales (46%)[85] and Scotland (65.7%)[86] fare better but there are still significant numbers of watercourse where water quality is a concern.

4.1 Directives, schemes and policies addressing water quality

i. The EU Water Framework Directive was the first legislation of its kind to tackle water pollution in Europe. It was also the first piece of legislation to address emerging chemicals like pharmaceuticals. For chemicals not explicitly included in the priority chemical list, the WFD is, in essence, just a monitoring scheme. It sets goals for reducing pollution but leaves it to Member States to implement their own legislation. In the UK this is implemented through The Water Environment (Water Framework Directive) (England and Wales) Regulations 2003 in England and Wales, in Scotland it is implemented through The Water Environment and Water Services (Scotland) Act 2003, and in Northern Ireland through The Water Environment (Water Framework Directive) Regulations (Northern Ireland) 2003.

ii. The Chemical Investigation Programme is a UK wide monitoring scheme, delivered by the water industry, aimed at assessing the levels of contamination in waterbodies. It is based on WFD guidelines but is independent of the WFD. It covers several chemicals not included on the WFDs watch list or priority list.

iii. River basin management plans based on the WFD classification sampling are aimed at tackling significant water management issues like nitrate pollution[87,88]

iv. Catchment Sensitive Farming and Countryside Stewardship were established in 2006 in England to address diffuse agricultural pollution. In 2018 the Water Environment Grant was launched. These schemes are entered into on a voluntary basis and largely cover pollutants like pesticides and fertilisers.

v. The UK Government's 25 Year Environment Plan aims for 75% of waters to be in good condition by 2027.

4.3 Ecological toxicity standards

There are several methods used for measuring and evaluating chemical toxicity. Each has its own benefits and limitations and are briefly discussed below:

i. ECx – regression-based measurements that evaluate the level of effect on a given percentage of the population (e.g., EC50 means the concentration that affects 50% of test organisms, EC10 means 10% are affected). It allows for calculations of confidence intervals. Like LC50s, ECx values are dependent on exposure time and vary between species.

ii. LC50s – can be more straight forward because they purely focus on lethal effects which is an obvious, indisputable outcome. But LC50s for pharmaceuticals tend to be well above ecologically relevant concentrations and disregard the sub lethal impacts of low concentrations found in some pharmaceuticals[89,51]. LC50s can also vary with exposure time, where lower concentrations still cause death, but over a longer period of

exposure[90]. LC50s are not useful as an environmental toxicity standard for pharmaceuticals due to the unrealistically high concentrations required.

iii. No Observed Effect Concentrations (NOECs) / Lowest Observed Effect Concentrations (LOECs) are a simple, relatively straight forward metric to measure, but this simplicity can lead to misinterpretation. They vary between species even within the same order and can depend on the measure used to evaluate the "effect", and no observable effect does not always mean no effect at all. Effects can be expressed in other ways than the measured effect (usually growth or reproduction). NOECs and LOECs are generally regarded as unreliable tools for evaluating substance impact in the ecological community as they can be misleading if not used correctly and do not account for variability or concentration-response relationships. While not fully rejected, there has been a move towards regression-based evaluations.

iv. Predicted No Effect Concentrations (PNECs) are the concentration at which a substance is unlikely to cause long or short-term effects. They are derived from NOECs and/or ECx or LC50s. Several assessment factors often need to be calculated for a PNEC to be considered trustworthy.

v. Predicted environmental concentrations (PECs) are used together with PNECs to determine potential risk in the environment.

vi. Risk Quotients (RQs) are a ratio of environmental concentrations to PNEC. It is a combination of measurable standards and a standardised safety limit aiming to give an overall idea of how dangerous a chemical might be to a given environment. A Risk Quotient greater than 1 indicates that environmental harm may be possible and further evaluations are required for that chemical.

vii. Annual Average Environmental Quality Standards (AA-EQS) are the target concentrations a substance must stay below in a given year to minimise ecological impact and for a water body to be considered to be of "good" status[14] at the WFD classification point. EQS only exist for substances addressed in the WFD priority substance lists and are aimed at monitoring long term impact.

viii. Maximum Allowable Concentrations (MAC-EQS) is the maximum release concentration allowed. The standard is set by the EU as a measure for short term impacts and is mostly used for monitoring batch releases of waste. It generally only exists for WFD priority substances.

ix. Other risk assessment values exist (e.g., the Relative Hazard Index), but they are not widely used in practice.

5 – Summary of pharmaceuticals of concern commonly identified in freshwater

In this report we have identified 18 chemicals for which we explore the possible effect on freshwater invertebrates (table 1 and 2). Sixteen of these are found on the WFD watch lists or have been identified as a chemical of concern in CIP2 (Table 1). The other two (paracetamol and naproxen) are commonly identified in freshwater monitoring schemes and literature[91].

Table 1. Legislation and current standards for commonly recurring pharmaceuticals in freshwater. PNEC values are taken from EU and Environment Agency literature.as the standards currently in use. Concentration values highlighted in bold indicate where median or mean values exceed the safety limit.

Pharmaceutical name	Addressed in current (2020) UK legislation?	Official PNEC (ng/L)	CIP2 Upstream median (range) (ng/L)	CIP2 Downstream median (range) (ng/L)	Known freshwater concentration ranges in the UK in literature (ng/L)
STEROID HORMONES					
17-Alpha-ethinyloestradiol (EE2)	- CIP2 - Previously on the WFD watch list (2018)	0.1 [13]	0.015 (<0.015-3.36)	0.015 (<0.015-4.9)	**<0.4–3.4** [93]
17-Beta-oestradiol (E2)	- CIP2 - Previously on the WFD watch list (2018)	1 [13]	0.15 (<0.15-6.2)	0.15 (<0.15-11.9)	**<0.4–4.3** [93]
Oestrone (E1)	- CIP2 - Previously on the WFD watch list (2018)	3.6 [16] 3 [13]	0.3 (<0.3-54.2)	0.7 (<0.3-65.6)	**<0.4 to 12.2 (water column) 34-388 (sediment)**[93]
ANTIBIOTICS					
Erythromycin	- CIP2 - Previously on the WFD watch list (2018)	200 [13,16]	5 (<5-710)	50 (<5-780)	**2.5-1378** [12]
					<0.5 – 351 [94]
					<0.5-141 [94]
					<10-57 upstream of WWTWs <10-1022 downstream of WWTWs [95]
					32 – 790 [37]
Clarithromycin	- CIP2 - Previously on the WFD watch list (2018)	130 [13]	6 (<0.5-722)	64.5 (<0.5-1250)	5.7 – 500 ng/L [37]
Azithromycin	- CIP2 - Previously on the WFD watch list (2018)	19 [16] 90 [13]	0.1 (<0.1-184)	6.45 (<0.1-221)	**73** [37]
Amoxicillin	- WFD watch list (2020)	78 [16]	n/a	n/a	<10 – 622 94
Ciprofloxacin	- WFD watch list (2020)	89 [13,16]	*n/a*	*n/a*	*No UK examples found*

Pharmaceutical name	Addressed in current (2020) UK legislation?	Official PNEC (ng/L)	CIP2 Upstream median (range) (ng/L)	CIP2 Downstream median (range) (ng/L)	Known freshwater concentration ranges in the UK in literature (ng/L)
Sulfamethoxazole	- WFD watch list (2020)	400 [15]	*n/a*	*n/a*	<0.5-2 [94] <0.5-4 [94] <50 upstream of WWTWs <50-132 downstream of WWTWs [95] 10 – 35 [37]
Trimethoprim	- WFD watch list (2020)	500 [15]	*n/a*	*n/a*	<1.5-126 [94] <1.5-183 [94] <10-36 upstream of WWTWs <10- 42 downstream of WWTWs [95] 3.4 – 350 [37] <5-44.4 [96]
NON-STEROIDAL ANTI-INFLAMMATORY					
Diclofenac	- CIP2 - Monitored, but not restricted - Previously on the WFD watch list	50 [13]	3 (<1-277)	33 (<1-372)	**2.5-2990.7** [12] <0.5-85 [94] **<0.5-261** [94] <20 upstream of WWTWs **<20- 154 downstream of WWTWs** [95] 5.9 – 380 [37] <10-79.47 [96]

Pharmaceutical name	Addressed in current (2020) UK legislation?	Official PNEC (ng/L)	CIP2 Upstream median (range) (ng/L)	CIP2 Downstream median (range) (ng/L)	Known freshwater concentration ranges in the UK in literature (ng/L)
Naproxen	None	128,000 [49]	*n/a*	*n/a*	<0.3-146 [94] <0.3-113 [94] 27 – 150 [37] 4.85-44.4 [96]
Ibuprofen	CIP2	10 [13]	**17 (<2.5-2850)**	**37 (<2.5-6600)**	**12.5-4838** [12] **<0.3-100** [94] **<0.3-93** [94] **<20-155 upstream of WWTWs <20-5044 downstream of WWTWs** [95] **30 – 450** [37] **<2-38.4** [96]
ANALGESICS					
Paracetamol (acetaminophen)	None	9,200 [49]	*n/a*	*n/a*	<1-2382 [94] <1.5-1379 [94] 8.2 ng/L – 1200 [37]

Pharmaceutical name	Addressed in current (2020) UK legislation?	Official PNEC (ng/L)	CIP2 Upstream median (range) (ng/L)	CIP2 Downstream median (range) (ng/L)	Known freshwater concentration ranges in the UK in literature (ng/L)
BETA-BLOCKERS					
Propranolol	CIP2	100 [13,49]	1.7 (<0.1-280)	31.2 (<0.1-340)	2.5-165 [12]
					<10-115 upstream of WWTWs <10-215 downstream of WWTWs [95]
					6.5-67 [37]
ANTIDEPRESSANTS					
Fluoxetine	None	47 [13]	0.4 (<0.1-62.7)	6.5 (<0.1-86.9)	<5 [96]
					6.2-7.9 [97]
					9.0 (mean}, 13.5 (max) [98]
Venlafaxine (and O-desmethylvenlafaxine)	WFD watch list (2020)	38.35 [15] (6.1 [15])	*n/a*	*n/a*	35.1 (mean), 75.6 (max) [5,98]
					0.9-85.5 [97]
ANTIEPILEPTICS					
Carbamazepine	None	2500 [13]	4 (<0.5-1230)	58 (<0.5-1340)	<0.5 -356 [94]
					<0.5-684 [94]
					5.6 – 200 [37]
					16.4-555 [96]

Table 2. Substances and their known sub-lethal effects. This is a summary of examples of effects, it does not include events where no effect was found, and it is not an exhaustive list of all literature for all substances. Its aim is to provide examples of the variety of effects and the diversity of species they affect in freshwater. It is noted where little or no literature is available for a substance. Numbers in bold indicate values that occur below concentrations found in the natural environment

Pharmaceutical name	Recorded concentrations of sub-lethal effects (ng/L, unless otherwise stated)	Sub-lethal Effect	Freshwater invertebrate species affected	Citation
STEROID HORMONES				
17-Alpha-ethinyloestradiol (EE2)	10 to 10,000	Mouthpart deformities with the highest incidence occurring at low to intermediate concentrations 10 ng to 10,000 ng/L	Non-biting midge (*Chironomus riparius*)	32
	1,000,000	Moulting delayed and wet weight reduced.	Non-biting midge (*Chironomus riparius*)	32
	1 to 50	Increase in the number of emerging adults and altered sex ratio towards 2:1 males to females in *Chironomus riparius.* No effect on egg viability.	Non-biting midge (*Chironomus riparius*)	21
	>78	Delayed emergence above 78 ng/L.	Non-biting midge (*Chironomus riparius*)	21
	100 to 320	No effect on parent generation, followed by smaller male gnathopods and altered gonad development in subsequent generations.	Freshwater amphipod (*Hyalella azteca*)	100
	1 to 100	Increase in stimulation of reproduction between 1 ng/L and 25 ng/L, and reduction in reproductive stimulation at 100 ng/L.	Mud snail *Potamopyrgus antipodarum*	101
	3,290.3 and 17,600	Above 3,290 ng/L there is an increased number of eggs per clutch and an increase in abnormal egg production and at 17,600 ng/L decrease in number of egg clutches per individual.	Pond snail *Lymnaea stagnalis*	102
	100, 1,000 and 10,000	Alters sex ratio to 2:1 females to males. At 10,000 ng/L there is an increase in population size due to an increase in juvenile recruitment.	Freshwater shrimp (*Gammarus pulex*)	34
17-Beta-oestradiol (E2)	50 and 250 nmol injection and exposure to municipal waste effluent	Increased vitellogenin production, in injected and effluent exposed individuals. Effluent exposed individuals also exhibited reduced shell growth and weight, and increase soft tissue growth and weight but there is no way to attribute this effect to just one chemical.	Freshwater mussel (*Elliptio complanata*)	29
	>10000	Oxidative stress in the earthworm in soil exposed to sewage effluent.	Earthworm (*Eisenia fetida*)	103
Oestrone (E1)	*n/a*	No recorded effects in freshwater invertebrates but does readily convert into E2 in freshwater fish [104].	*n/a*	*n/a*
ANTIBIOTICS				

Pharmaceutical name	Recorded concentrations of sub-lethal effects (ng/L, unless otherwise stated)	Sub-lethal Effect	Freshwater invertebrate species affected	Citation
Erythromycin	>179,000	Erythromycin thiocyanate results in higher mortality, reduced reproduction, and impaired growth.	Water flea (*Daphnia magna*)	[105]
	940,000 (EC50)	Growth inhibition.	Rotifer (*Brachionus calyciflorus*)	[24]
	220,000 (EC50)	Growth inhibition.	Water flea (*Ceriodaphnia dubia*)	[24]
	110, 220 and 550	Effects on immune function: decreased thiol production, increased phagocytosis by haemocytes and decreased lysozyme activity.	Freshwater mussel *Elliptio complanata*	[106].
Clarithromycin	12,210,000 (EC50)	Growth inhibition.	Rotifer (*Brachionus calyciflorus*)	[24]
	8,160,000 (EC50)	Growth inhibition.	Water flea (*Ceriodaphnia dubia*)	[24]
Azithromycin	*n/a*	No literature found.	*n/a*	*n/a*
Amoxicillin	*n/a*	No effects observed in the Cnidarian, *Hydra vulgaris*, at concentrations of 10,000-10,000,000 ng/L which appears to be the only freshwater invertebrate tested for this substance.	*n/a*	[107].
Ciprofloxacin	>500,000	Indirectly alters food consumption, growth, and energy storage through changes in the microbial community, especially fungi. Fungi drive the response, but effects may be positive or negative for the shrimp depending on the composition of the microbial community.	Freshwater shrimp (*Gammarus fossarum*)	[108].
	1,100	Effects on immune activity: increased ROS production, decreased lysozyme activity and increased phagocytosis by haemocytes.	Freshwater mussel (*Elliptio complanata*)	[106]
Sulfamethoxazole	9,630,000 (EC50)	Growth inhibition.	Rotifer (*Brachionus calyciflorus*)	[24]
	210,000 (EC50)	Growth inhibition.	Water flea (*Ceriodaphnia dubia*)	[24]
	25,000,000: 5,000,000 Sulfamethoxazole:Trimethoprim combination	Increased susceptibility to insecticidal proteins. No effect on mortality with each antibiotic alone.	Black fly (*Simulium vittatum*)	[109]
	22, 110, and 550	Effects on immune activity: increased ROS production, decreased thiol production, increased lysozyme activity and cyclooxygenase activity.	Freshwater mussel (*Elliptio complanata*)	[106]
	633 to 25,328	Increase in immunotoxic responses.	Freshwater mussel (*Elliptio complanata*)	[110]

Pharmaceutical name	Recorded concentrations of sub-lethal effects (ng/L, unless otherwise stated)	Sub-lethal Effect	Freshwater invertebrate species affected	Citation
Trimethoprim	25,000,000: 5,000,000 Sulfamethoxazole:Trimethoprim combination	Increased susceptibility to insecticidal proteins. No effect on mortality with each antibiotic alone.	Black fly (*Simulium vittatum*)	109
	20, <22, 22 and 110	Effects on immune activity in freshwater mussel *Elliptio complanata*: increased ROS production, decreased thiol production, increased phagocytosis by haemocytes, and decreased lysozyme activity.	Freshwater mussel (*Elliptio complanata*)	106
	400,000,000	Decrease in larval activity over 96 hours.	Midge larvae (*Diamesa zernyi*)	111
	725 to 29,037	Immunotoxic responses.	Freshwater mussel (*Elliptio complanata*)	110
	50,000,000	Inhibition of regeneration.	Hydra (*Hydra attenuata*)	112
NON-STEROIDAL ANTI-INFLAMMATORY				
Diclofenac	**637**	Weak reduction in lysozyme membrane stability in haemocytes after 96hr exposure.	Freshwater zebra mussel (*Dreissena polymorpha*)	113
	60,000, 156,000 and 250,000	Genotoxic effects resulting in DNA damage with cytotoxic effects above 156,000 ng/L.	Freshwater zebra mussel (*Dreissena polymorpha*)	114
	1000, 10,000, 100,000, 1,000,000 and 10,000,000	Cytotoxicity in gill, blood, and gastric tissues, with toxicity increasing with length of exposure.	Freshwater zebra mussel (*Dreissena polymorpha*)	115
	500,000	This was the lowest concentration tested in the study. It caused a significant reduction in egg production.	Water flea (*Daphnia magna*)	90
	34,000	Decreased emergence ratio.	Non-biting midge (*Chironomus riparius*)	116
	>50,000	Delay in time to first egg production and alteration in gene expression related to growth, development, reproduction, and metabolism. Responses were time dependant, longer exposure at lower concentrations leads to the same responses as short exposure at high concentration.	Water flea (*Daphnia magna*)	117
	2,000,000 to 25,000,000	Increasing concentrations of diclofenac lead to reduction in population increase rates and decreases density.	Rotifer (*Plationus patulus*) and cladoceran (*Moina macrocopa*)	35
	100,000	Induces immune response shown as an increase in phagocytosis by haemocytes but does not affect immunocompetence.	Pond snail (*Lymnaea stagnalis*)	118

Pharmaceutical name	Recorded concentrations of sub-lethal effects (ng/L, unless otherwise stated)	Sub-lethal Effect	Freshwater invertebrate species affected	Citation
Naproxen	>26,000	Breakdown products reduce growth and reproduction and have greater toxic effects at lower concentrations than naproxen itself on small crustacea and rotifers.	Rotifer (*Brachionus calyciflorus*), Fairy shrimp (*Thamnocephalus platyurus),* Water flea *Ceriodaphnia dubia*)	44,50
	20,000	Reduces eclosion from eggs and pupation but does not affect larval mortality. Naproxen also reduces the mean fecundity of females, and subsequent generations appear to be more resilient to exposure. At concentrations over 164 mg/L emergence is reduced.	Mosquito (*Aedes aegypti*)	119
	560,000 (EC50)	Growth/reproduction inhibition.	Rotifer (*Brachionus calyciflorus*)	24
	330,000 (EC50)	Growth/reproduction inhibition.	Water flea *Ceriodaphnia dubia*)	24
	76,600,000 and 339,200,000 ng/kg in sediment	Oxidative stress and genotoxicity.	Amphipod (*Hyalella azteca*)	120
	575-2,302.6	Induction or inhibition of various immunotoxic effects in haemocytes.	Freshwater mussel (*Elliptio complanata*)	110
	40,000,000	Contraction of the body column and tentacles (a stress response) and alterations in gene transcription over 24 hours.	Hydra (*Hydra magnipapillata*)	121
	>1,100,000	Reduced fecundity.	Water flea (*Moina macrocopa*)	122
	300,000 – 30,000,000	Reduction in population growth over the range of concentrations and a reduction in fecundity at 30,000,000 ng/L.	Water flea (*Daphnia magna*)	122
	1,000,000, 5,000,000 and 10,000,000	Inhibition of regeneration.	Hydra (*Hydra attenuata*)	112
Ibuprofen	**>1**	Dual effect of increased ventilation (a sign of stress) at 1, 10 and 100 ng/L and decreased locomotion at all other concentrations measured.	Freshwater shrimp (*Gammarus pulex*),	19
	>1230000000 µg/L	Reduction in reproductive success.	Water flea (*Daphnia magna* and *Moina macrocopa*)	123
	100000000000	Initial increase followed by a decrease in larval activity over 96 hours.	Midge (*Diamesa zernyi*)	111
	45000, 450000, 909000	Genotoxic effects resulting in DNA damage and cytotoxic effects above 450,000 ng/L.	Freshwater zebra mussel (*Dreissena polymorpha*)	114

Pharmaceutical name	Recorded concentrations of sub-lethal effects (ng/L, unless otherwise stated)	Sub-lethal Effect	Freshwater invertebrate species affected	Citation
	400,000	Lowest concentration tested caused a significant reduction in egg production.	Water flea (*Daphnia magna*)	90
	5,000,000 and 10,000,000	Inhibition of regeneration.	Hydra (*Hydra attenuata)*	112
	200, 2,000 and 8,000	Cytotoxicity and genotoxicity, increasing in strength with concentration.	Freshwater zebra mussel (*Dreissena polymorpha*)	124
	100 to 50,000	Oxidative stress.	Freshwater clam (*Corbicula fluminea*)	125
	>1,020,000 and >5,360,000	LOEC for reduced growth and hatching rates, respectively.	Ramshorn snail (*Planorbis carinatus*)	126
ANALGESICS				
Paracetamol (acetaminophen)	160,000,000 and 800,000,000	Poorer development and flight indexes, with effect on flight dependant on arachidonic acid availability.	Mosquito (*Culex pipiens*)	127
	2,000,000 to 32,000,000	Increasing concentrations of paracetamol lead to reduction in population increase rates and decreases population density.	Rotifer (*Plationus patulus*) and water flea (*Moina macrocopa*)	35
	30,000, 150,000 and 450,000	Cytotoxic effects and genotoxic effects resulting in DNA damage at all concentrations.	Freshwater zebra mussel (*Dreissena polymorpha*)	114
	80,000	Lowest concentration tested caused a significant reduction in egg production.	Water flea (*Daphnia magna*)	90
	>12,200,000 (EC50)	Reduced population growth and number of neonates and an increase in total offspring at 26,700,000 ng/L.	Water flea (*Daphnia longispinosa*)	128
	>4,000,000	Reduced population growth.	Water flea (*Daphnia magna*)	128
	3,880 to 61,950	Oxidative stress after 28 days.	Freshwater clam *Corbicula fluminea*	129
BETA-BLOCKERS				
Propranolol	>2,593	Alters nerve response to light in skin tissue, mediated by serotonin.	Pond snail (*Lymnaea stagnalis*)	130
	100,000	Reduction in reproduction.	Amphipod (*Hyalella azteca*)	131
	250,000	Reduction in reproduction.	Water flea *Ceriodaphnia dubia*)	131
	50,000 to 800,000	Increases reproduction in *Daphnia magna* between 50,000 and 400,000 ng/L before decreasing reproduction at 800,000 ng/L.	Water flea (*Daphnia magna*)	132
	1 to 100	Increases reproduction.	Water flea (*Daphnia magna*)	89

Pharmaceutical name	Recorded concentrations of sub-lethal effects (ng/L, unless otherwise stated)	Sub-lethal Effect	Freshwater invertebrate species affected	Citation
	100,000,000 and 153,000,000	Significant change over 24 hours in production of various metabolites, amino acids, and other metabolic products, with significant change in concentration for 46% of metabolites.	Freshwater shrimp (*Gammarus pulex*)	133
ANTIDEPRESSANTS				
Fluoxetine	>89,000	Reduced reproduction.	Water flea (*Ceriodaphnia dubia*)	134
	>32,000 (NOEC = 470 and EC10 = 810)	Reduced reproduction and disrupted development.	Mud snail (*Potamopyrgus antipodarum*)	25
	20 and 200	Decreases oocyte and spermatozoan density and at 200ng/L alters oestradiol production. May induce gamete release.	Freshwater zebra mussel (*Dreissena polymorpha*)	26
	>10	Dual effect, increased ventilation (a sign of stress) at 10 and 100 ng/L and decreased locomotion at 10,000 and 1,000,000 ng/L.	Freshwater amphipod (*Gammarus pulex*)	19
	>300,000	In freshwater mussels (*Lampsilis fasciola* and *Lampsilis cardium)* it induces lure behaviour and release of nonviable offspring in females at 300,000 ng/L and 3,000,000 ng/L, increase male *Elliptio complanata* spawning at 3,000,000 ng/L. *Elliptio complanata* also bioaccumulates fluoxetine.	Freshwater mussel (*Lampsilis Fasciola, Lampsilis cardium* and *Elliptio complanata)*	61
	>3,700 to 100,000	Transgenerational impacts on reproduction and development. Fluoxetine alters hormone levels at 33,300 ng/L, decreases fecundity in the first generation at 100,000 ng/L, and increases size and length of time until first spawning in the second generation at 3,700 ng/L. The second generation are also more likely to die at 33,300 ng/L and 100,000 ng/L. The number of embryos found in the brood pouch increases at lower concentrations and decrease at higher concentrations. The same concentrations had no effect on the valve snail *Valvata piscinalis.*	Mud snail (*Potamopyrgus antipodarum*)	135
	1,000	Induces parturition.	Freshwater swan mussels (*Anodonta cygnea*)	136
	36,000	Increases fecundity.	Water flea (*Daphnia magna*)	22
	30,933	Increase in immunotoxic responses.	Freshwater mussel (*Elliptio complanata*)	110
	1	Increased phototactic behaviour.	Water flea (*Daphnia magna*)	89
	100 to 10,000,000	Reduced starvation tolerance and immobilisation at the highest concentration.	Water flea (*Daphnia magna*)	137

Pharmaceutical name	Recorded concentrations of sub-lethal effects (ng/L, unless otherwise stated)	Sub-lethal Effect	Freshwater invertebrate species affected	Citation
	100 and 1,000	Reduced response to light and stimulated aggregation.	Water flea (*Daphnia magna*)	138
Venlafaxine and O-desmethylvenlafaxine	**> 0.313**	Causes foot detachment in freshwater snails at concentrations as low as 0.313ng/L for *Leptoxis carinata* and 31.3 ng/L in *Stagnicola (=Lymnaea) elodes.*	Freshwater snail (*Leptoxis carinata*) and Pond snail (*Stagnicola (=Lymnaea) elodes*)	28
ANTIEPILEPTICS				
Carbamazepine	>234,000 ng/kg	Reduced emergence rate in non-biting midge.	Non-biting midge (*Chironomus riparius*)	36
	>100	Dual effect on increased ventilation (a sign of stress) at 100 ng/L and decreased locomotion at all other concentrations measured up to 1,000,000 ng/L.	Freshwater shrimp (*Gammarus pulex*)	19
	31,400	Reduced growth and altered sex ratio.	Non-biting midge (*Chironomus riparius*)	116
	1000, 10,000, 100,000, 1,000,000 and 10,000,000	Cytotoxicity gill, blood, and gastric tissues, with toxicity increasing with length of exposure.	Freshwater zebra mussel (*Dreissena polymorpha*)	115
	700 and 14,000	Immunotoxic effects including increased intracellular esterase activity, phagocytosis, and reduced haemocyte adherence.	Freshwater mussel (*Elliptio complanata*)	110
	100 to 50,000	Oxidative stress, cytotoxicity, and genotoxicity.	Freshwater clam (*Corbicula fluminea*)	125
	25,000,000	Increased regeneration.	Hydra (*Hydra attenuata*)	112
	1 to 1,000	Increased phototactic behaviour.	Water flea (*Daphnia magna*)	89
	100 to 1,000	Reduced response to light and stimulated aggregation.	Water flea (*Daphnia magna*)	138

5.1 Analgesics

Analgesics is the broad classification for painkillers which include some of the most used and available class of pharmaceuticals. NSAIDs are a specific subclass of analgesics which have been separated out in this report due to their specific properties and known environmental toxicity.

Paracetamol (also known as acetaminophen) is a widespread over-the-counter drug. Its effects in freshwater invertebrates are varied even in related species[128]. Effects range from reducing population growth to cytotoxic and genotoxic effects (Table 2). Similarly to NSAIDs, toxicity of paracetamol increases with time and concentration which results in LC50s being reached over longer periods of time at lower concentrations[90]. It is pseudo-persistent in waterways due to its high level of use. Paracetamol is sometimes wrongly classed as an NSAID in literature.

5.2 Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs are common, essential drugs, which are often found in chemical assessments of freshwater. NSAIDs are a class of analgesics that specifically inhibit the enzyme cyclo-oxygenase (COX) in vertebrate animals, resulting in anti-inflammatory effects. They can cause sub-lethal toxicity at environmentally relevant concentrations in freshwater indicator species (Tables 1 and 2). NSAID toxicity is time and concentration dependant, for example 21-day exposure at lower concentrations can reach 50% mortality for diclofenac at 2mg/L compared to 24 hours at 486mg/L[90]. The effects of NSAIDs (and paracetamol) in freshwater invertebrates have been reviewed by Parolini (2020)[139].

Diclofenac is a widely used drug that is commonly found in waterways globally and found in relatively high concentrations. It is slow to degrade and difficult to remove using standard water treatment. It has also been detected in groundwater and, to a lesser extent, in drinking water (reviewed in Heberer, 2002)[39]. Diclofenac is notoriously destructive for some avian species through bioaccumulation from their food sources[1,140]. Toxic effects in invertebrates have been recorded but are limited in frequency and species covered[139]. In some UK waterbodies, it is found at concentrations on average 3 or more times higher than the PNEC[12] (see table 1), yet lacks strict regulation.

Ibuprofen is almost ubiquitous in waterways globally. It is also poorly metabolised by humans resulting in 50% or more of the original compound being expelled from the body. While relatively fast to degrade in the environment, its high usage rates result in a virtually constant presence in freshwater. Recent research suggests that ibuprofen could have significant impacts on our waterways. For instance, in some fish, ibuprofen can cause lasting disruption to reproduction at levels as low as 100 ng/L[123]. For the few invertebrate species tested, measured effects for survival, growth and reproduction tend to occur above 1 mg/L[126], but most of these studies do not account for the disproportionately high levels of sub-lethal effects observed at low, ecologically relevant concentrations seen with other substances[19] or in combination with other drugs[52]. Algae, diatoms, and cyanobacteria tend to be far more sensitive to ibuprofen[38], which could result in secondary effects on invertebrates who rely on these biofilms.

Naproxen is commonly found in waterways, though to a lesser extent than diclofenac and ibuprofen[91]. Naproxen has the highest PNEC of all substances addressed in this report. Its toxicity is time and concentration dependant[121] and its breakdown products can be 4 to 16-fold more toxic than naproxen itself[44,50].

5.3 Antidepressants

Antidepressants are neurohormones that work by modulating the neurotransmitters serotonin, dopamine, and norepinephrine. The systems for these chemicals are evolutionarily ancient, with

both dopamine and serotonin also used by molluscs, crustaceans and even plants. Antidepressants are known to affect molluscs and crustaceans[20,26,51] which have been used as test organisms in neurological studies for more than 50 years. Serotonin and dopamine are known to influence:

- Egg maturation, spawning and other forms of reproduction in bivalves (serotonin increases, dopamine acts against serotonin induced spawning)[51].
- Reproductive behaviour in the pond snail (*Lymnaea stagnalis*) is reduced by serotonin and embryonic behaviour is altered[51].
- Reproductive behaviour in the ramshorn snail (*Biomphalaria glabrata*) is increased by serotonin[51].
- Larval development in freshwater and marine snails and nudibranchs is altered by serotonin and dopamine[51].

In crustacea, neurohormones control a variety of processes including:

- Increased reproductive development and hatching rates in various crayfish, crab, and shrimp species (Red swamp crayfish *Procambarus clarkii*, the White Pacific shrimp *Litopenaeus vannamei*, the freshwater giant prawn, *Macrobrachium rosenbergii*, the fiddler crab *Leptuca pugilator,* Black tiger shrimp *Penaeus monodon)*, while in other species it inhibits maturation (reviewed in Fong & Ford, 2014)[51].
- Dopamine reduces ovarian and testicular maturation in water fleas, fiddler crabs and red swamp crayfish (reviewed in Fong & Ford, 2014)[51].
- Serotonin influences phototaxis and geotaxis behaviour in various crustaceans, a function that is manipulated by acanthocephalan parasites to ensure their current host is eaten by their next host[20].
- Serotonin (but not fluoxetine) induces changes in aggressive behaviour in Noble crayfish, (*Astacus astacus*), causing fights to last longer[141].

The influence of antidepressants appears to be highest at lower concentrations, and effects vary between species[20,51]. Low concentrations of these substances naturally found in the environment may be more relevant to the impacts of these substances than LC50s and some current PNEC values.

Fluoxetine is one of the top 5 antidepressants prescribed in England and its use is steadily increasing each year[142]. It recurs in literature and is commonly detected in waterways. It has also been shown to have significant sub-lethal effects in crustacea, bivalves and gastropods (Table 2).

Venlafaxine is a commonly prescribed antidepressant that is listed on the 2020 WFD watch list. It can cause foot detachment in freshwater snails at concentrations as low as 313 pg/L, disrupting ecology and resulting in inadvertent migration for affected individuals[28]. This is well below the current PNEC value of 38.35 ng/L[15].

5.4 Antiepileptics

Carbamazepine is used to treat epilepsy, trigeminal neuralgia, schizophrenia, and bipolar disorder. It has been flagged as a chemical of concern due to its sub-lethal effects (Table 2) and its highly toxic break down products (in particular acridine, a known carcinogen and mutagen)[43]. It can cause malformations and reduced emergence rates in developing non-biting midges[36] as well as alter behaviour in water fleas[89,138]. In several cases it also appears to induce effects in invertebrates at concentrations below the PNEC of 2,500 ng/L[13] (Table 2).

5.5 Steroid hormones

Steroid hormones are thought to affect the development and reproduction of many different species of molluscs[101,143]. Metabolism of steroid hormones including testosterone, oestradiols, and progesterones by molluscs has been documented since the 1970s[144], so it is logical to think that these chemicals might have non-target effects outside of vertebrate animals. However, the effects of vertebrate steroid hormones in molluscs are highly debated and the necessary pathways and receptors for vertebrate steroid hormones are currently fragmented or entirely absent[31,144]. Scott (2013) also suggests that many studies on endocrine disruption by oestrogens in molluscs are not up to standard, and that it is unlikely that vertebrate steroid hormones directly affect the reproductive system of molluscs in any way. Currently, conflicting evidence alongside the absence of complete endocrine pathways leave direct impacts on reproduction and endocrine disruption up for debate[31,143]. However, it is still feasible that exposure to oestrogens leads to malformations[145] or cause toxic effects on other systems that indirectly lead to changes in reproduction[102]. Oestrogens (E1, E2 and EE2) are known to feminise male fish at concentrations as low as 1 ng/L[146]. At similarly low levels in invertebrates, EE2 alters sexual development and general growth in molluscs, crustacea and insects, with transgenerational effects in several species[21,100,143]

5.6 Antibiotics

Antibiotics are common in our rivers, relatively stable in the environment and are capable of bioaccumulation in invertebrates. They are used in human medicine, livestock care and in fish farming. They are a growing focus of freshwater monitoring programmes - the only four pharmaceuticals on the current WFD watch list are antibiotics.

Antimicrobial resistance is a particular cause for concern. Bacteria need a constant presence of antibiotics in the environment to develop and maintain resistances. In most cases this occurs in healthcare situations or intensive farming where there is constant use. Otherwise, resistance can occur in bacteria living in people and animals who do not complete courses of antibiotics. If antibiotics are present in the environment, it is more likely for multiple resistances to occur in bacteria outside high use settings at greater frequency.

Several antibiotics are addressed in this review:

- Macrolide antibiotics (erythromycin, clarithromycin, azithromycin) are commonly prescribed and pervasive in the environment. Macrolides are considered to be the most environmentally toxic antibiotics, especially erythromycin[24,105].
- Trimethoprim and sulfamethoxazole are two antibiotics often used together to treat various infections.
- Amoxicillin is a beta-lactam antibiotic used in the treatment of various infections in humans and animals.
- Ciprofloxacin is a fluoroquinolone antibiotic and a last resort antibiotic for treating serious infection.

It has been suggested that length of exposure, rather than increases in concentration, is the major factor in chronic toxicity effects[147].

Some studies suggest that antibiotics are more detrimental to photosynthetic species when compared to crustaceans and rotifers. For example, for the algae *Pseudokirchneriella subcapitata* the EC50 for growth inhibition was 0.02 and 0.002 mg/L for erythromycin and clarithromycin respectively compared to 0.22 mg/L and 8.16 mg/L in the crustacean *Ceriodaphnia dubia*[24].

There is evidence that antibiotics disrupt microbial communities and alter the ecosystem services they can provide, including the breakdown of other pollutants[148]. This is likely the most important route of effect for invertebrates because changes in the microbial community will change availability of nutrients for detritivores. Ciprofloxacin for example appears to have little to no effect on freshwater macroinvertebrates, but significantly disrupts the fungal community. This changes the quality of diet of freshwater shrimps and affects their feeding and growth rates[108]. The authors suggest that whether the effect is positive or negative on the growth and food consumption of the shrimp likely depends on the microbial community which varies with time and location. In studies for ciprofloxacin (the only antibiotic addressed in this report with these data) impacts are usually found in the microbial community, but not in the macroinvertebrate community of streams, and effects are usually found at relatively high concentrations[108,148–150]. Ciprofloxacin has also been observed to disrupt nematode communities in marine sediments[151].

One area for concern for insects aside from immediate toxic and chronic effects is the impact on bacterial communities that live inside them. Insect microbiota are increasingly recognised as an incredibly important aspect of insect biology. An estimated 50% of insects are thought to harbour symbiotic bacteria that live in their tissues and can be inherited between generations[152]. These bacteria can play hugely important roles in regulating reproduction, protection from natural enemies, or processing of nutrients[153–156]. Most knowledge of symbiotic bacteria comes from terrestrial invertebrates because freshwater invertebrates currently lack the breadth and depth of research into their symbionts. However, recent studies suggests that symbiotic bacteria are similarly common in freshwater. Several species of deronectid water beetle, midge, dragonfly, and damselfly have all been found to harbour symbiotic bacteria though their purpose is not yet known[157–159].

In research, antibiotics like rifampicin and tetracycline are routinely used to cure insects of their bacteria to investigate their effects[160]. In some cases, a lack of symbiont can result in sterilisation, accelerated death rates from the absence of vital nutrients or, in one case, the restoration of the missing sex in asexual species[161–163]. These methods usually use relatively large doses that are not relevant to environmental concentrations, unless they are aiming for a partial cure, and specifically use antibiotics that affect the insect host as little as possible.

Despite this, very little literature exists on the effects of antibiotics on invertebrates in general, especially for freshwater invertebrates. The studies that do exist all use concentrations well above what is found naturally in the environment. It is possible that the direct impacts on invertebrates at low concentrations are negligible, as observed for ciprofloxacin, but unfortunately for most substances there is not enough literature available to conclude either way.

5.7 Beta-blockers

Beta-blockers can interact with molluscan hormone systems. They work by interfering with the receptors of neurological hormones involved in β-adrenergic signalling pathways.

Propranolol is a beta-blocker commonly found in freshwater that is capable of blocking serotonin sites and interfering with β-adrenergic signalling pathways in molluscs[164]. The β-adrenergic and serotonin pathways have several vital roles in development, behaviour, and reproduction in invertebrates[51,130,131,165]

6 – Examination of CIP2 data

6.1 – Methodology

Selection of pharmaceuticals.

The 18 pharmaceuticals discussed in this review were based on their inclusion on WFD watch lists, whether they were considered a potential risk in CIP2, or their frequency of occurrence in scientific literature. Of the 18 substances discussed in the review, 12 were included in CIP2 sampling alongside the metabolites of two of the substances (Table 3). These 14 substances were examined for their patterns of prevalence in the environment from available CIP2 data.

Table 3. the 12 pharmaceuticals and two metabolites addressed in this section

Class	Pharmaceuticals	Metabolites
Antibiotics	Azithromycin Clarithromycin Erythromycin Ciprofloxacin	Norerythromycin
Beta-blockers	Propranolol	
Steroid hormones	Oestrone (E1) 17-beta oestradiol (E2) 17-alpha ethinyloestradiol (EE2)	
Non-steroidal anti-inflammatory	Diclofenac Ibuprofen	
Anti-epileptic	Carbamazepine	10,11-epoxycarbamazapine
Anti-depressant	Fluoxetine	

Chemical concentration data

Chemical concentration values were obtained from the UK's second chemical investigation programme (CIP2). This programme addressed the chemical concentrations in WWTW processes and receiving waters. Differences in data quality are discussed below. CIP2 specifically targeted treatment plants where there were concerns, to pinpoint problem plants and examine worst case scenarios. As such, it does not necessarily reflect UK waters or WWTWs as a whole.

Data for England and Wales was retrieved from the UKWIR data portal[166], and Scottish data was obtained on request from Scottish Water. Technical details of data collection for UKWIR CIP2 can be found in Combers et al (2018) and UKWIR (2018), or on the UKWIR data portal website[166]. CIP2 data collection in Scotland followed the same methods but adopted different minimum required Levels of Detection (LOD) and carried out data collection in a different timeframe.

Data was filtered for WWTWs with more than 100 datapoints per sampling location to remove sites that were not sampled for the full duration of the study. Across the combined datasets there was an average of 502 data points per pharmaceutical in waterbodies and an average of 1,460 data points per pharmaceutical in WWTW samples.

Scotland CIP2 data covered 20 WWTWs and consisted of 6,556 and 6,919 data points for influent and effluent values respectively; 6,690 and 6,934 for upstream and downstream data; and an average of 484 for individual pharmaceuticals per sample location. Further descriptive data can be found in Table 4.

The number of datapoints per WWTW covered in UKWIR data varied between pharmaceuticals at 45 (EE2), 50 (E1 and E20), and 51 (all other substances) with a total of 13,147 and 13,101 for influent and effluent values, respectively. The full data set for upstream and downstream pharmaceutical concentrations will not be available until March 2022, so unfortunately this cannot be included in these analyses.

UKWIR CIP2 data has previously been assessed for removal efficiencies of WWTWs in England and Wales by Comber et al (2018)[17]. Comber et al (2018) also attempted to predict instances where wastewater treatment plants might lead to downstream contamination from influent and effluent concentrations compared with available wastewater dilution data. They do not assess the upstream and downstream data itself due to these data being unavailable. These analyses were also examined in the UKWIR report[13].

Concentrations across sample locations were compared in relation to the PNEC values listed in Table 1. Concentration measurements were classed as 'exceeding the limit' if the concentration was more than the PNEC. Where two or more values exist for a PNEC in policy reports and legislation, the lowest is used.

Statistical analysis was applied to all CIP2 Scotland data and to influent and effluent data for UKWIR CIP2. Data was non-normal and over-dispersed, so a GLM with a negative binomial link function was applied with stats model[167] in python 3.7[168]using the minimum effective model of:

$$'GLM' = 'Concentration' \sim C('Sample\ Location')$$

Concentrations upstream and downstream of WWTWs for all available data were directly compared to visualise the influence of WWTWs on pharmaceutical concentrations in freshwaters. All data is from the Scottish data set, and was filtered for upstream and downstream

concentrations collected on the same day then marked for breaches of the safety limits. Concentrations were plotted against each other with Seaborn[169].

Influent and effluent concentrations in Scottish and UKWIR data was compared at each WWTW for each pharmaceutical to examine the variation in removal success and to assess the number of breaches of PNECs across the UK. PNEC would normally be measured at the WFD Classification sampling point so these results will show a worst-case scenario of water quality. The average of each WWTW influent and effluent concentration was plotted against each other.

Level of Detection (LOD) varies between the two data sets. Each data set was treated separately according to their own LODs during cleaning and preparation. Any value marked as falling below the LOD was taken to be half the LOD for data analysis purposes. Where data is combined, the lowest LOD is indicated on figures. Unfortunately, these LOD values do not always reflect the true LOD values and in some cases appear to be substantially higher than those used by the laboratory that tested the water samples. Original LOD values used in CIP2 can be found in Appendix 1.

Risk quotients

Maps were drawn in python using geopandas and matplotlib[168,170,171]. UK river basin boundaries were obtained from each country's respective websites[172–174].

Risk quotients for each substance at a given sample location was calculated from median concentration values for each WWTW, divided by the PNEC. For each substance, the risk quotients for each WWTW were plotted on a map of the UK.

6.2 – Results

CIP2 Scotland data demonstrated a general pattern of concentrations of: influent>effluent> downstream>upstream (table 4). UKWIR CIP2 followed similar trend for effluent and influent.

Full resolution images of figures included in this report can be found at https://doi.org/10.6084/m9.figshare.16628968.v2.

Analysis of monitoring data: Upstream vs Downstream

The change in concentration between upstream and downstream values indicates the level of impact WWTWs have on surface water contamination. In the Scottish data, the increase in concentration between upstream and downstream shows that WWTWs contribute significantly to increased downstream pollution (though not always above the PNEC value) for 7 of 14 substances ($p = 0.005$ to $p<0.001$ for each): clarithromycin, erythromycin, ibuprofen, diclofenac, carbamazapine, 10,11-epoxy-carbamazapine, propranolol (see Figure 3).

The impact of WWTWs is further illustrated in Figure 5, which shows that the concentrations downstream of WWTWs tend to be higher than upstream values on the same day, and are more likely to exceed the PNEC. This agrees with predictions from UKWIR effluent data by Comber et al. (2018) that WWTWs can pose a significant risk of downstream contamination for chemicals like ibuprofen and diclofenac.

Some chemicals do not follow the trend of increasing concentrations between upstream and downstream. Azithromycin shows a strong correlation for high concentrations occurring downstream (Figure 5), but is only very weakly higher than upstream values ($p = 0.088$). E1, E2, EE2 and norerythromycin show no statistical difference between downstream values and upstream concentrations suggesting WWTWs have little impact on surface water concentrations for these substances.

Analysis of monitoring data: Influent vs Effluent

Removal rates are poor in many examples and vary enormously between WWTW and substance and in several cases, pharmaceuticals increase through some WWTWs (Figures 3, 4 and 6). Across all Scottish samples, the median concentration for carbamazepine and norerythromycin increases between the influent and effluent (Figure 3, table 4). The slight but significant increase in carbamazepine from a median of 304 ng/L in influent to 429 ng/L in effluent agrees with recent literature on similar small increases in this chemical through municipal wastewater treatment[175]. In the UKWIR data, epoxy-carbamazepine shows a significant overall decrease while carbamazepine shows no significant change (Figure 4).

At several individual WWTWs across the UK we also see increases in steroid hormone and propranolol concentrations (Figure 6). Norerythromycin and the three steroid hormones are known breakdown and transformation products of erythromycin and steroid hormones. Different conditions and bacteria will breakdown and alter pharmaceuticals in different ways, so variations in WWTW function may favour the production of certain products. This may explain the increases in substances through some treatment plants and reductions through others.

Table 4. Summary data for all data for each substance in Scottish and UKWIR datasets

Scotland CIP2 Data	Downstream				Upstream				Influent				Effluent			
Pharmaceutical	Count	Median (ng/L)	25th percentile (ng/L)	75th percentile (ng/L)	Count	Median (ng/L)	25th percentile (ng/L)	75th percentile (ng/L)	Count	Median (ng/L)	25th percentile (ng/L)	75th percentile (ng/L)	Count	Median (ng/L)	25th percentile (ng/L)	75th percentile (ng/L)
10,11-epoxy-Carbamazepine	**488**	31.6	5.25	69.325	**469**	0.1	0.1	12.3	**481**	193	56.7	369	**490**	276	143.25	410.75
17-alpha ethinyloestradiol (EE2)	**527**	0.015	0.015	0.05	**510**	0.015	0.015	0.015	**511**	0.15	0.15	0.35	**525**	0.12	0.05	0.29
17-beta oestradiol (E2)	**508**	0.15	0.15	0.425	**487**	0.15	0.15	0.15	**424**	10.1	5.575	15.625	**500**	0.4	0.15	1.7
Azithromycin	**489**	6.4	2	16.4	**472**	0.1	0.1	1.525	**482**	70.15	8.375	181.75	**489**	67.7	23.8	158
Carbamazepine	**489**	56	15	132	**472**	3	0.5	26	**482**	304	131	536.5	**489**	429	221	666
Ciprofloxacin	**481**	1	1	5	**464**	1	1	1	**470**	196.5	58.5	469	**483**	28	11	56.5
Clarithromycin	**489**	62	26	131	**472**	6	0.5	32.25	**481**	409	130	851	**489**	475	238	739
Diclofenac	**489**	32	12	70	**472**	3	1	15	**480**	332.5	133	644.25	**489**	208	119	340
Erythromycin	**489**	50	10	100	**472**	5	5	20	**456**	315	90	742.5	**488**	370	140	660
Fluoxetine	**489**	6.5	3	14.2	**472**	0.4	0.1	2.025	**482**	121	62.5	197	**490**	66.65	34.1	107.75
Ibuprofen	**492**	37	14.75	106.5	**476**	16.5	2.5	46	**487**	8,510	4,325	13,300	**492**	66.5	7	384.25
Norerythromycin	**488**	1	1	3.25	**471**	1	1	1	**407**	1	1	1	**482**	16	5	33
Oestrone (E1)	**527**	0.7	0.3	2.2	**509**	0.3	0.3	0.7	**432**	21.9	11.7	33.325	**523**	3.3	0.7	12.6
Propranolol	**489**	31.2	13.5	63.4	**472**	1.75	0.1	12.85	**481**	187	93.3	317	**490**	249.5	161.25	366.75
TOTAL	**6,934**				**6,690**				**6,556**				**6,919**			

UKWIR CIP2 data	Influent				Effluent			
Pharmaceutical	Count	Median (ng/L)	25th percentile (ng/L)	75th percentile (ng/L)	Count	Median (ng/L)	25th percentile (ng/L)	75th percentile (ng/L)
10,11-epoxy-Carbamazepine	**971**	150	50	360	**992**	110	50	263.5
17-alpha ethinyloestradiol (EE2)	**574**	0.37	0.24	0.54	**646**	0.13	0.07	0.25
17-beta oestradiol (E2)	**947**	13.9	10	20	**728**	0.3	0.15	1.4
Azithromycin	**970**	250	118	510	**993**	200	88.7	369
Carbamazepine	**971**	501	308	780	**993**	610	394	810
Ciprofloxacin	**961**	570	240	1290	**981**	80	32	200
Clarithromycin	**971**	900	433.5	1680	**993**	360	190	650
Diclofenac	**972**	450.4	261.5	730	**992**	290	173.75	420
Erythromycin	**965**	580	305	980	**990**	330	150	530
Fluoxetine	**971**	80	50	130.5	**993**	44.5	30	70
Ibuprofen	**968**	16,450	10,300	23,125	**988**	20	5	310.75
Norerythromycin	**967**	50	26.5	80	**991**	50	25	50
Oestrone (E1)	**969**	40	25	56	**828**	5	1	12.045
Propranolol	**970**	224.5	130	380	**993**	162	110	234
TOTAL	**13,147**				**13,101**			

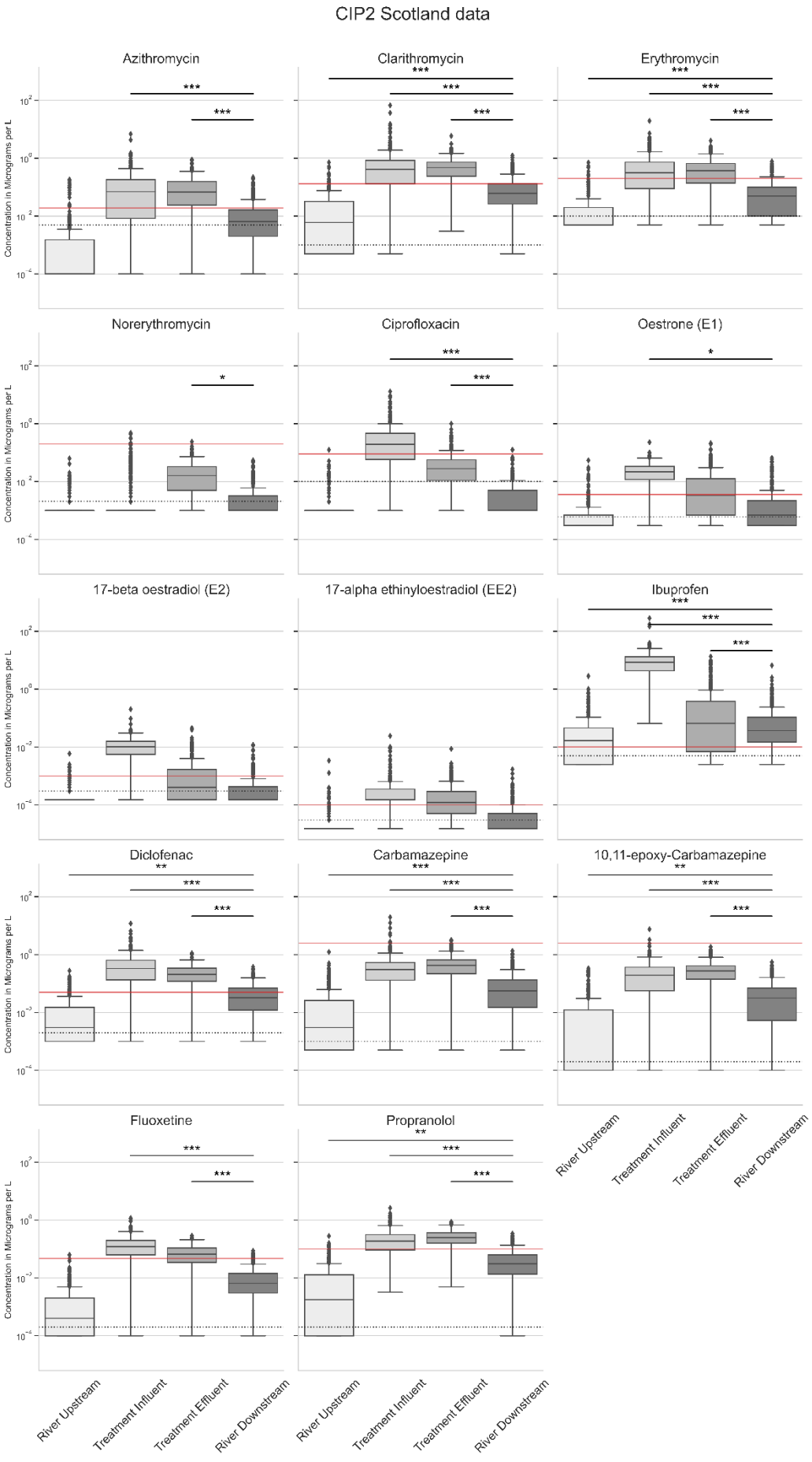

*Figure 3. Concentration across all values in micrograms per litre on a logarithmic axis for each sampling location (upstream, influent, effluent and downstream) and pharmaceutical. Each group has an average of n=500. specific n number can be found in Table 4. Significance values for a GLM with a negative binomial link function are plotted as follows: *** = p<0.001, **=p<0.01, *=p<0.05. Limit values are shown as follows: red solid line = PNEC and black dotted line = LOD. Box plots illustrate the median (centre line), the interquartile range (the box), the maximum and minimum values that are within 1.5 times the interquartile range (the whiskers), and outliers that are outside 1.5 times the interquartile range (diamonds).*

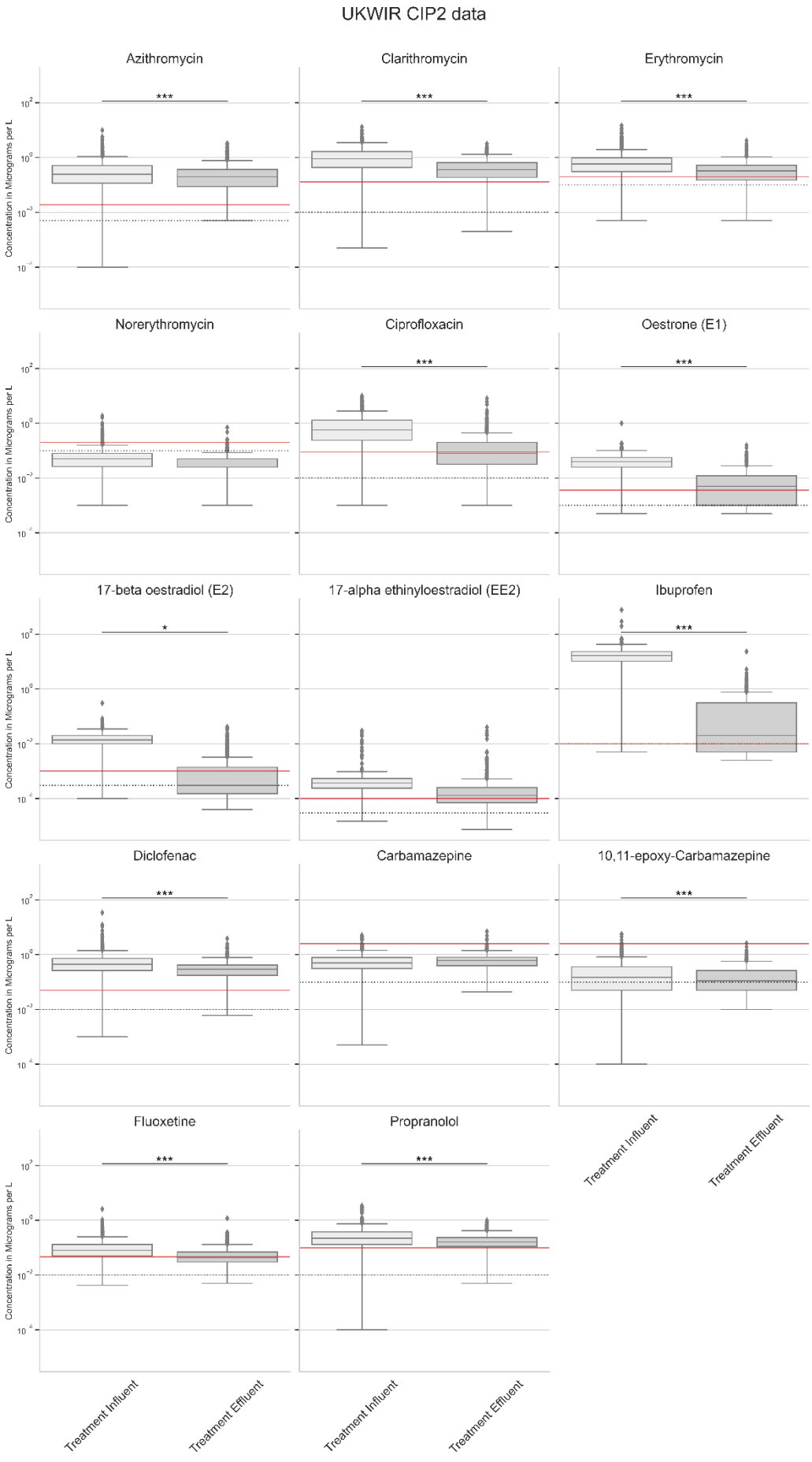

*Figure 4. UKWIR CIP2 Concentration across all values in micrograms per litre on a logarithmic axis for each sampling location and pharmaceutical. Average Sample size for influent/effluent is n=937.Significance values for a GLM with a negative binomial link function are plotted as follows: *** = p<0.001, **=p<0.01, *=p<0.05. Limit values are show as follows: red solid line = PNEC black dotted line = LOD. Box plots illustrate the median (centre line), the interquartile range (the box), the maximum and minimum values that are within 1.5 times the interquartile range (the whiskers), and outliers that are outside 1.5 times the interquartile range (diamonds).*

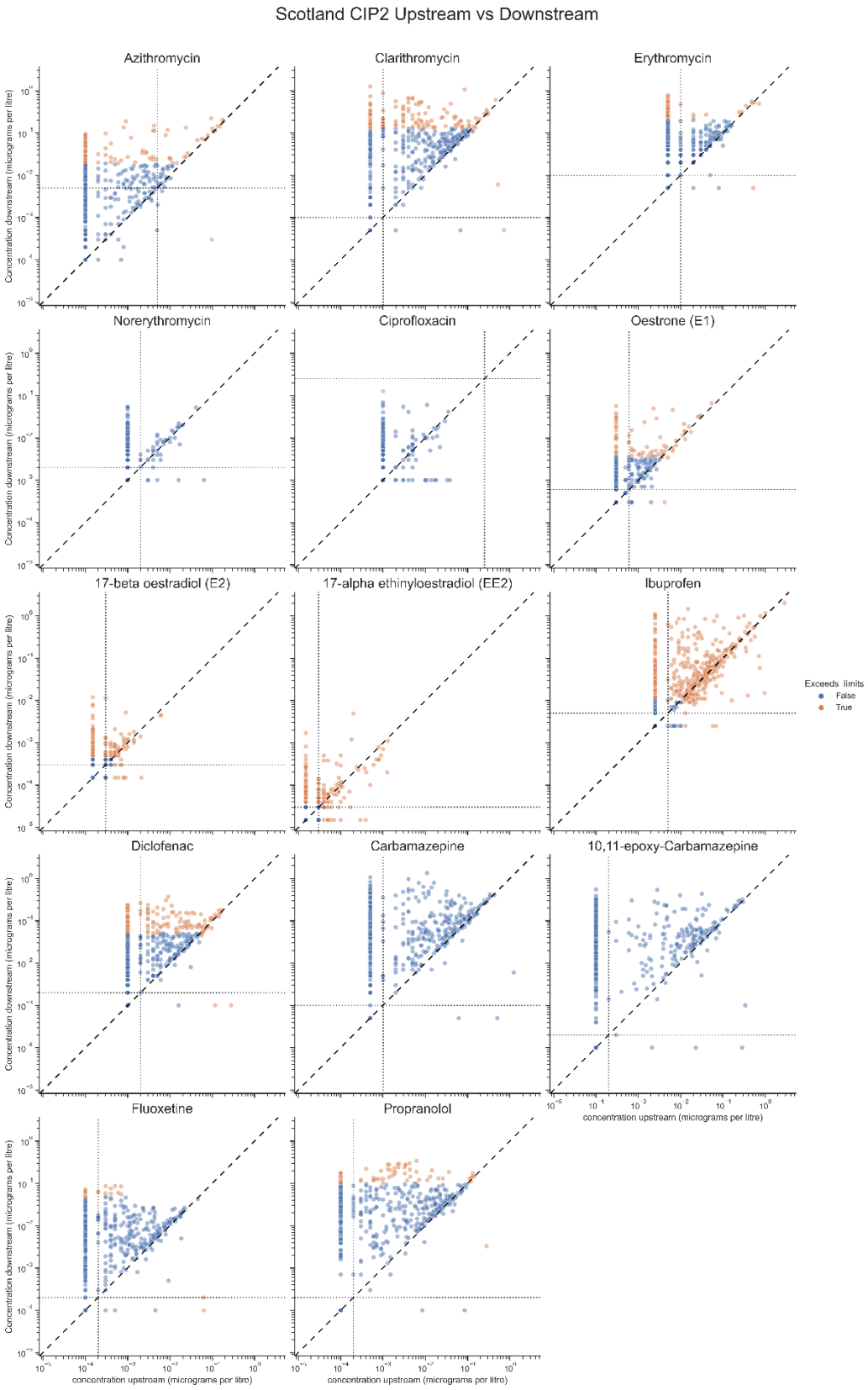

Figure 5. Correlation of upstream and downstream concentrations for Scotland CIP2 samples (average n = 500). The diagonal dotted line indicates the expected line of fit if there were no difference between them. Above this line indicates greater contribution by the WWTW, below this line indicates greater contribution by an upstream source. Each point represents an up and downstream concentrations at a given WWTW on the same day. Orange indicates values where the up or downstream value is higher than the PNEC for that chemical. The black dotted line indicates the LOD provided for Scottish data.

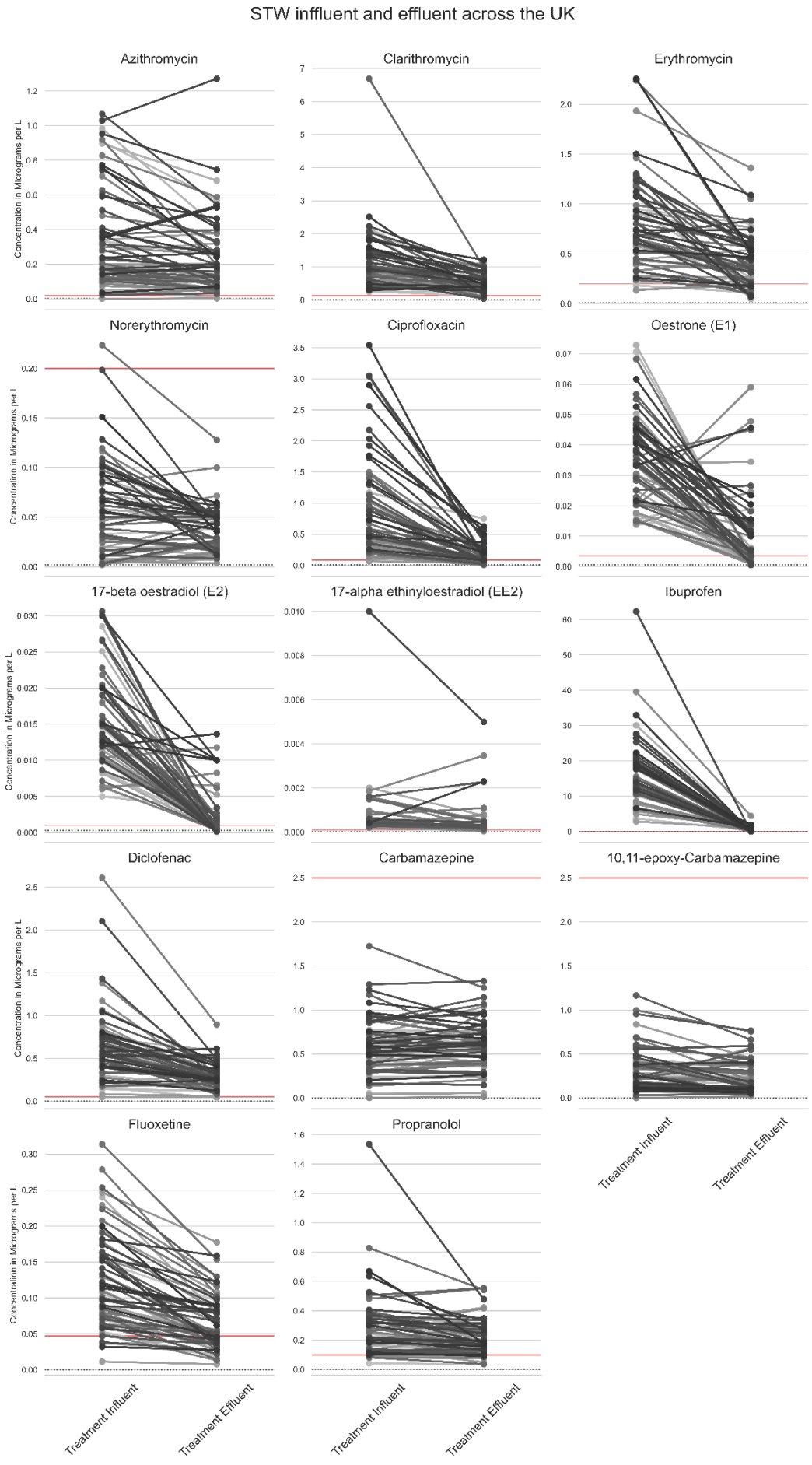

Figure 6. Changes in mean concentration (micrograms per L) between influent and effluent for all 71 WWTWs across the UK. Limit values are show as follows: red solid line = PNEC and black dotted line = LOD.

Analysis of monitoring data: Influent and Downstream concentrations in CIP2 Scotland data

For E1, upstream and effluent concentrations are similar to downstream concentrations. E1 is naturally excreted by humans and other animals, however it is also a transformation product of biologically produced E2 and the synthetic EE2. Only influent concentrations are significantly higher than downstream concentrations ($p=0.011$). This indicates some success in removal, transformation, or dilution of contaminants to levels similar to environmental concentrations before release in effluent. However, concentrations for E1 also regularly exceed safety limits by more than 52% in effluent samples, so this similarity is not a marker of success.

Analysis of monitoring data: Effluent and Downstream concentrations in CIP2 Scotland data

Eleven of 14 substances exceeded environmentally safe limits in 35% to 94% of effluent samples (Figure 7). The highest being Diclofenac (94%) followed by Azithromycin (91%) and Clarithromycin (82%). 10 of 14 substances exceeded environmental safe limits in 3% to 84% of downstream samples, with a top three of ibuprofen (84%), diclofenac (34%) and EE2 (31%). Ibuprofen is the only substance to increase frequency of breaches between effluent and downstream, rising from 62% to 84% of all samples exceeding the PNEC (Figure 7).

Eleven of 14 chemicals in both CIP2 UKWIR and CIP2 Scotland show significant reductions in concentration between effluent and downstream waters (GLZM, $p=0.21$ to $p<0.001$) which indicates another source of removal. This could be the result of dilution in the river itself, adsorption of the chemical into sediment, or further breakdown of the chemical in the natural environment.

The three steroid hormones (E1, E2, EE2) show no statistical difference between downstream values and either effluent or upstream concentrations in Scottish data suggesting that WWTWs have negligible contribution to downstream concentrations of these substances. However, E1, E2, EE2 often exceed safety limits in effluent (52%, 35% and 69% of samples) and downstream waters (15%, 25% and 31% of samples) (Figure 7). The similarity of concentrations in each sampling location and the frequency of breaches suggest that concentrations are often very close to exceeding the safety limit.

Norerythromycin is a metabolite whose effluent values are higher than downstream values (GLZM, $X^2_{wald} = 2.307$ df = 3, $p = 0.021$). Downstream values do not differ strongly from upstream and influent values ($p = 0.575$ and $p = 0.090$ respectively). This suggests that the concentrations of Norerythromycin increase though the WWTW during the breakdown of parent compounds, but that this does not strongly impact downstream concentrations. The median concentration of norerythromycin increases through Scottish WWTWs from 1 ng/L to 16 ng/L (table 4, figure 3), but the same trend is not seen in UKWIR data where the median remains at 50ng/L through the WWTW (Table 4, Figure 4). Understanding the reasons for this difference are beyond the limits of the data in this study but could be due to underlying chemical processes or differences in pharmaceutical usage over time.

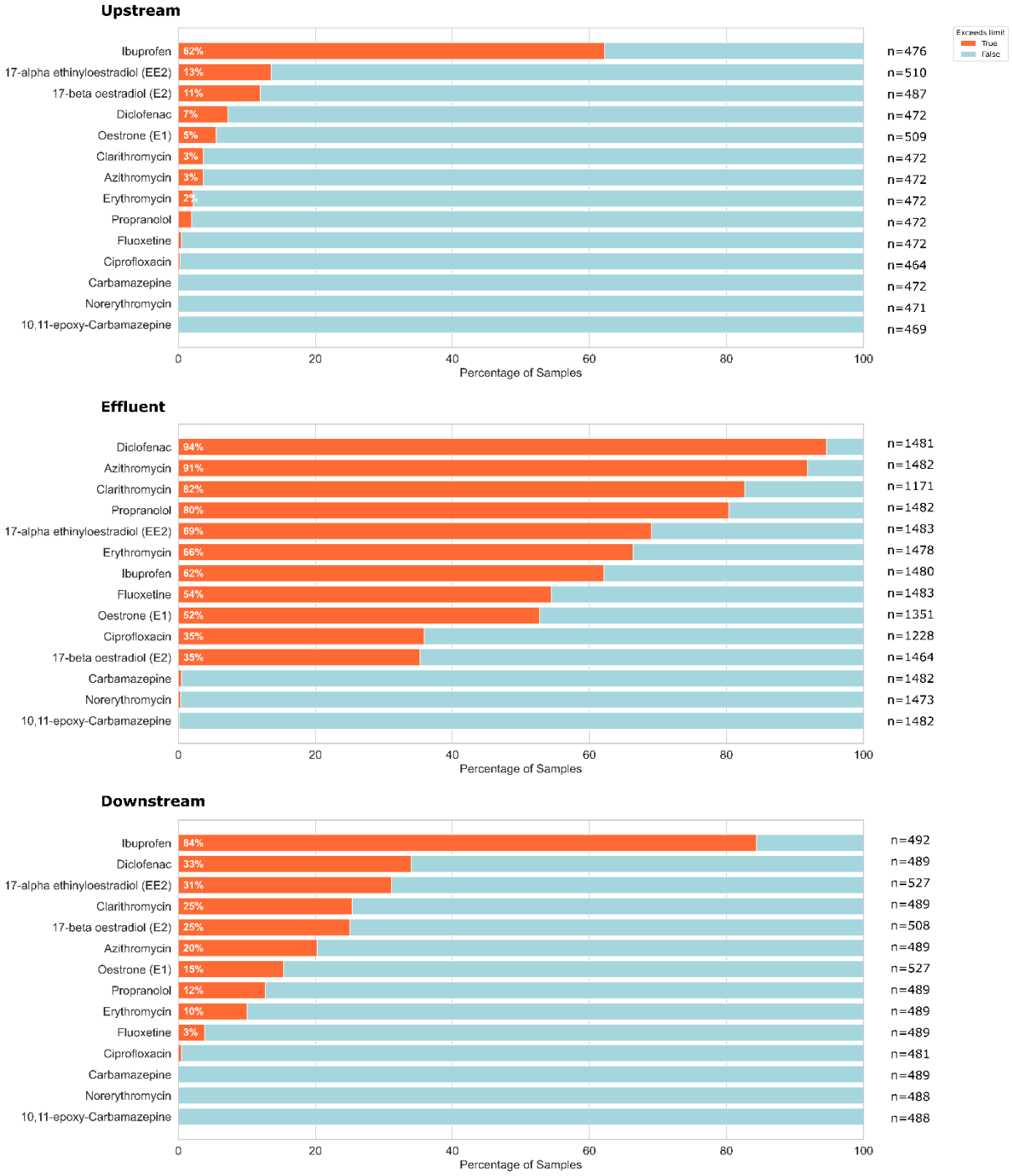

Figure 7. Percentage of effluent, upstream and downstream concentration values that exceed the PNEC for a given pharmaceutical across all UK samples. Downstream and upstream are CIP2 Scotland data, please see table 4 for sample sizes for individual data sets.

Analysis of monitoring data: the questionable successes

WWTWs contribute to significantly higher carbamazepine levels in downstream waters in CIP2 Scotland, though they remain below the current PNEC (Figure 5). Carbamazepine does occasionally breach the PNEC (Figures 3 and 4) but only in effluent at individual WWTWs (Figure 4). However, the effects of carbamazepine on invertebrates have been observed below the PNEC (table 2) so this should not be regarded as a "safe" level for this substance.

The two breakdown products, norerythromycin and 10,11-epoxycarbamazepine, also rarely exceed safety levels across sampling locations. However, their safety limits are those of their parent compound. Breakdown products are often more toxic than parent compounds and little literature exists on their impacts, so these values should be considered with caution.

Risk quotients by location

RQs lower than 1 indicate that PNEC values are not exceeded. The number of instances resulting in risk quotients (RQs) of more than one in effluent is dispersed across the UK (figure 8 and 9). In several cases there are RQs much greater than 10 and very few fall below 1 (figure 8 and 9). These high values are not associated with particular river basins or countries, leading to the conclusion that pharmaceutical contamination in effluent is a nationwide issue.

Downstream RQs for Scotland show that contamination is diluted out or otherwise removed in the receiving waters resulting in an RQ of less than 1 in many instances (figures 10 to 12). However, 19 of 20 sites have an RQ higher than 1 for ibuprofen. The next lowest is diclofenac with 6 of 20 exceeding 1, followed by E2 and EE2 with 5 of 20, then clarithromycin with 4 of 20.

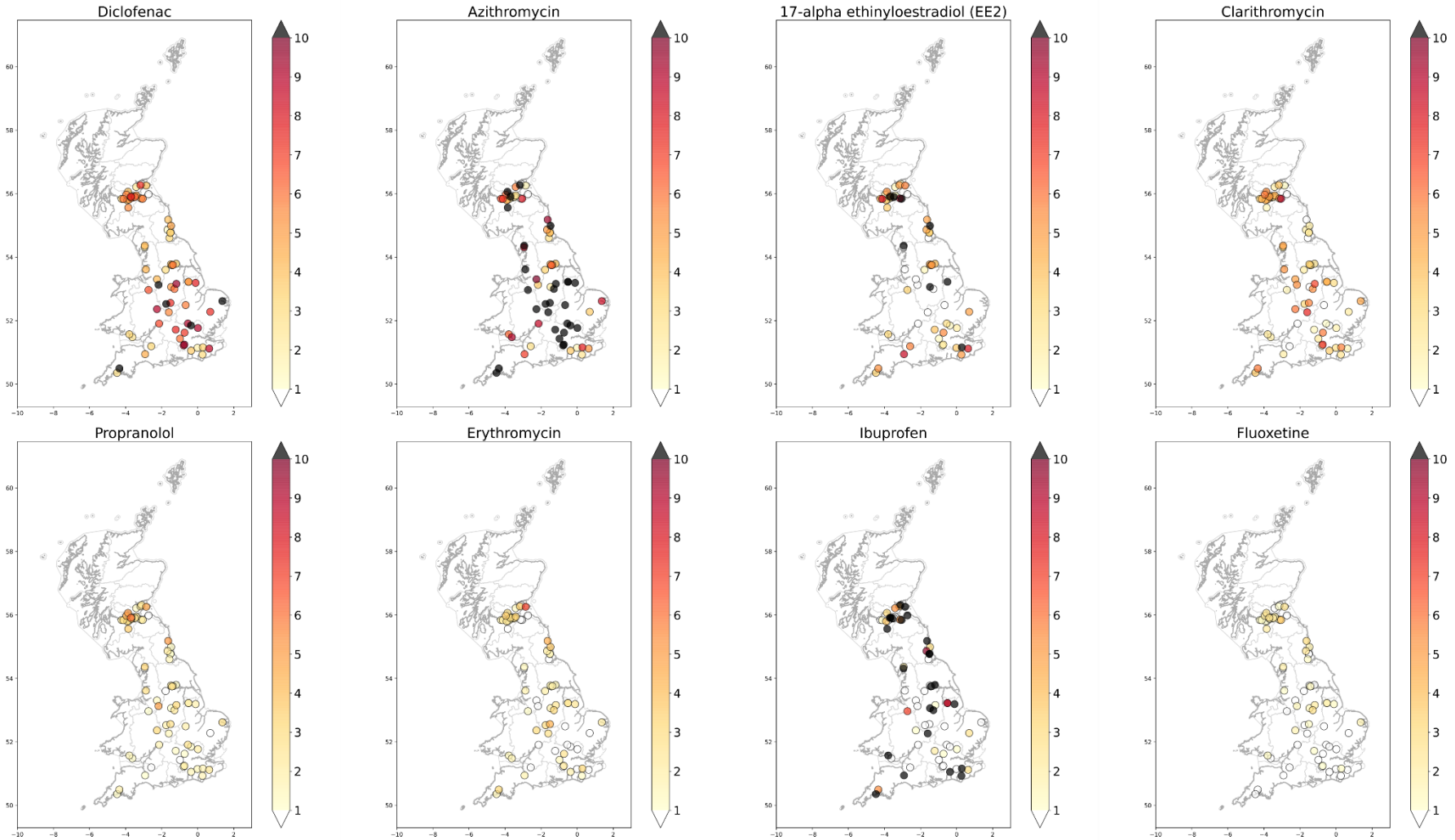

Figure 8. Risk quotient of pharmaceuticals in WWTW effluent. White circles indicates that the RQ is below 1, black indicates the RQ is more than 10.

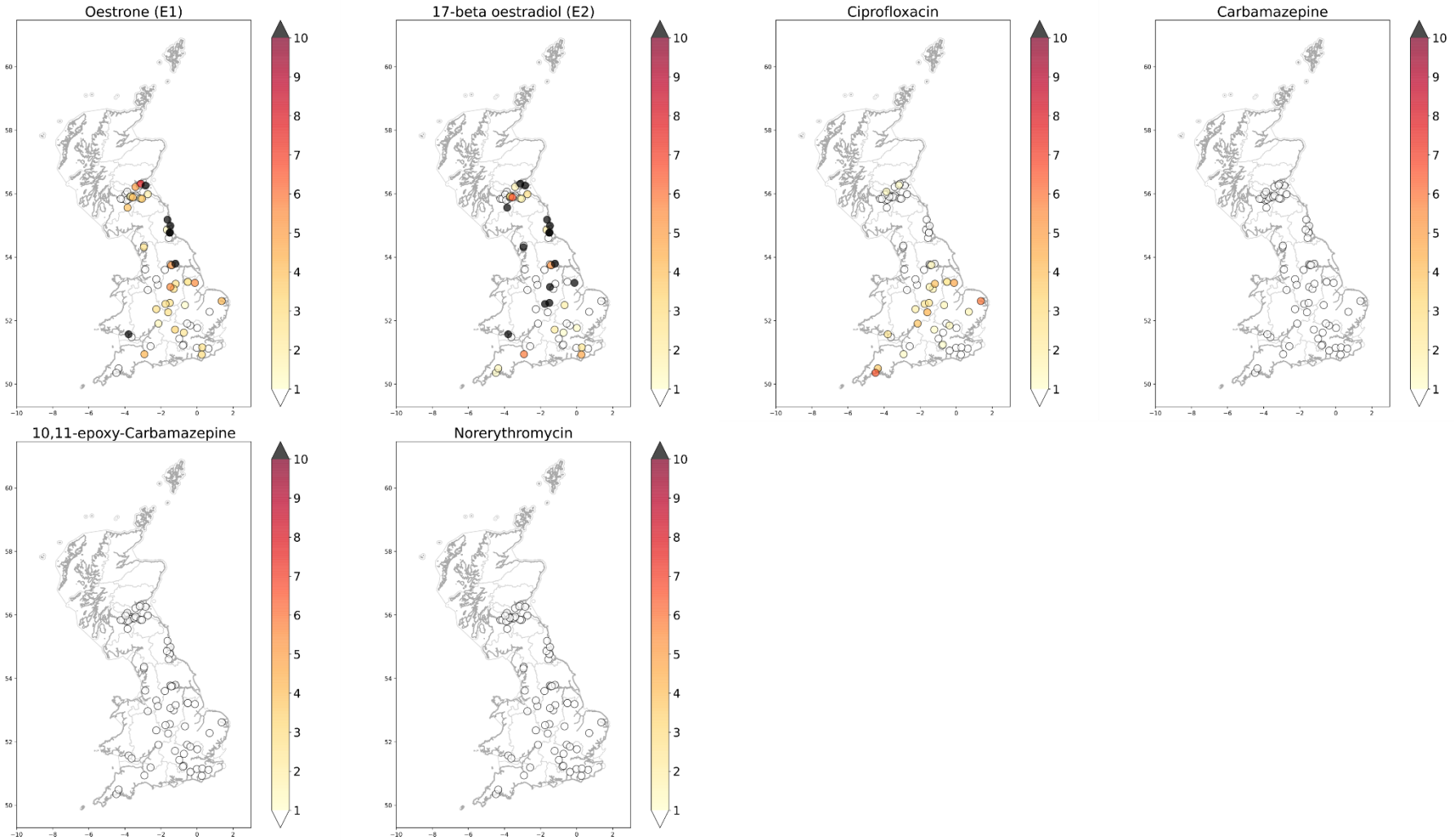

Figure 9. Risk quotient of pharmaceuticals in WWTW effluent. White circles indicates that the RQ is below 1, black indicates the RQ is more than 10.

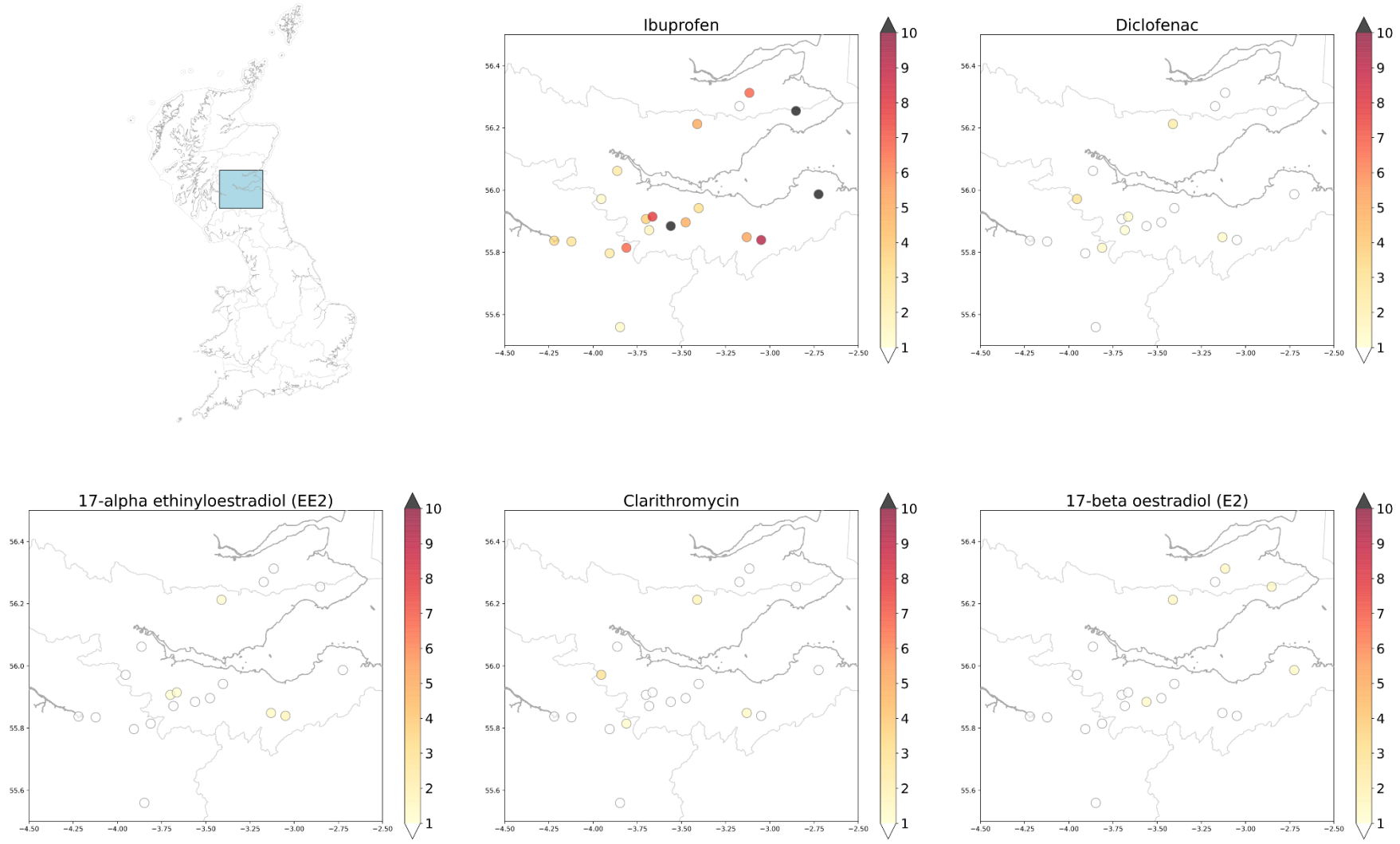

Figure 10. Risk quotient of pharmaceuticals downstream of Scottish WWTW. White circles indicates that the RQ is below 1, black indicates the RQ is more than 10.

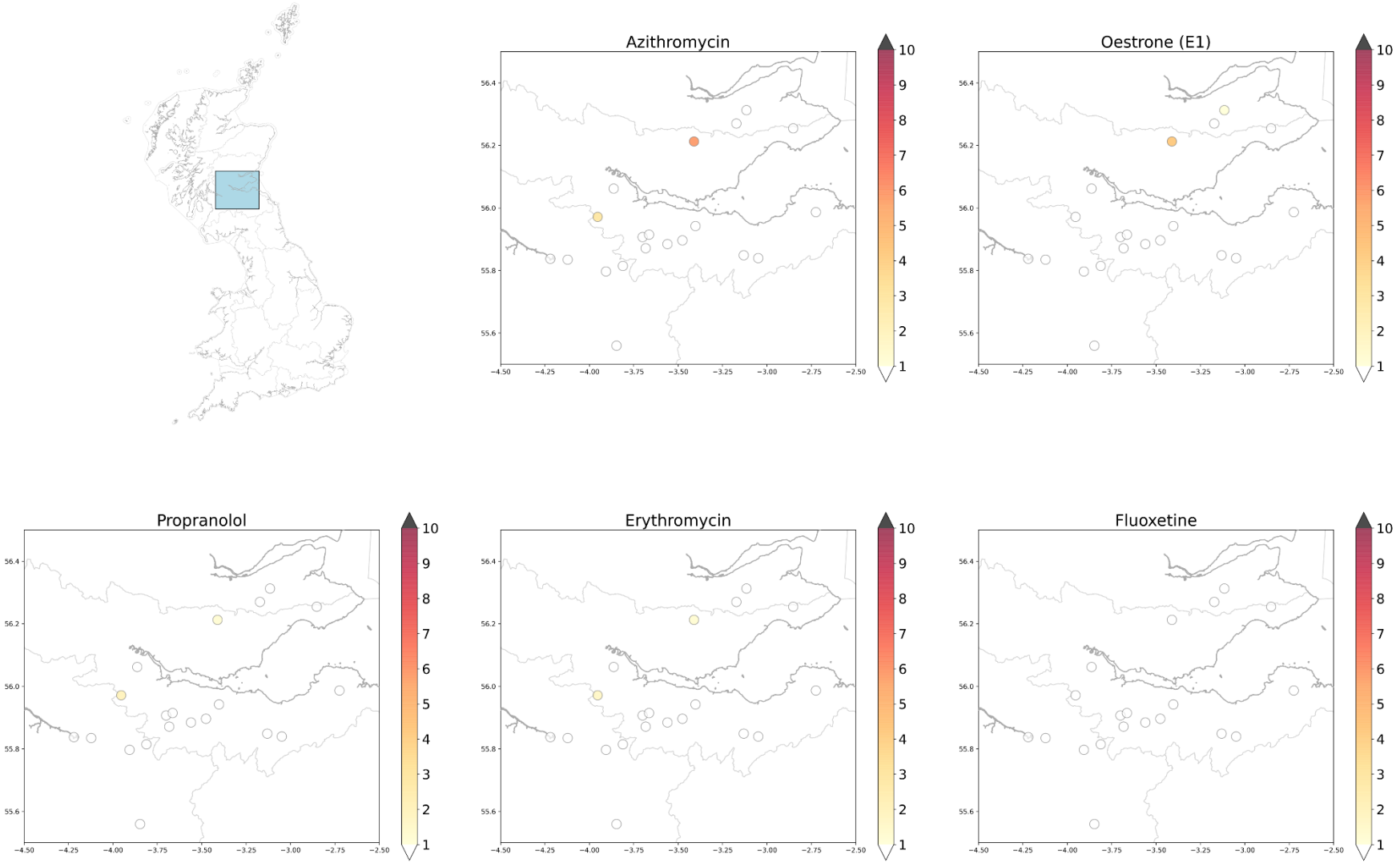

Figure 11. Risk quotient of pharmaceuticals downstream of Scottish WWTW. White circles indicates that the RQ is below 1, black indicates the RQ is more than 10.

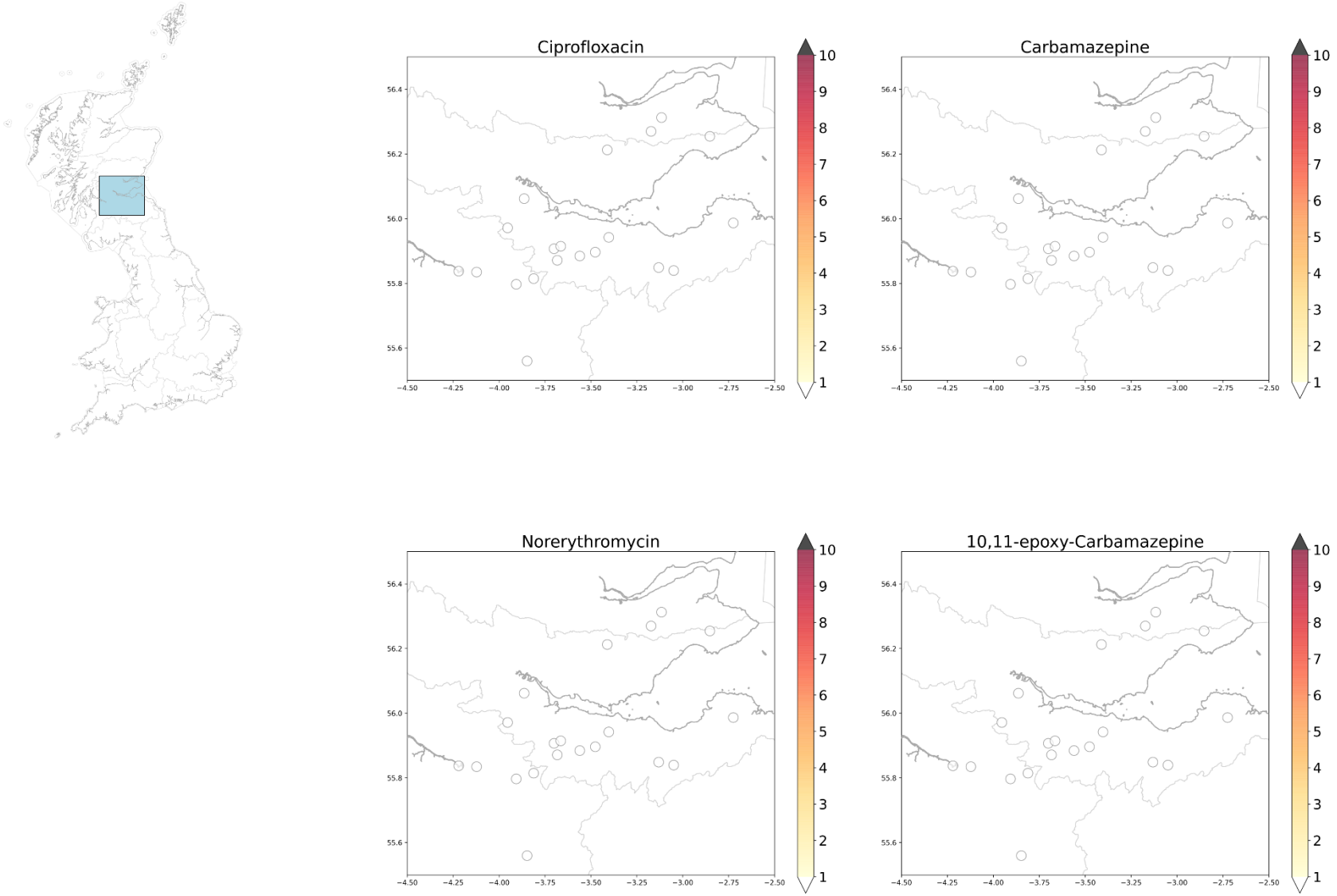

Figure 12. Risk quotient of pharmaceuticals downstream of Scottish WWTW. White circles indicates that the RQ is below 1, black indicates the RQ is more than 10.

7 – Discussion

Chemical contamination of waterbodies significantly influences the composition of the freshwater macroinvertebrate community[55]. Pharmaceuticals are some of the most detected emerging chemicals, yet many lack substantial literature on their direct impacts in freshwater. From the limited data available, we see that the response of invertebrates to pharmaceuticals is incredibly variable. It is species, time, and concentration dependant, and can also vary based on environmental conditions such as pH and presence of other chemicals[52,90,176].

From CIP2 data, it is clear that many pharmaceuticals are present in the UK environment at potentially dangerous levels. These concentrations are generally lower than most recorded concentrations that cause alterations in biological or behavioural function in invertebrates. However, large spikes in concentrations regularly occur, resulting in every single substance in this study exceeding its PNEC at some point in time across environmental and WWTW samples. In several cases spikes take concentrations to levels that have been observed to impact the growth, behaviour, and reproduction of freshwater invertebrates (Table 1 and 2).

From the review of current literature and CIP2 data, the main chemicals of concern appear to be:

- Ibuprofen – pervasive and regularly found in all waterbodies exceeding the PNEC, with high-risk quotients for 19 of 20 Scottish WWTWs in downstream waters. It has also been recorded to occur at concentrations that impact invertebrates.
- Carbamazepine – appears to affect invertebrates below the current PNEC at levels that occur in the environment.
- Fluoxetine – occurs in the environment at concentrations that have been observed to alter invertebrate behaviour and reproduction, though to a lesser extent than carbamazepine. It is also known to bioaccumulate.
- Venlafaxine - not included in CIP2 sampling but causes stress responses in the freshwater snail, *Leptoxis carinata,* at concentrations as low as 0.313ng/L which is well below the PNEC 38.35 ng/L and concentrations previously recorded in UK freshwaters (Table 1).
- Diclofenac – poorly eliminated through WWTWs, commonly occurs in waterbodies above the PNEC. It is known to bioaccumulate in invertebrates and can impact some avian species.

The impacts of pharmaceuticals are difficult to discern and study in the environment and very few studies measure the effects of pharmaceuticals at environmentally relevant concentrations or conduct long term studies. Length of exposure can dramatically alter conclusions of toxicity data where effects are time dependant, as seen with the LC50 of NSAIDs[90]. In general, the impacts of pharmaceuticals are often not recorded until there is a large visible effect, for example: the decimation of vulture populations by diclofenac[140]; or the slowing of dung decomposition due to long term use of veterinary parasiticides disrupting dung beetle communities[177].

Several substances do have the potential to impact invertebrates in the environment, but data for many substances are sparse. For example, effects of antibiotics in general are extremely poorly represented in the literature. The most commonly observed effect of pharmaceuticals in invertebrates are alterations in reproduction and growth, with some research suggesting peaks in effect at low concentrations for substances such as fluoxetine and naproxen[51,89]. Though changes in reproduction might not sound concerning, it can potentially lead to long term impacts on the ecosystem. The invasive zebra mussel spawns more readily under the influence of fluoxetine[26],

which in theory could make it a more efficient invader. Other effects include: Snails detaching themselves to move away from venlafaxine contaminated areas at extremely low concentrations[28]; algae, rotifer and small crustacean populations being stunted by the presence of naproxen and its more toxic breakdown products[44]; and insects emerging less successfully in the presence of carbamazepine[36].

Invertebrates are also capable bioaccumulators, posing the risk of contamination and poisoning for insectivorous vertebrates which may be more sensitive to drug classes designed for humans or domesticated animals[3]. At this time, there are no studies that directly address the transfer of pharmaceuticals from invertebrates to terrestrial insectivores. Some studies have examined the bioaccumulation capacities of fish, where the main mode of accumulation seems to be through the gills rather than through diet[76]. Biomagnification is very unlikely for pharmaceuticals, but this does not rule out potential for direct effects between single trophic levels.

The CIP and WFD monitoring systems in the UK have so far given us a good impression of the state of our river basins and is an important tool that should continue to be used and refined. In the last decade, the state of UK rivers has seen little improvement. As of 2020, 0% of England's rivers are in "good health"[83], suggesting the goal of 75% in "good condition" by 2027 is unlikely to be achieved. Lack of progress is not helped by stark differences in management strategies between countries within the UK[178]. This report shows that pharmaceutical contamination is a nationwide issue and, at the very least, any instance with an RQ greater than 1 deserves to be examined and dealt with appropriately. It may also be useful to examine the differences in ecology between sites of high RQ and low RQ, as well as differences before and after WWTW improvement, if such improvements are made.

8 – Conclusion

Pharmaceuticals are a widespread and common occurrence in freshwaters across the UK. They can occur above the current recommended PNECs in the environment in up to 84% of tested samples. However, their impacts on the environment are poorly understood.

Based on a review of existing literature, antidepressants like fluoxetine, carbamazepine, and venlafaxine appear to be the most dangerous pharmaceuticals to freshwater invertebrates. But strong data is sparse to non-existent, especially for drug classes like antibiotics. Further research must be carried out to fully understand how widespread their impacts are in freshwaters.

CIP2 data shows that PNEC values are at times exceeded in individual influent and effluent samples for all substances addressed in the second half of this report as well as in up and downstream samples for most substances measured during CIP2 Scotland. Based on the frequency of concentrations occurring in excess of PNEC limits in up and downstream waters, ibuprofen provides the greatest concern for freshwater environments, followed by diclofenac and EE2. We strongly recommend that a similar examination of up and downstream water concentrations is carried out when data is released by UKWIR to provide a more detailed understanding of the problem in the UK.

Our report highlights WWTWs as a major source of contamination that are not equipped to fully deal with pharmaceutical substances. Wastewater treatment facilities must be improved to prevent novel pollutants such as pharmaceuticals entering the environment alongside controls to reduce pharmaceuticals entering WWTWs. Policy driving improvements in water quality must address

pharmaceuticals and their impact on the environment. For example, encouraging consumers to return unused medicines to the pharmacy for safe disposal.

We also encourage further examination of other sources of contamination beyond WWTWs – such as septic tanks, river and pond sediments, landfill run off, and agricultural runoff. Long term examinations of effects on invertebrates in the environment or, at the very least, environmentally relevant concentrations are also required.

Monitoring our waterways for contamination is extremely important, however updated Environmental Risk Assessments must be taken into consideration and applied to Water Framework Monitoring programmes as soon as practically possible. Improved evaluation of the environmental risks posed by pharmaceutical products should include breakdown products as well as the parent pharmaceutical. All chemicals should undergo an assessment of risk linked to chemicals mixing in the environment. Retrospective environmental risk assessments should be carried out on pharmaceuticals already in use, to further identify risks to the environment and allow for a more informed choice of the most suitable pharmaceutical for use.

Finally, methods to control pathways must be explored to create sustainable options for reducing the number of pharmaceuticals in the environment. This might include increased education on the correct usage and disposal of pharmaceuticals, regulating the availability of the most prevalent/worst impacting pharmaceuticals, prescribing fewer damaging drugs where the option exists, and if appropriate, offering alternate treatments to pharmaceuticals such as blue-green prescribing.

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Appendices

Appendix 1 – LOD values by data source (all value in micrograms per litre)

Pharmaceutical	Scotland CIP2	UKWIR CIP2
10,11-epoxy-Carbamazepine	0.0002	0.1
17-alpha ethinyloestradiol (EE2)	0.00003	0.00003
17-beta oestradiol (E2)	0.0003	0.0003
Azithromycin	0.005	0.005
Carbamazepine	0.001	0.1
Ciprofloxacin	0.25	0.01
Clarithromycin	0.001	0.01
Diclofenac	0.002	0.01
Erythromycin	0.01	0.1
Fluoxetine	0.0002	0.01
Ibuprofen	0.005	0.01
Norerythromycin	0.002	0.1
Oestrone (E1)	0.0006	0.001
Propranolol	0.0002	0.01

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