



## Quantification and risk assessment of polar organic contaminants in two chalk streams in Hampshire, UK using the Chemcatcher passive sampler

Rosamund F.A. Robinson<sup>a</sup>, Graham A. Mills<sup>b</sup>, Roman Grabic<sup>c</sup>, Adam Bořík<sup>c</sup>, Gary R. Fones<sup>a,\*</sup>

<sup>a</sup> School of the Environment, Geography and Geosciences, University of Portsmouth, Burnaby Road, Portsmouth PO1 3QL, UK

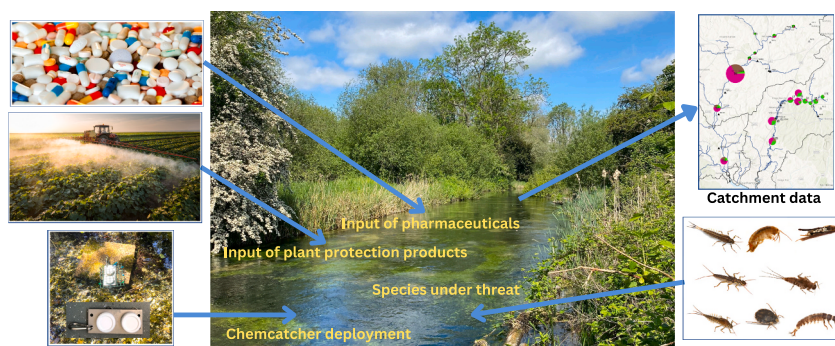
<sup>b</sup> School of Pharmacy and Biomedical Sciences, University of Portsmouth, White Swan Road, Portsmouth PO1 2DT, UK

<sup>c</sup> University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátíší 728/II, CZ-389 25 Vodňany, Czech Republic

### HIGHLIGHTS

- Chemcatcher used to sequester polar pollutants in river catchments in southern England.
- 121 compounds quantified using a targeted mass spectrometric workflow.
- Waste water treatment plant effluents were the main contributor of pharmaceuticals.
- Five compounds were found at or above their predicted no effect concentrations.
- Presence of imidacloprid could impact macroinvertebrate communities.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Freshwater systems are facing a number of pressures due to the inputs of polar organic contaminants from a range of sources including agriculture, domestic and industry. The River Itchen and River Test are two sensitive chalk streams in Southern England that are experiencing a decline in invertebrate communities. We used Chemcatcher passive samplers to measure time-weighted average concentrations (14 days) of polar pollutants at nine sites on the River Itchen and eight sites on the River Test over a 12-month period. Sampler extracts were analysed using a targeted LC/MS method. In total, 121 plant protection products and pharmaceutical and personal care products were quantified (range of  $\log K_{ow}$  from -1.5 to 7). Concentrations (sub  $\text{ng L}^{-1}$  to  $>500 \text{ ng L}^{-1}$ ) in both rivers showed spatial and temporal variations. A greater number of compounds and higher concentrations were found in the River Test. The chemical profile was dominated by inputs from wastewater treatment plants and legacy plant protection products. On the River Itchen, high concentrations ( $\sim 100 \text{ ng L}^{-1}$ ) of caffeine were observed directly downstream of a fish farm. Using the NORMAN database, the predicted no effect concentration (PNEC) freshwater values were exceeded by only five contaminants (2-hydroxy-terbutylazine, alprazolam, azithromycin, diclofenac and imidacloprid). In addition, venlafaxine was detected above its EU Watch List concentration. These exceedances were mainly downstream of direct inputs from treatment plants. These compounds are known to have ecotoxicological effects on a range of aquatic biota including

\* Corresponding author.

E-mail address: [gary.fones@port.ac.uk](mailto:gary.fones@port.ac.uk) (G.R. Fones).

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macroinvertebrates. Of concern is the ubiquitous presence of the ectoparasiticide imidacloprid, highlighting the need to control its use. The impact of the cocktail of pollutants found in this study on the long-term effects on chalk stream ecosystems remains unknown and needs further investigation.

## 1. Introduction

Anthropogenic polar organic contaminants such as pharmaceuticals and personal care products (PPCPs) and plant protection products (PPPs) are frequently detected in surface waters (Wilkinson et al., 2023; Taylor et al., 2020). These chemicals originate from several sources and can enter the aquatic environment via a number of different pathways. Point sources include discharge from wastewater treatment plants (WWTPs) (Heberer, 2002), leakage from septic tanks, (Richards et al., 2017) combined sewer overflow pipes (CSOs) (Petrie, 2021) and from aquaculture activities, (Alvarado-Flores et al., 2021). Diffuse sources include run-off of PPPs from treated fields (Haith, 2010), as aerosols during spray application (Bonmatin et al., 2015) and through leachate entering the groundwater aquifer (Marsala et al., 2020). An important and often overlooked pathway of polar organic contaminants is wastewater from WWTPs, septic tanks and on-site treatment plants which discharge directly into the subsurface strata. This will have an impact on surface water primarily fed by groundwater (Standley et al., 2008). Polar organic chemicals present a threat to the aquatic environment and can jeopardize biodiversity (Malaj et al., 2014). Some compounds (including metabolites and transformation products) have been shown to cause behavioural changes (Brodin et al., 2013; Al-Badran et al., 2019), endocrine-disrupting effects (Metcalf et al., 2013), physical changes (Fong and Molnar, 2013) and changes to reproductive responses (Godoi et al., 2024).

Chalk streams are unique and rare aquatic environments. These are groundwater fed and make an important contribution to global biodiversity (Rangeley-Wilson, 2021). The majority of the world's chalk streams are located in the south of England (Robinson et al., 2022), with the remainder being found in northern and western France (Maréchal and Rouillard, 2020). They are characterised by a high alkalinity ( $> 50 \text{ mg L}^{-1} \text{ CaCO}_3$ ) with a pH ranging from 7.4 to 8 and a small temperature range between 5 and 21 °C, (Mainstone, 1999). Chalk streams are a valuable habitat for rare invertebrates and plants. Many of these have been legally protected and designated as Sites of Special Scientific Interest and others as European sites of Special Areas of Conservation (SAC), under the EU Habitats Directive 92/43/EEC (European Commission, 1992). Recently, it has been noted that the macroinvertebrate population has declined in many chalk streams in the UK (Salmon and Trout, 2016 and 2019; WildFish, 2021). The reasons for this decline are still unknown but the presence of PPCPs and PPPs in the streams could be a contributory factor (Miller et al., 2021). It is important therefore to identify sources of pollution to enable mitigation strategies to be undertaken in order to reduce the loss of species biodiversity in these important environments.

Currently, water quality monitoring strategy for organic contaminants in the UK is based on the collection of spot water samples at variable frequencies. This approach provides only an estimate of the pollutants present at the time of sampling and may miss stochastic events. To overcome this limitation passive sampling has been proposed as an alternative or complementary method to spot water sampling (Bernard et al., 2019; Valenzuela et al., 2020). Passive sampling has a number of advantages, including *in situ* pre-concentration of analytes, estimation of time weighted average (TWA) concentration over the sampling period, low cost, and ease of deployment (Vrana et al., 2005). Several designs of passive samplers have been developed for monitoring different classes of pollutants (Vrana et al., 2005). For polar organic pollutants three main types of sampler have been used to date; these include Chemcatcher (Moschet et al., 2015), organic-diffusive gradient in thin-films (o-DGT) (Guibal et al., 2019) and polar organic compound

integrative sampler (POCIS) (Vrana et al., 2021). These devices can be used to detect the presence or absence of a compound (Rimayi et al., 2019; Taylor et al., 2020) or, if the uptake rate is known, a TWA concentration can be calculated (Vrana et al., 2005; Petrie et al., 2016; Römerscheid et al., 2023). These concentrations can be used subsequently in risk assessment studies (Dulio and von der Ohe, 2013; Molnar et al., 2021). To date, there has been limited studies on the concentration of polar contaminants in chalk streams. Robinson et al. (2022) identified a number of substances using a suspect screening approach, but with no quantification of the chemicals present. [35].

In this study we deployed Chemcatcher passive samplers in two chalk streams (River Itchen and River Test) in the south of England, UK. Samplers were deployed (8 discrete time points covering all four seasons) at various locations along the catchments including the headwaters. These sites covered a number of inputs (point and diffuse) from anthropogenic activities and where regular ecological monitoring had been conducted. Sampler extracts were analysed using a liquid chromatography-mass spectrometry (LC-MS) targeted analysis approach for a suite of PPPs and PPCPs. Extract concentration data were subsequently converted to TWA concentrations using a generic sampling uptake rate obtained from Römerscheid et al. (2023). Multivariate statistical analysis was performed to identify spatial and temporal trends within the data set. The TWA concentration data were compared against lowest predicted no-effect concentration (PNEC). These values were used to estimate the hazard quotient in order to assess the risk posed by the polar organic chemicals present. Results are expected to yield insights into chemical mixtures present and their potential for producing adverse effects on lotic ecosystems.

## 2. Methods

### 2.1. Catchment description and sampling sites

The catchment was located in the south of England and characterised by chalk downland (Allen, 2017). The two main watercourses are the River Itchen (42 km long) and the River Test (64 km long), both groundwater fed. Further details on the catchments are provided in (Robinson et al., 2022). Both rivers have stable flow and temperature regimes and support a high number of indigenous species (Mainstone, 1999). The study area (Fig. 1) was mostly rural, with both rivers flowing through small towns and villages; however, the River Itchen flows through the city of Winchester which is a main urban area in the catchment.

There were nine sampling sites (I1-I9) on the River Itchen and its tributaries (Fig. 1). Sites comprised the early headwaters, upstream and downstream of a WWTP outlet and upstream and downstream of a commercial fish farm. There were eight monitoring sites (T1-T8) on the River Test and its tributaries (Fig. 1). Sites encompassed the early headwaters, upstream of a WWTP outlet, downstream of a number of WWTP outlets and downstream of a commercial watercress farm. Description of each site is given in Table S1 (Itchen) and Table S2 (Test).

There were a number of WWTPs on the River Itchen that discharged directly into the river (three plants) (Table S3) or into the subsurface strata (three plants) (Table S4). In contrast, on the River Test five plants discharged into the main river (Table S5), five into its tributaries (Table S5), whilst eight discharged to the subsurface strata (Table S6). The location of the WWTPs in relation to the sampling sites are shown in Fig. S1.

Some sampling sites coincided with macroinvertebrate sampling that had been undertaken over a number of years. In the River Itchen these



were sites I3, I4, I5, I8 and I9. Sites in the River Test were T1, T2, T6, T7 and T8.

## 2.2. Materials and reagents

All reagents and pure water were of analytical grade or purer. Methanol (MeOH) and ultra-pure water (UPW) were obtained from Thermo Fisher Scientific (Loughborough, Leicestershire, UK). Toluene, dichloromethane, formic acid, and ammonium acetate used in LC mobile phases were purchased in LC-MS grade quality (Merck, Darmstadt, Germany). UPW ( $>18.0 \text{ M}\Omega \text{ cm @ } 25 \text{ }^\circ\text{C}$ ) was produced using an in-house Milli-Q® purification system (Merck, Burlington, USA) or Aqua-MAX-Ultra system (Younglin, Korea). UPW was used in all laboratory procedures. Glassware was soaked in a 5 % Decon 90 solution (Decon Laboratories Ltd. Hove, UK) overnight, rinsed with UPW then MeOH before use. A mix of isotopically labelled analytical standards, (Nováková et al., 2023) were prepared by dissolving them in MeOH and for working solutions diluting to  $1 \mu\text{g L}^{-1}$  and used for targeted analysis Table S7.

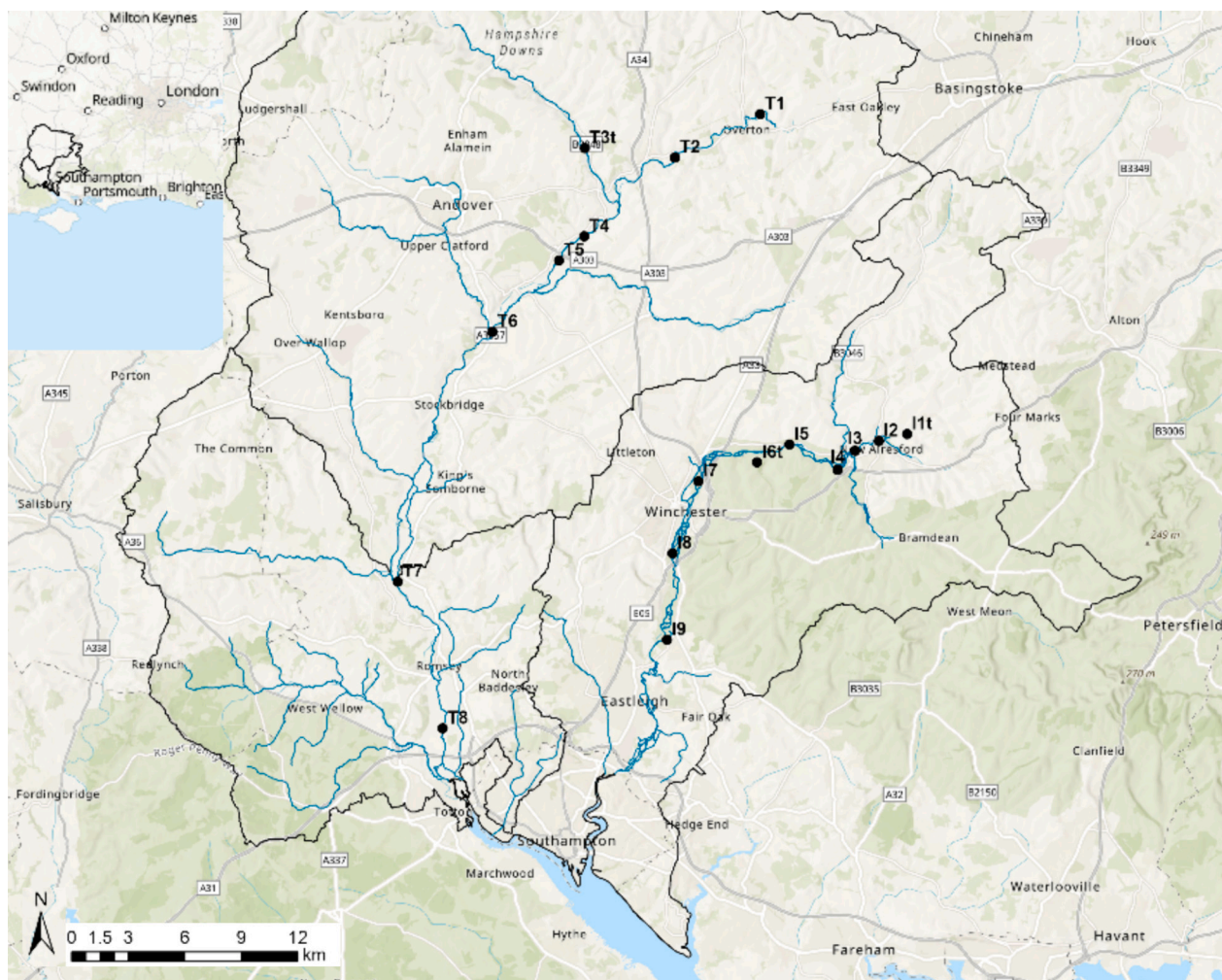
## 2.3. Chemcatcher passive sampler preparation

The Chemcatcher comprises two components, a polytetrafluoroethylene (PTFE) base and a PTFE retaining ring (AT Engineering, Tadley, UK) and were cleaned and prepared according to the protocol (Robinson et al., 2023). Briefly the components were soaked in a 2 %

Decon 90 solution and rinsed with UPW water. Supor polyethersulfone (PES) 0.2 mm pore size membranes (Pall Europe, Portsmouth, UK) were cut to size (52 mm) and soaked in MeOH and UPW. Hydrophilic-lipophilic balanced (HLB-L) sorbent (47 mm diameter) disks were purchased from Biotage (Uppsala, Sweden) soaked and conditioned in MeOH and UPW. The Chemcatcher samplers were prepared by placing the HLB disk onto the sampler body, overlaying with a PES membrane and securing in place with the retaining ring. Prepared samplers were stored in UPW water before deployment (Robinson et al., 2023).

## 2.4. Field deployment of Chemcatcher

Duplicate Chemcatcher samplers were deployed on the river bed at 17 sites on the two rivers (Fig. 1). Samplers were deployed between November 2021 and August 2022. Dates of deployment and recovery of samplers are given in Table S8. The dates for the four seasons were based on the astronomical calendar. In the autumn and winter seasons, sampling occurred over two consecutive 14-day periods, but during the spring and summer seasons, the consecutive sampling periods were split due to set timings of macrophyte cutting (sampling was not permitted by the riparian owners during this period). At each sampling site, samplers were fastened to stainless steel plates using R pins and affixed to concrete slabs and then lowered to the river bed. The full deployment apparatus and protocol is given in (Robinson et al., 2023). Field blank samplers were exposed during all deployment and retrieval operations.



**Fig. 1.** Map showing the location of the nine sites in the River Itchen (I) and eight sites in the River Test, (T) (The 't' indicates the site is in a tributary of the main river). The inset shows the location of the Itchen and Test catchments in the UK. Further details of the sampling sites are given in Tables S1 and S2.

## 2.5. Water quality parameters and additional site data

Water quality parameters (pH, water temperature [°C], dissolved oxygen [%], conductivity [ $\mu\text{S cm}^{-1}$ ]) were measured during each Chemcatcher deployment and retrieval operation using a YSI EXO1 multiparameter sonde (Xylem Analytics UK Limited). The river flow rate

( $\text{m s}^{-1}$ ) and depth (m) were measured using a handheld electromagnetic flowmeter (Valeport Ltd., Totness, UK) and depth measuring stick respectively. Rainfall data for the sampling area were collected from the Met Office Hadley Centre observations datasets for SE England (<http://www.metoffice.gov.uk/hadobs/hadukp/>).

**Table 1**

Plant protection products identified with CAS numbers and log  $K_{ow}$  values together with PNEC (lowest freshwater value obtained from the NORMAN Ecotoxicology Database). Data for the range of Chemcatcher TWA concentrations ( $\text{ng L}^{-1}$ ) are given for each river and each season.

Compound	CAS RN	log $K_{ow}$	PNEC ng $\text{L}^{-1}$	River Itchen ng $\text{L}^{-1}$				River Test ng $\text{L}^{-1}$			
				Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
2,4-D	(94-75-7)	-0.82	600				0.3-1.3	0.6-1.3	1.1-2.4	4.3	0.3-2.3
Dichlorprop	(120-36-5)	3.43	1000					0.6-21.3	1.2		0.2-1.1
2-hydroxy-terbuthylazine	(66753-07-9)	0.3	7.3	0.2-6.7	1.7-5.1	0.9-4.6	0.2-8.5	0.8-6.1	1.8-5.9	0.3-7.3	1.7-7.8
Alachlor	(15972-60-8)	3.52	300	0.7							
Atrazine	(1912-24-9)	2.61	600	2.1-9.3	2.2-6.2	0.2-4.9	0.4-2.7	0.4-1.5	2.1-6.2	0.7-4.4	0.5-2.6
Atrazine-2-hydroxy	(2163-68-0)	0.1	10,000	0.3-1.7	0.5-1.9	0.3-1.5	0.2-0.9	0.4-1.1	0.4-2.0	0.3-1.3	0.2-0.7
Atrazine-desethyl	(6190-65-4)	1.51	260	5.3-24.3	6.2-16.7	0.7-11.6	1.2-7.6	3.0-24.3	4.4-12.8	1.8-11.4	1.7-5.2
Atrazine-desethyl-desisopropyl	(3397-62-4)	-0.1	600		3.7-15.2				5.0-13.7		
Atrazine-desisopropyl	(1007-28-9)	1.15	390	0.6-5.5	2.4-5.7	1.8-2.9	1.4-92.7	0.6-3.6	1.5-7.4	1.5-2.7	16.7-88.1
Azoxystrobin	(131860-33-8)	2.5	200						0.2-0.5	0.2-1.7	
Bentazone	(25057-89-0)	2.34	270,000	0.7-3.3	0.4-1.2	0.3-1.0	0.2-0.9	0.5-9.9	0.4-4.4	0.2-1.2	0.2-2.6
Carbendazim	(10605-21-7)	1.52	440		0.3-0.6		0.06-0.1		0.4-1.7	0.2-1.8	0.1-1.0
Chlorantranilprole	(500008-45-7)	2.76	250		0.2			1.2-19.8	0.2-2.1	0.2-1.4	
Chloridazon-desphenyl	(6339-19-1)	-0.3	250,000					12.5			
Chloridazon-methyl-desphenyl	(17254-80-7)	-0.2	37,000	0.3-1.2	0.2-1.1			0.3-0.8	0.3-0.8		
Chlorotoluron	(15545-48-9)	2.41	600	0.5	0.1-0.3	0.1-0.2		0.5-5.6	0.2-1.8	0.1-0.9	0.3-0.7
Chlorotoluron-desmethyl	(22175-22-0)	2.6	3710					0.5			
Cyproconazole	(94361-06-5)	2.9	1300		0.1-0.2			0.2-0.4	0.2-0.3	0.2	
DEET (Diethyltoluamide)	(134-62-3)	2.02	88,000	2.7	0.7-1.6	1.7-3.1	4.2	2.0-18.0	2.7-34.0	33.0-39.4	4.4-24.0
Diazinon	(333-41-5)	3.81	10						0.1	0.4	
Dimethenamid ESA	(205939-58-8)	1.89	16,500					1.0			
Dimethenamid OA	(380412-59-9)	2	7860					0.8-1.0			
Dimethoate	(60-51-5)	0.78	70						6.7		
Dimethomorph	(110488-70-5)	2.63	5600					0.4-4.3	0.1-1.1	0.3	1.8
Diuron	(330-54-1)	2.68	70					0.7-4.9	0.4-7.4	0.4-7.3	0.3-0.5
Diuron-desmethyl	(3567-62-2)	2.9	1240					0.5-1.1	0.5-2.1	0.3-2.1	
Epoxiconazole	(133855-98-8)	3.2	200	0.3	0.1-0.3	0.2-0.3	0.3	0.3-0.8	0.1-0.6	0.2-0.7	
Fenuron	(101-42-8)	0.98	1450						0.1-0.3	0.2-0.3	
Flusilazole	(85509-19-9)	3.7	2300		0.03				0.04-0.1		
Foramsulfuron	(173159-57-4)	4.01	17	0.5							
Imidacloprid	(138261-41-3)	-0.57	13	0.6-1.7	1.0-1.2	0.3-1.1	0.3-1.2	0.9-24.3	0.6-27.4	0.4-22.8	0.3-12.8
Ioxynil	(1689-83-4)	0.9	130							0.1	
Isoproturon	(34123-59-6)	2.87	640		0.05-0.1			0.5-2.0	0.1-1.0	0.3-0.5	0.3
Isoproturon-didemethyl	(56046-17-4)	2.3	5780							0.5	
Lenacil	(2164-08-1)	3.09	340					0.7-1.1			
MCPA	(94-74-6)	3.25	660								0.4
MCPP (Mecoprop-P)	(16484-77-8)	-0.19	3600					0.6-7.1	1.3-18.2	4.1-9.4	5.2
Metalaxyl	(57837-19-1)	1.65	100					0.5			
Metazachlor	(67129-08-2)	2.7	20	0.6-1.8	0.3-1.3	0.3-0.9	0.2-0.4	0.5-1.8	0.3-2.3	0.3-1.4	0.2-0.6
Metazachlor ESA	(172960-62-2)	1	91,900	3.0-21.3	3.2-6.6	1.2-10.0	0.7-4.0	4.9-16.7	3.8-8.4	1.7-5.6	0.3-2.9
Metazachlor OXA	(1231244-60-2)	2.49	25,700	1.0-5.8	1.2-2.7	1.4-2.9	0.3-1.7	1.4-4.4	1.4-3.0	1.4-2.6	0.3-1.0
Methoxyfenozide	(161050-58-4)	3.72	86					0.6	0.3-0.7		
Metolachlor	(51218-45-2)	3.13	200					1.8			
Metolachlor ESA	(171118-09-5)	1.4	21,700					1.2			
Metolachlor_OA	(152019-73-3)	2.5	16.6					0.8			
Metsulfuron_methyl	(74223-64-6)	2.2	10			0.08-0.1	0.1-0.13		0.1-0.2	0.1-0.13	
Monolinuron	(1746-81-2)	2.3	150	1.8-2.6							
Prometryn	(7287-19-6)	3.51	500						0.1-0.2		
Propazine	(139-40-2)	2.93	180		0.1-0.3			0.9	0.2-0.4	0.2-0.6	
Propazine-hydroxy	(7374-53-0)	2.51	70				2.6				
Propiconazole	(60207-90-1)	3.72	1400		0.1-0.4	0.1-0.3		0.5-0.8	0.1-1.7	0.1-3.5	0.2-0.6
Pyrimethanil	(53112-28-0)	2.84	1500							0.7	
Simazine	(122-34-9)	2.18	1000	1.0-9.9	1.7-4.9	0.3-4.9	0.4-2.9	1.7-8.2	1.8-4.1	0.6-5.8	0.6-1.8
Simazine-hydroxy	(2599-11-3)	-0.3	180	0.5-0.7	0.1-1.0	0.3-1.0	0.2-0.4	0.5-1.0	0.3-1.1	0.3-0.7	0.2-0.3
Tebuconazole	(107534-96-3)	3.7	240				0.2	1.5		1.0-1.7	0.2-0.4
Terbuthylazine	(5915-41-3)	3.4	220	1.0-1.2			0.06-0.1	1.0-2.3			0.1-0.14
Terbuthylazine-desethyl	(30125-63-4)	2	250	0.6-1.2	0.8-2.0		0.1-1.9	0.5-1.3	0.8-2.0		0.1-1.1
Terbutryn	(886-50-0)	3.74	65			0.4		0.6-2.0	0.1-0.6	0.2-1.0	0.3
Triticonazole	(131983-72-7)	3.29	1000							0.2	
Warfarin	(81-81-2)	2.7	1200						0.4		

## 2.6. Chemcatcher disk extraction

The extraction procedure is described fully in (Robinson et al., 2023). Briefly, the Chemcatcher samplers were disassembled and the PES membranes discarded. The HLB disks were air dried overnight. Disks were eluted into glass vials (60 mL) using MeOH (40 mL) under gravity. Eluates were dried using a Genevac centrifugal rotary evaporator (SP Scientific, Ipswich, UK) and the resultant extracts transferred into 2 mL deactivated (silanized) vials (Agilent, Santa Clara, USA). The volume was adjusted to 1 mL with MeOH, weighed then stored at  $-18^{\circ}\text{C}$  before instrumental analysis. Due to financial constraints of the project, only one deployed sampler and one field blank were extracted. The other set of disks were used as back-up samples in case of loss during analysis.

## 2.7. Instrumental Analysis

Prior to instrument analysis the vials were removed from the freezer, homogenized (Vortex V-1 plus Personal Vortex, Biosan) and weighed. Samples from each vial were diluted with UPW and spiked with two isotopically labelled internal standards mixtures (plant protection products and pharmaceuticals) (Nováková et al., 2023), Table S7. Two seven-point calibration curves (0.05 to 50  $\mu\text{L}$ ) were prepared in UPW water: methanol (1:1, v:v) for quantification purposes using the same internal standards and native plant protection products and pharmaceuticals. Randomly selected extracts were fortified with the standard mixtures for quality control (Grabic et al., 2012; Švecová et al., 2021). Field and extraction blank samples were prepared together with each set of the samples to assure QA/QC.

## 2.8. HPLC-ESI-MS/MS target analysis

Targeted analysis was accomplished by a TSQ Quantiva Triple-Stage Quadrupole MS coupled with an Accela 1250 pump (Thermo Fisher Scientific, USA) and an HTS XT-CTC autosampler (CTC Analytics AG, Switzerland), using reversed-phase chromatography separation with Hypersil GOLD aQ column (2.1 mm  $\times$  50 mm, particle size 5  $\mu\text{m}$ ) (Thermo Fisher Scientific, San Jose, CA, USA). The sample extract injection volume was 10  $\mu\text{L}$ . The method used was as described in (Švecová et al., 2021; Vrana et al., 2021) and in Table S9. The MS/MS data was quantified using internal standards (Nováková et al., 2023). A list of target analytes used is given in Table S7.

## 2.9. Data processing

The instrument was operated using Xcalibur Software and obtained data were processed by TraceFinder 4.1 Software (both Thermo Fisher Scientific) expressed as ng/Chemcatcher (Grabicová et al., 2022). The instrumental LOQ reflects the response corresponding to half the concentration of the lowest calibration point in the curve that shows deviation from the mean response factor lower than 30 % (Vrana et al., 2021). The sample LOQs were calculated from the instrumental limit of quantification by correcting to the internal standard response and aliquot/volume of individual samples. The mean seasonal LOQs for all analytes are reported in ESM 2 Tables 1 and 2.

## 2.10. Statistical analysis

Statistical analyses were accomplished using the Minitab statistical package (Minitab v. 21.3.0). Normality of data was first tested by the Kolmogorov-Smirnov test. Then because of the non-normal distribution of the data, a non-parametric one-way analysis of variance (ANOVA) plus *post hoc* multi-comparison tests, Tukey pairwise comparisons, Dunnett multiple comparisons and Fisher pairwise comparisons with a significance level set at 0.05 and a 95 % confidence interval were completed.

For visualization all compound and concentration data were

imported in Microsoft Excel format to Agilent Mass Profiler Professional software (MPP, B14.91. Agilent Santa Clara, USA) for statistical analysis. The programme was used to produce Venn diagrams separating the compounds and seasons for each river (Taylor et al., 2021).

## 2.11. Time-weighted average concentration calculation

The theory of uptake of an analyte by the Chemcatcher is well understood and has been described in detail elsewhere (Vrana et al., 2005). Briefly, accumulation of a pollutant by the receiving phase of the Chemcatcher follows first-order kinetics, occurring in linear, then curvilinear regimes, ending at equilibrium. In the linear uptake regime, accumulation is time integrative; hence, an analytes TWA concentration over the exposure period can be calculated using Eq. (1).

$$C_w = \frac{M_{S(t)} - M_0}{R_S \times t} \quad (1)$$

where:  $C_w$  = concentration ( $\text{ng L}^{-1}$ ) of analyte in water;  $M_{S(t)}$  = mass (ng) of analyte in Chemcatcher HLB disk after exposure time  $t$  (day);  $M_0$  = mass (ng) of analyte in HLB disk of Chemcatcher field blank;  $R_S$  = sampler uptake rate of analyte ( $\text{L day}^{-1}$ )

$R_S$  is a theoretical volume of water sampled per unit time and must be determined for each analyte. Values vary with environmental parameters such as water temperature, flow rate and the physicochemical characteristics of the analyte (Vrana et al., 2005).  $R_S$  is measured in laboratory-based calibration experiments (Grodtko et al., 2021) or in the field (Allinson et al., 2023). Determining  $R_S$  for a large number of pollutants is both expensive and time-consuming. For the Chemcatcher the  $R_S$  does not vary greatly for a wide range of polar contaminants. Römerscheid et al. (2023) have proposed that a generic  $R_S$  value can be used to calculate TWA concentrations for these chemicals. In this study, we used their median  $R_S$  of  $0.047 \text{ L day}^{-1}$  to calculate the TWA concentration of the polar chemicals found in the River Itchen and Test.

## 2.12. Hazard quotient calculation

The PNEC value of a compound is based on existing environmental quality standards (EQS) but should not be based on a single toxicity test (Chapman and Elphick, 2015). The value from the NORMAN ecotoxicology database is derived from acute test data from each trophic level, provisional data from quantitative structure activity relationship (QSAR) models and chronic data from standard and non-standard endpoints (Dulio and von der Ohe, 2013). The lowest PNEC value for each compound quantified was obtained from the NORMAN Ecotoxicology Database for freshwater (<https://www.norman-network.com/nds/ecotox/lowestPnecIndex.php>).

The PNEC can be utilised to estimate the environmental risk of a compound on aquatic ecosystems by determining the hazard quotient compound index (HQ) using Eq. (2). Where the HQ is  $<0.5$  the risk is evaluated as being a low risk, where the HQ is  $0.5 < \text{HQ} < 1$  represents a medium risk and where the HQ is  $>1$  indicates a high ecological risk to aquatic organisms (Syberg et al., 2009; Ginebreda et al., 2010; Molnar et al., 2021).

$$HQ_{ij} = \frac{C_{ij}}{PNEC_j} \quad (2)$$

where:  $HQ_{ij}$  = the hazard quotient of compound  $j$  in sample  $i$ ;  $C_{ij}$  = the concentration of compound  $j$  in sample  $i$ ;  $PNEC_j$  = the predicted no-effect concentration for compound  $j$ .

As more than one compound is likely to be present in a sample there are two effect levels to consider, the concentration addition (CA) for similarly acting chemicals and the independent action (IA) for dissimilarly acting chemicals (Syberg et al., 2009; Escher et al., 2020). Many chemicals may have different modes of action and possibly more than one mode of action depending on the organism exposed, therefore,

**Table 2**  
Pharmaceutical and personal care products identified with CAS numbers and log  $K_{ow}$  values together with PNEC (lowest freshwater value obtained from the NORMAN Ecotoxicology Database). Data for the range of Chemcatcher TWA concentrations ( $\text{ng L}^{-1}$ ) are given for each river and each season.

Compound	CAS RN	log $K_{ow}$	PNEC $\text{ng L}^{-1}$	River Itchen $\text{ng L}^{-1}$				River Test $\text{ng L}^{-1}$			
				Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
1H-benzotriazol	(95–14-7)	1.44	19,000		9.1–39.2	14.7–31.3	11.4–58.5	12.2–283.7	369.3	53.1–591.2	69.1–398.5
1H-benzotriazol_ (5/4)-methyl	(136–85-6)	1.4	150,000	0.8–13.5	1.9–17.9	1.8–12.9	1.5–9.9	6.0–257.2	7.8–243.2	2.4–440.7	1.5–243.2
1H-benzotriazol_1-methyl	(13351–73-0)	1.1	13,200	4.4						4.4–5.0	1.2
Alprazolam	(28981–97-7)	2.12	77				51.7				62.3
Amitriptyline	(50–48-6)	4.92	140		0.3–0.5			0.6–7.3	0.3–2.6	11.1	0.7
Atenolol	(29122–68-7)	0.16	150,000	0.5	0.1–0.3	0.2		0.3–4.0	0.3–3.3	0.2–3.0	0.1–0.8
Atorvastatin	(134523–00-5)	6.36	8500					0.5–1.4			
Azithromycin	(83905–01-5)	4.02	19		0.7–16.8			95.7–104.9	1.0–15.0	0.3–3.8	
Bezafibrate	(41859–67-0)	4.25	2300	1.0–3.5	0.2–0.4	0.5–1.1	0.5	2.6–19.8	2.3–25.8	0.4–10.5	0.2–5.2
Bisoprolol	(66722–44-9)	1.87	92,000	0.3–0.4	0.2–0.5	0.2		0.6–7.4	0.3–4.4	3.0–3.5	0.2–2.4
Caffeine	(58–08-2)	0.16	1200	18.5–129.2	3.8–120.6	14.9–71.7	9.9–136.2	11.4–16.1	2.3–23.7	15.5–22.3	5.6–26.4
Carbamazepine	(298–46-4)	2.25	2000	0.4–9.9	0.5–8.3	0.3–10.0	0.3–9.1	3.6–63.8	1.7–89.7	1.1–118.5	2.1–68.4
Carbamazepine 10,11-epoxide	(36507–30-9)	1.3	2570	0.3–1.2	0.1–1.8	0.2–1.0	0.2–1.7	0.7–22.8	0.7–10.5	0.4–12.0	0.2–10.8
Cetirizine	(83881–51-0)	–0.61	410	0.3–1.5	0.3–1.4	0.1–3.0	0.2–2.3	3.0–33.4	1.0–24.3	0.2–38.0	1.7–19.8
Citalopram	(59729–33-8)	3.74	16,000		0.3–0.9			19.8–21.3	0.5–9.3	4.3–8.2	0.8–2.0
Clarithromycin	(81103–11-9)	3.16	120	0.9–1.2	0.5–1.3	0.9		1.5–17.6	0.4–4.6	1.0–2.6	0.300
Clindamycin	(18323–44-9)	2.16	44	0.3				0.3–1.0			
Clindamycin sulfoxide	(22431–46-5)	1.1	4000	0.5–1.0	0.7–0.8			0.5–5.8	0.9–1.4	1.5–1.8	0.5–2.3
Clomipramine	(303–49-1)	5.19	30	4.3				4.3			
Codeine	(76–57-3)	1.28	7190	0.3–0.7	0.2	0.2		0.4–3.6	0.2–0.7	1.4	0.2–1.1
Diclofenac	(15307–86-5)	4.02	50	0.3–7.0	0.5–2.9	0.8–2.6	0.2–2.4	0.3–51.2	0.5–69.9	0.8–51.7	0.3–31.9
10,11 Dihydrocarbamazepine	(3564-73-6)	2.3	2390						0.5	0.4–0.5	0.3–0.33
Dihydroxy carbamazepine 10,11	(35079–97-1)	0.3	1910	4.7–7.1	0.2–11.6	9.3–14.3	2.0–7.9	2.4–304.0	1.2–243.2	0.7–516.7	0.7–197.6
Diltiazem	(34933–06-7)	2.7	230					0.7			
Diphenhydramine	(58–73-1)	3.27	991						0.6–0.7	0.5–2.0	0.05–0.1
Disopyramide	(3737–09-5)	2.58	690	0.1–0.2				0.1–2.0	0.2–2.7	2.7	0.1–1.0
Donepezil	(120014–06-4)	4.7	480								0.3
Erythromycin	(114–07-8)	3.06	300	0.4–0.8				0.4–3.8	0.6–1.8	0.6	0.6
Fenofibrate	(49562–28-9)	5.2	200		0.2–0.3						
Fexofenadine	(83799–24-0)	2.81	$2 \times 10^5$	0.3–18.2	2.4–13.4	3.0–14.0	0.2–14.4	34.3–348.9	0.6–395.1	0.8–167.2	0.2–167.2
Gabapentin	(60142–96-3)	–1.1	$1 \times 10^6$	0.5	0.3–0.6	0.3–0.4		0.5–9.1	0.4–7.1	0.4–7.3	0.4–2.7
Glibenclamide	(10238–21-8)	3.754	63						0.2	0.2	
Irbesartan	(138402–11-6)	5.31	$7 \times 10^5$	0.4–0.8	0.3–0.6	0.3–0.5	0.3–0.4	3.3–36.5	2.3–36.5	0.3–28.9	2.1–18.2
Lamotrigine	(84057–84-1)	0.99	8000	1.2–11.7	0.2–8.3	0.7–7.4	0.9–11.1	2.3–304.0	1.1–125.9	0.4–182.1	1.5–167.2
Loperamide	(53179–11-6)	5.15	$1.4 \times 10^5$		0.4			0.5–0.6	0.4	0.3–1.3	
Memantine	(19982–08-2)	3.28	1840				0.3	0.5–1.7	0.4–2.1	0.4–3.5	0.3–1.8
Metoprolol	(51384–51-1)	1.88	8600		0.3–0.4				0.1–0.3	0.3	
Metoprolol acid	(56392–14-4)	–1.5	47,700	0.6–0.7	0.3–1.2	0.5–1.0	0.2–0.4	1.1–11.9	1.2–13.1	0.5–36.5	0.4–6.4
Miconazole	(22916–47-8)	6.1	25							0.6	
Mirtazapine	(61337–67-5)	2.9	1000	0.4–0.6	0.3			0.7–9.7	0.2–3.2	1.2–1.5	1.1–1.7
N-Desmethylcitalopram	(62498–67-3)	2.8	500	0.4–0.8	0.6–1.4	0.3–0.5	0.4–0.6	1.4–19.8	1.0–30.4	0.8–14.1	0.8–9.0
Norsertaline	(87857–41-8)	4.3	140		0.1–0.5			2.3	0.2–6.7	1.4–11.4	0.6–1.0
O-Desmethylvenlafaxine	(93413–62-8)	2.72	880	2.1–3.2	0.9–2.0	2.1–3.8	1.4	6.7–96.7	1.0–40.9	0.6–71.4	3.2–54.7
Oxazepam	(604–75-1)	2.24	370		0.35			0.5–2.73	0.6–4.6	0.6–4.7	0.4–3.0
Oxcarbazepine	(28721–07-5)	1.11	2950	0.7	0.2–0.6	0.4–0.8	0.4–0.6	0.7–13.4	0.8–27.4	0.6–27.4	0.4–10.6
Propranolol	(525–66-6)	3.48	411	0.9–1.7	0.4–1.4	0.4–0.7	0.29–0.3	2.4–16.7	1.0–22.8	0.4–13.7	0.3–4.4
Rosuvastatin	(287714–41-4)	2.48	1800		0.4–0.5		0.1	0.9–2.9	1.1–1.5	0.5	0.1
Sertraline	(79617–96-2)	5.29	9.4		0.1–0.4			4.9–7.4	0.2–7.1		0.8–1.0
Sotalol	(3930-20-9)	0.24	6520	0.5–1.4	0.5–0.6	0.7–1.2	0.2–0.6	1.1–8.2	0.9–5.8	0.8–9.4	0.4–5.2
Sulfadiazine	(68–35-9)	–0.09	1000					0.4			

(continued on next page)



Table 2 (continued)

Compound	CAS RN	log $K_{ow}$	PNEC ng L <sup>-1</sup>	River Itchen ng L <sup>-1</sup>				River Test ng L <sup>-1</sup>			
				Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
Sulfamerazine	(127-79-7)	0.14	680					0.3			
Sulfamethazine	(57-68-1)	0.14	30,000					0.5-0.6	0.4-0.5		
Sulfamethoxazole	(723-46-6)	0.89	600	0.4-0.9	0.3-3.2	0.2-1.8	0.2-1.2	0.6-12.9	0.3-25.8	0.3-59.3	0.2-15.2
Sulfapyridine	(144-83-2)	0.35	460	3.8-5.9	0.1-4.3	1.7-2.6	1.2-2.9	10.3-74.5	0.1-89.7	0.1-121.6	6.1-65.4
Tamoxifen	(10540-29-1)	7.1	77							0.3	
Telmisartan	(144701-48-4)	3.5	49,000					0.4-10.6	0.6-12.3	4.9-5.3	0.5-1.7
Tramadol	(27203-92-5)	3.01	8650		0.02-1.1	1.1-1.7	0.3-0.8		0.03-24.1	22.8-31.9	0.3-19.8
Trimethoprim	(738-70-5)	0.91	120,000	0.5-1.0	0.4-0.6		0.2-0.3	0.7-3.0	0.5-4.3	2.4-3.6	0.4-1.8
Valsartan	(137862-53-4)	4	$5.6 \times 10^5$	0.9-3.2	0.8-1.3	0.6-0.9	0.2-0.24	1.8-9.4	0.8-12.3	0.6-12.0	0.3-8.1
Venlafaxine	(93413-69-5)	3.28	880	1.3-2.6	0.4-1.2	0.8-1.3	0.5-0.7	6.5-46.8	1.3-30.4	0.9-27.4	1.4-16.7
Verapamil	(52-53-9)	3.79	2530		0.4-0.5					0.5	

grouping chemicals on their mode of action is likely to be unfeasible (Syberg et al., 2009). Where environmental mixtures are at low effect levels, the CA concept is the most conservative and the default model for mixture toxicity assessment (Syberg et al., 2009). The CA effect equation (Eq. 3) was used to calculate the total HQ for a mixture of compounds in a sample for each site and season (Ginebreda et al., 2010). Throughout the manuscript this is referred to as  $\sum HQ$ .

$$\sum HQ_i = \sum^j hq_{ij} \quad (3)$$

where:  $\sum HQ_i$  = the hazard quotient of the sample  $i$ ;  $i$  = the sample;  $hq_{ij}$  = the HQ of the compound  $j$  in sample  $i$ .

### 3. Results and discussion

#### 3.1. Chemcatcher deployments

Chemcatcher devices were deployed in duplicate for two 14-day periods over four seasons at 17 sites on the two rivers; totalling 136 deployments and 272 Chemcatcher devices. The first set of samplers deployed in the winter in the River Itchen remained in the water for 19 days as storms prevented their retrieval (Table S8). One of the duplicate samplers was lost in the field due to damage to the PES membrane. This low loss rate (0.4 %) was comparable to other monitoring programmes that used passive samplers (Taylor et al., 2021; Bernard et al., 2019). The degree of biofouling on the PES membrane was limited and varied between seasonal deployments. The PES membrane has been shown to be resistant to biofouling due to its low surface energy. Furthermore, the relatively short deployment periods (14 days) minimised the impact of biofouling. Across the seasons the flow rate in the two rivers varied between 0.09 and 0.66 m s<sup>-1</sup> (Table S10 and Table S11). Hence, we were able to use the generic Chemcatcher median sampling rate of 0.047 L day<sup>-1</sup> calculated by Römerscheid et al. (2023), where their flow velocity for the median uptake rate laid between 0.1 and 0.5 m s<sup>-1</sup>.

#### 3.2. Rainfall, river flow and water quality parameters

The daily rainfall during the deployment periods is shown in Fig. S2. There were a number of heavy periods of rainfall during the winter sampling period, however, this did not appear to impact the river flow rates. The mean river flow rates across the seasons for each river catchment are shown in Tables S10 and S11. The Itchen and Test are groundwater fed rivers (Mainstone, 1999), where the flow rate is usually greater in the winter season and lowest in the spring season. The depth of water at the sampling sites only varied by ~25 cm over the seasons in line with changes in river flow and being groundwater fed (Tables S12 and S13).

Water temperature (Tables S14 and S15) varied throughout the year from a minimum of ~8 °C in late autumn to ~19 °C in the summer. No large variations across sampling sites were observed. Dissolved oxygen (Tables S16 and S17) increased in the spring and summer due to photosynthesis. In the River Itchen there were pronounced declines at sites I5 which was downstream of a fish farm and I6t which was a shallow tributary. The pH (Tables S18 and S19) varied throughout the year from a minimum of 7.3 in the winter to a maximum of 8.6 in the summer in line with a chalk stream catchment. A decrease in pH was seen at sites I5 and I6t which had also observed decreased oxygen saturation. A decrease in pH across the seasons was observed at T6 which was downstream of two WWTP discharge pipes. Conductivity (Tables S20 and S21) showed similar trends to pH but with an increase at sites I5, I6t and T6.

#### 3.3. Polar contaminants quantified using Chemcatcher

Across the study, 121 polar organic compounds (see Tables 1 and 2 for list) including metabolites and transformation products (range of log

$K_{ow}$  – 1.5 to 7) were identified and quantified using internal standard technique (Nováková et al., 2023). Of these, 58 were classified as pharmaceuticals (National Health Service (NHS) website: <https://www.nhs.uk/>), three classified as personal care products (EU REACH information on chemicals website: <https://echa.europa.eu/information-on-chemicals>) and 60 were classified as plant protection products (University of Hertfordshire Pesticide Database website: (<https://sitem.herts.ac.uk/aeru/ppdb/>)).

The mean LOQ ( $\text{ng L}^{-1}$ ) for each seasonal sampler deployment and compound is given in ESM 2 Tables 1–2. The mass (ng) of pollutant accumulated per Chemcatcher HLB disk across the sampling campaign is given in ESM 2 Tables 3–10. The calculated TWA concentrations ( $\text{ng L}^{-1}$ ) using the Chemcatcher median sampling rate of  $0.047 \text{ L day}^{-1}$  (Römerscheid et al., 2023) for all the pollutants detected are given in ESM 2 Tables 11–12. Spatial and temporal variations of the compounds detected and quantified are discussed in the subsequent sections.

### 3.4. Temporal variation of pollutants

To assess the temporal variation the contaminants were grouped into the four seasons for PPCPs and PPPs across the two river catchments. The percentage detection for each compound over the seasons is given in ESM 2 Tables 13 and 14. We investigated the compounds where the percentage detection was  $>50\%$  in any season for each river.

Out of the 61 PPCPs (16 therapeutic categories) quantified, six had a detection percentage  $>50\%$  in both rivers and three in individual rivers across all seasons. The anti-epileptic drugs carbamazepine (and its two metabolites) and lamotrigine were detected in every season in both rivers. This detection frequency was similar to Burns et al. (2018) who detected carbamazepine in every season in a study on two rivers in Yorkshire, UK. White et al. (2019) sampling only in the winter detected carbamazepine in 95 % of samples from the River Thames, UK. The antibiotic sulfamethoxazole was detected at a frequency  $>50\%$  for all seasons across both rivers, except in the summer in the River Itchen. Sulfapyridine was detected  $>50\%$  in the River Test in the winter and spring and  $<50\%$  in the summer and autumn indicating the seasonal use and potential higher removal during the wastewater treatment warm season (Golovko et al., 2014). The other antibiotics quantified were found predominately in the autumn and winter. This is in contrast to a study (Moreno-González et al., 2014) where the highest concentrations of antibiotics were found in spring.

Caffeine was detected in all seasons with the highest frequency observed in the winter for both rivers. This compound is a ubiquitous pollutant and is often used a tracer for indicating anthropogenic activities (Buerge et al., 2006). The antihistamines, fexofenadine and cetirizine, were detected in all seasons with the highest frequency observed in spring and summer highlighting their seasonal use when the pollen count is expected to be high (Golovko et al., 2014). Another important drug, venlafaxine (antidepressant) and its metabolite *o*-desmethylvenlafaxine (both on the EU Commission Implementing Decision WL, (European Commission, 2022)) was detected in all seasons for both rivers. Both of these were detected previously by Robinson et al. (2022) in the River Itchen and River Test.

The transformation product 5-methyl-1H-benzotriazole of the anti-corrosive agent 1H-benzotriazole (classified as a personal care product) was detected in every season in both rivers at a mean frequency  $\sim 80\%$ . The parent product was only detected at a mean frequency of  $\sim 25\%$ .

This agrees with other studies conducted on UK and European rivers (Giger et al., 2006; Janna et al., 2011). A likely source of this anti-corrosive agent is road run-off in addition to WWTP effluents (Reemtsma et al., 2010).

Out of the 60 PPPs quantified, there were: 19 % fungicides, 39 % herbicides, 32 % herbicide transformation products and 10 % insecticides. The domination by herbicides was expected in this rural catchment where cereal crops and improved grassland predominate. Of those PPPs quantified, 12 were found at a frequency  $>50\%$ . The banned herbicides atrazine and simazine (European Commission, 2004 (EU 91/414/EEC)) together with their transformation products were detected in every season in both rivers. The parent herbicides had 100 % detection frequency. These legacy PPPs are ubiquitous in groundwater and are continuously entering the rivers via springs. These findings agree with other studies in rivers in Germany (Römerscheid et al., 2024) and the UK (Robinson et al., 2022). Another banned herbicide, diuron, was detected in all seasons, but only in the River Test. The highest detection frequencies were in the autumn and winter.

The herbicide chlorotoluron was detected across all seasons with the highest detection frequency occurring in winter ( $\sim 90\%$ ). This is used to control broad-leaved weeds and grasses with a number of applications including winter wheat (plant protection products usage statistics FERA website: <https://pusstats.fera.co.uk/published-reports>). The herbicides bentazone used to control annual weeds ( $\sim 96\%$  frequency) and metazachlor (and associated transformation products) were detected across all seasons. Metazachlor detection frequencies were lower (28–50 %) in the summer compared to the autumn and winter (72–94 %) as this product is used to protect oilseed rape and is applied predominately in the autumn (<https://pusstats.fera.co.uk/published-reports>). This agrees with Warner et al. (2021) who detected metazachlor in the late autumn and winter in a catchment in Germany. The transformation product metazachlor-ethane sulfonic acid (ESA) was detected at higher frequencies than the parent compound. This indicates the importance of measuring both parent and environmental breakdown products in the analysis. This is further indicated by the herbicide, terbutylazine, which had low detection frequencies across the seasons (0–33 %) compared to the transformation product 2-hydroxy-terbutylazine (89–100 %).

The insecticide, imidacloprid, was detected throughout the year in both rivers. This product is currently used as an ectoparasiticide to control fleas on companion animals (Perkins et al., 2024). Imidacloprid is no longer approved for outdoor use (European Commission, 2018 (EU 2018/783)), but is permitted for use in greenhouses (European Commission, 2018 (EU 2018/783)) and used in bait products (Goulson, 2013). This compound has been detected as a ubiquitous pollutant in a number of rivers throughout England (Perkins et al., 2021; Preston-Allen et al., 2023). In the winter there were a number of fungicides (carbendazim, epoxiconazole, propiconazole and herbicides (isoproturon, propazine) that were detected predominately in this period with a high (range of 56–94 %) frequency indicating the seasonal use of these PPPs.

To assess the seasonal variation of both PPPs and PPCPs across the two catchments the data was entered into the Agilent Mass Profiler Professional software (MPP) to undertake multivariate statistical analysis. This enabled Venn diagrams to be produced for the River Itchen (Fig. 2) and the River Test (Fig. 3). For the River Itchen a total of 83 compounds were detected with thirty-five compounds present throughout the year. The greatest number of compounds were found in

**Table 3**  
Sum of average hazard quotient ( $\sum \text{HQ}$ ) for each season and each site on the River Itchen.

Season	I1t	I2	I3	I4	I5	I6t	I7	I8	I9
Autumn	0.31	0.45	0.32	0.78	0.83	0.51	0.62	0.81	0.58
Winter	0.46	0.61	0.34	0.70	0.59	1.08	0.70	1.00	0.84
Spring	0.01	0.29	0.18	0.58	0.62	0.38	0.35	0.66	0.41
Summer	0.01	0.55	0.31	0.50	0.35	0.20	0.18	0.59	1.05



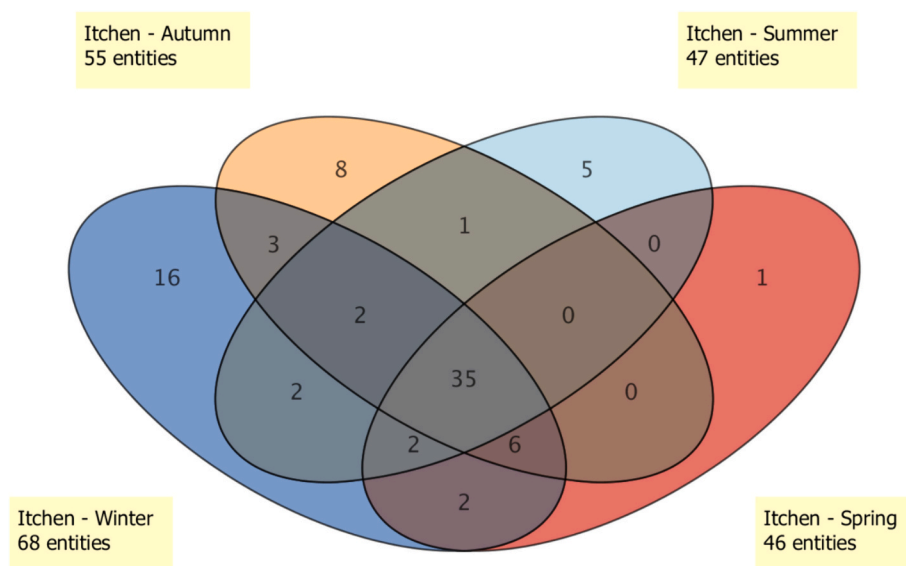


Fig. 2. Venn diagram of the number of compounds detected in each season (spring, summer, autumn and winter) for the River Itchen.

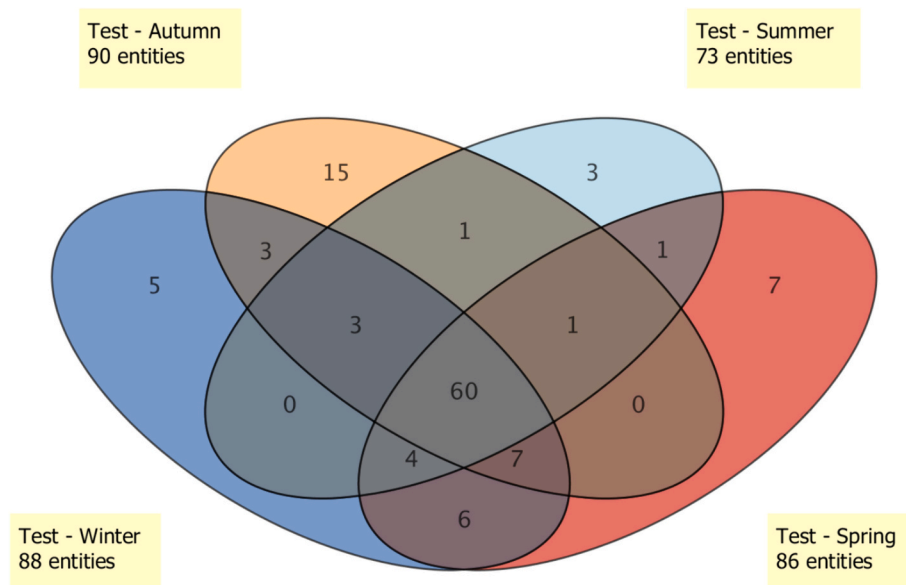


Fig. 3. Venn diagram of the number of compounds detected in each season (spring, summer, autumn and winter) for the River Test.

winter (68 compounds) followed by autumn (55 compounds), summer (47 compounds) and spring (46 compounds). In winter, 16 contaminants were present that were not seen in any other season. Further details on the compounds seasonal detections are given in ESM 2 Table 13. In contrast, a total of 116 compounds were detected in the River Test, with sixty occurring throughout the year. The greatest number of compounds were found in autumn (90 compounds) followed by winter (88 compounds), spring (86 compounds) and summer (73 compounds). In autumn, 15 contaminants were present that were not seen in any other season. Further details on the compounds seasonal detections are given in ESM 2 Table 14. The data has highlighted the seasonal variation of PPPs and PPCPs in both catchments showing the need for monitoring campaigns to be all year round.

### 3.5. Spatial variation of pollutants

To assess the spatial variation of the contaminants (PPP and PPCPs

combined) across both catchments the annual mean number of detections and concentrations ( $\text{ng L}^{-1}$ ) were plotted. The mean annual concentration for each site is shown in Fig. 4. as a pie chart. The mean number of detections are shown in Fig. S3.

For the River Itchen, at the uppermost sampling sites (I1t–I4) the contribution to the total concentration was mainly from PPPs, the greatest contribution at I4. This was as expected due to the rural nature of the catchment area. From site I5 onwards the main contribution switched to PPCPs due to a more urbanised catchment with a higher population density with inputs from point sources including WWTPs and septic tanks and various non-point sources (Standley et al., 2008). This was evidenced by the presence of greater concentrations of caffeine than detected at I8. Site I5 was directly downstream of a fish farm which may be a contributing factor (Alvarado-Flores et al., 2021). Baldiserra et al. (2019) reported caffeine being used as a dietary supplement and an antibiotic for farmed fish. Decreased oxygen levels and pH were observed at this site. The concentration of PPPs was similar across the

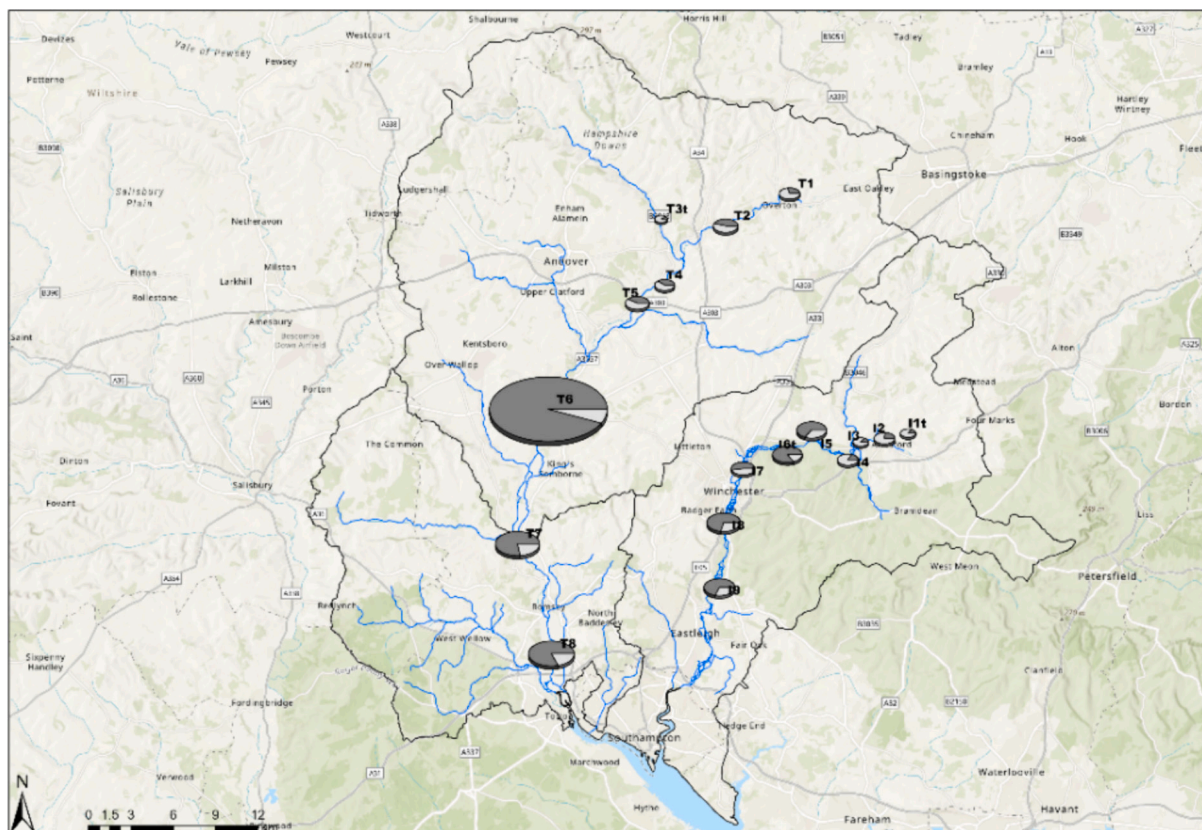


Fig. 4. Pie chart showing annual mean concentration ( $\text{ng L}^{-1}$ ) of PPCPs (dark grey) and PPPs (white) for each sampling location on the River Itchen and River Test.

river with a mean of  $37 \pm 10 \text{ ng L}^{-1}$  and for PPCPs a mean of  $50 \pm 5 \text{ ng L}^{-1}$ . ANOVA analysis and the multi-comparison *post hoc* tests showed sites I5, I6t, I8 and I9 were statistically significantly different to other sites on this river.

For the River Test, only the source site (T1) and the Bourne Rivulet tributary (T3t) had the highest contribution from PPPs. Further down the catchment the total concentration was dominated by PPCPs due to the numerous WWTP inputs both into the subsurface strata and directly into the river. The chemical profile of the River Test was dominated by the large WWTP input (Fullerton) which was upstream of site T6. This site also had the highest number of detections. ANOVA analysis and the multi-comparison *post hoc* tests showed that this site was statistically significantly different to the other sites on this river. The mean annual concentration of PPPs ranged from 33 to  $100 \text{ ng L}^{-1}$ , whilst for PPCPs the range was 2– $1770 \text{ ng L}^{-1}$ .

We detected carbamazepine ( $1\text{--}119 \text{ ng L}^{-1}$ ) and its metabolites (10,11 dihydroxy carbamazepine [ $1\text{--}517 \text{ ng L}^{-1}$ ]; 10,11-epoxide carbamazepine [ $1\text{--}23 \text{ ng L}^{-1}$ ]) and caffeine ( $2\text{--}136 \text{ ng L}^{-1}$ ) in both rivers across all seasons. These compounds are known to be markers of anthropogenic activity particularly from wastewater pollution (Buerge et al., 2006; Dvory et al., 2018). Carbamazepine is not removed in WWTPs either by degradation or by adsorption (Clara et al., 2004) and in some WWTPs the concentrations in the effluent are similar or greater than the concentration in the influent, (Golovko et al., 2014). Carbamazepine was detected at sites on both rivers not directly impacted by discharge from municipal WWTPs, indicating other sources and pathways for this chemical. Other sources include septic tanks or on-site package treatment plants discharging to the river or drain (Comber et al., 2019; Macedo et al., 2022) and aging sewer system infrastructure leaking to the subsurface strata, (Wolf et al., 2012). The highest concentrations of caffeine were observed at two sites on the River Itchen (I5 and I6t) which were downstream of a fish farm. As noted previously, these are the sites where pH and dissolved oxygen concentrations were at

their lowest indicating that fish farms have potential to impact on water quality (Alvarado-Flores et al., 2021).

It was interesting to observe that the herbicides atrazine and simazine which were banned for use in the EU in 2003 were detected in every sample. The likely source of these legacy compounds was from contaminated groundwater as has been reported by others (Sassine et al., 2017; Allen, 2017). Concentrations of atrazine ( $0.2\text{--}18 \text{ ng L}^{-1}$ ) and simazine ( $0.2\text{--}10 \text{ ng L}^{-1}$ ) were low and did not vary considerably across the catchment. The transformation products of atrazine (desethyl-atrazine; desisopropyl-atrazine; desethyl-desisopropyl-atrazine; 2-hydroxy-atrazine) and simazine (terbutylazine; propazine) were also frequently detected but with some seasonal variability.

### 3.6. PNEC and toxicity

The TWA concentrations of the contaminants found using the Chemcatcher were sub-divided into the two rivers and four seasons and the values compared to the PNEC values (lowest PNEC freshwater) obtained from NORMAN database (Tables 1 and 2).

Two of the PPPs (imidacloprid and 2-hydroxy-terbutylazine) detected were above the PNEC value. The imidacloprid PNEC value of  $13 \text{ ng L}^{-1}$  was exceeded in the River Test in all four seasons with concentrations reaching a maximum of  $24 \text{ ng L}^{-1}$ . In the River Itchen the concentrations were on average an order of magnitude lower. The highest concentrations found were at sites impacted by WWTPs. However, it was also detected at other sites not in close proximity to the direct discharge from a WWTP. Sources of imidacloprid include excretion from pets, washing of pets who have received treatment and the washing of pet bedding (Perkins et al., 2021; Wells and Collins, 2022). There have been a number of studies investigating the impact of imidacloprid in the aquatic environment.

These include a decline in abundance of invertebrate species (Yamamuro et al., 2019), a reduction in the abundance of flying insect

larvae (Van Dijk et al., 2013) morphological malfunctions in frog tadpoles (García Samojeden et al., 2022), and an impact on growth, moulting interval, survivorship, behavioural change and body colour change of brown shrimp (*Farfantepenaeus aztecus*) (Al-Badran et al., 2019). Recent work has shown that imidacloprid can adversely impact stream macroinvertebrate communities (Nowell et al., 2024; Schmidt et al., 2022).

2-Hydroxy-terbutylazine, is a transformation product of terbutylazine and is used as both an herbicide and a biocide on building facades (Paijens et al., 2019). The PNEC value of  $7.3 \text{ ng L}^{-1}$  was exceeded in the spring at T5 and the summer at I8 and T8. In the other instances the concentration found was near the PNEC value. Laboratory studies have shown this PPP can have an impact of the growth rate of common carp (*Cyprinus carpio L.*) (Velisek et al., 2014) and physiological damage to red swamp crayfish (*Procambarus clarkii*) (Stara et al., 2016).

A number of PPPs (alachlor, atrazine, diuron, isoproturon, simazine and terbuthryn) that were detected are priority substances listed in the EU Directive (2013/39/EU) (European Commission, 2013) and are required to be monitored. All of these PPPs detected in our study were below their respective maximum allowable concentration and also their PNEC value.

Two of the PPCPs (azithromycin and diclofenac) detected were above the PNEC value and a third (alprazolam) was near to its PNEC value ( $77 \text{ ng L}^{-1}$ ). For azithromycin the lowest PNEC ( $19 \text{ ng L}^{-1}$ ) (ESM 3 Table 4) was exceeded in the autumn at  $106 \text{ ng L}^{-1}$ . Studies of this PPCP, used to treat Covid-19, have reported it reduced the levels of protein in adult zebrafish (*Danio rerio*) (Mendonça-Gomes et al., 2021) and affected the growth rate, cell viability and photosynthesis of microalga (*Raphidocelis subcapitata*) (Almeida et al., 2021). The lowest PNEC for diclofenac was  $50 \text{ ng L}^{-1}$  this was exceeded in the autumn at  $51 \text{ ng L}^{-1}$ , the winter at  $70 \text{ ng L}^{-1}$  and the spring at  $52 \text{ ng L}^{-1}$  all at site T6. Diclofenac has been recorded to affect the reproduction in fish (*Astyanax lacustris*) (Godoi et al., 2024); the respiration rate, fecundity and development of amphipods (*Gmelinoides fasciatus*), and affects the heart rate of molluscs (*Unio pictorum*) (Berezina et al., 2022).

Alprazolam (a benzodiazepine) is a veterinary and human tranquiliser not approved for use in the UK or the EU, (<https://sitem.herts.ac.uk/aeru/ppdb/>) it is classified in the UK as a Class C drug under the Misuse of Drugs Act 1971. (<https://www.legislation.gov.uk/ukpga/1971/38/contents>). It was only detected in the summer at two sites (I8 and T5) probably from recreational use. Alprazolam has been found to affect embryo development in amphibians (Fogliano et al., 2022) and impact behaviour of freshwater fish (Brodin et al., 2017).

A number of PPCPs (azoxystrobin, clindamycin, miconazole and trimethoprim) detected are on the EU Watch List (EU 2022/1307). These were all below their maximum allowable concentrations and also below their PNEC values. However, venlafaxine and *o*-desmethylvenlafaxine which are also on the EU Watch List (EU 2022/1307) were detected above their maximum allowable concentration ( $6 \text{ ng L}^{-1}$ ) but below their PNEC value ( $880 \text{ ng L}^{-1}$ ). Elevated concentrations of these two compounds have been shown to affect the behaviour and mortality of fish (Sehonova et al., 2019), affect the locomotion of freshwater snails (*Lymnaea stagnalis*) (Raman et al., 2024), and both the parent and metabolite caused the malformation of zebrafish (*Danio rerio*) embryos (Rodrigues et al., 2023).

The HQ was calculated (Eq. 2) for the four chemicals (2-hydroxy-

terbutylazine, imidacloprid, azithromycin and diclofenac) that exceeded their PNEC value. The risk for each occasion (site, river, season) the PNEC was exceeded indicated there was a high ecological risk to aquatic organisms (Molnar et al., 2021). Although these four specific compounds were present at concentrations less than the lowest PNEC in other seasons and other sites, they were nevertheless always detected at the same sites. As Vijver and van den Brink (2014) stated that a single plant protection products is unlikely to cause adverse ecological effects, we calculated (Eq. 3 with the mean concentration for each season and site) for the  $\sum\text{HQ}$  for the mixtures detected (Tables 3 and 4).

There were three sites on the River Itchen (Table 3) where the result of eq. 3 was  $\sum\text{HQ} > 1$ . These were I6t and I8 in the winter and I9 in the summer and therefore considered to be a high ecological risk (Molnar et al., 2021). The result for I6t was driven by the presence of azithromycin and 2-hydroxy-terbutylazine. For I8 in the winter and I9 in the summer the result was driven by the presence of 2-hydroxy-terbutylazine. The sites where the  $\sum\text{HQ}$  was  $0.5 < \sum\text{HQ} < 1$  could be considered to be of medium risk. Such sites are I4, I5, I6, I6t, I7, I8 and I9 in the autumn, I2, I4, I5, I7 and I9 in the winter, I4, I5 and I8 in the spring, and I2, I4 and I8 in the summer. These results were also driven by the presence of 2-hydroxy-terbutylazine.

There were four sites on the River Test (Table 4) where the result of eq. 3 was  $\sum\text{HQ} > 1$ . These were T6 for all seasons, T7 and T8 for autumn, winter and summer and T5 for summer and were of an ecological risk (Molnar et al., 2021). At these four sites the results were driven by the presence of imidacloprid and the high concentrations of other pharmaceuticals, especially azithromycin in the autumn and winter. In addition, the  $\sum\text{HQ}$  of  $> 1$  at T5 in the summer was driven by the presence of 2-hydroxy-terbutylazine. Those sites of medium risk were T1, T2, T4, in the autumn and winter, T5 in the autumn, winter and spring.

By using the Chemcatcher we were able to monitor the concentration of polar contaminants for a total of 16 weeks over the four seasons. Hence our monitoring data is more representative than infrequent (e.g. annually) spot samples that are normally undertaken to assess ecological risk in riverine environments. It should be recognised that other contaminants are likely to be present that were not on our targeted list of chemicals quantified.

#### 4. Conclusions

This study provides the first quantitative characterisation of PPPs and PPCPs in two sensitive and protected chalk stream river catchments over a 12-month period. Using Chemcatcher and a LC/MS targeted analysis approach we quantified 121 polar organic compounds having a wide range of physico-chemical properties. Data indicated the presence of pollutants in both rivers at concentrations ranging from  $\text{sub ng L}^{-1}$  to  $> 500 \text{ ng L}^{-1}$ . We noted seasonal differences in the concentrations of PPCPs with the highest values in the winter and spring and for PPPs the highest concentrations were found in the autumn and winter. The number of contaminants detected was different between the two catchments. The River Test had 116 compounds compared to the 83 found on the River Itchen. This observation can be attributed to the larger number of WWTP discharges into the River Test. For both catchments, the headwater sampling sites had the lowest number of contaminants detected and their concentrations was also low. The major class found here was PPPs due to the rural landscape and limited waste water inputs. Further down the catchments, where direct waste water inputs occurred, the chemical profile of the rivers was dominated by PPCPs. Highest concentrations were found at sites I8 and T6 which were both downstream of direct waste water inputs. Removal of organic contaminants within WWTP processes are often substantial (i.e., a reduction in concentrations between influent and effluent flows). However, for many polar substances this is not the case, as highlighted by our data. This highlights the need for further investment in the treatment of effluents that discharge into protected and sensitive aquatic environments.

**Table 4**  
Sum of average hazard quotient ( $\sum\text{HQ}$ ) for each season and each site on the River Test.

Season	T1	T2	T3t	T4	T5	T6	T7	T8
Autumn	0.81	0.54	0.27	0.64	0.62	10.26	1.43	1.85
Winter	0.73	0.76	0.34	0.73	0.92	5.58	1.31	1.30
Spring	0.15	0.46	0.24	0.33	0.98	4.39	0.84	0.15
Summer	0.34	0.41	0.29	0.39	1.40	2.73	1.18	1.10



Five contaminants (2-hydroxy-terbutylazine, alprazolam, azithromycin, diclofenac and imidacloprid) were found to be above or close to their PNEC values according to the NORMAN database. In addition, venlafaxine was detected above its Watch List concentration. All of these individual compounds have been shown to have ecotoxicological effects on a range of aquatic biota. Of most concern was the presence of the ectoparasiticide imidacloprid which is designed to have a toxicological effect on invertebrates. Our findings highlight the need for this substance to be controlled in its use. It should be remembered that PNEC assesses the toxicity of an individual chemical over a short time interval and takes no account of either long term exposure or pollutant mixtures. The long-term effects on macroinvertebrates of the cocktail of pollutants found in our study are unknown and warrant further investigation.

### CRedit authorship contribution statement

**Rosamund F.A. Robinson:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Graham A. Mills:** Writing – review & editing, Supervision, Methodology. **Roman Grabic:** Writing – review & editing, Resources, Investigation, Data curation. **Adam Borík:** Writing – review & editing, Resources, Investigation, Data curation. **Gary R. Fones:** Writing – review & editing, Visualization, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

Data will be made available on request.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.173316>.

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