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Occurrence, patterns and previously overlooked sources of three veterinary ectoparasiticides in rural and urban Welsh rivers☆

Molly Hadley [a], Laura Rodwell [a], Matt Stewart [a], Daniel Crowther [a], Jasper Linley-Adams [a], Sian Craig [b], Suzanne Thomas [b], Anthony Gravell [b], Isabelle Durance [a], Ian P. Vaughan [a], S.J. Ormerod [a,*]

[a] Water Research Institute, School of Biosciences, Cardiff University, Cardiff, CF10 3AX, UK
[b] Natural Resources Wales, Swansea University, Singleton, SA2 8PP, UK

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ABSTRACT

Chemicals from pet flea-treatments or sheep-dips sometimes exceed no-effect concentrations in rivers. We investigated three such compounds – imidacloprid, fipronil and diazinon – in nine Welsh rivers during 2021–2023. We analysed 140 grab samples using ultra high-performance liquid chromatography with quadrupole-time-of-flight mass spectrometry (LC/Q-TOF-MS) to assess how concentrations varied i) within and among rural and urban rivers in relation to wastewater inputs; ii) with an indicator of wastewater contamination, caffeine, and iii) with flow. We assessed fish and macroinvertebrate communities along a concentration gradient in the most contaminated stream. Imidacloprid (0–76 ng/L) occurred in 77 % of samples and fipronil (0–35 ng/L) in 44 %. Odds of detection were 26X and 8X greater in urban than rural sites for imidacloprid and fipronil, respectively, exceeding predicted no-effect concentrations (PNECs) in 38 % and 44 % of urban samples. Both compounds increased downstream in urban reaches i) receiving wastewater outfalls and ii) where sewer misconnections apparently impacted invertebrate communities. Significant correlations with caffeine confirmed links with wastewater. Imidacloprid, fipronil and caffeine were modelled effectively from Wastewater Treatment (WWTP) discharge, but model residuals were consistent with additional effects from misconnected sewers. In contrast, diazinon occurred patchily linked to livestock farming in the Wye (174 ng/L), Tywi (29 ng/L) and Ely (94 ng/L). Flow effects on all concentrations were weak.

These data provide important support for the role of 'down the drain' routes through which compounds used as pet flea-treatments reach British rivers, for the first time revealing that misconnected sewers might increase imidacloprid concentrations sufficient for observable biological effects.

1. Introduction

Urban rivers across Europe and the UK improved in biological quality from the late 1980s/early 1990s onwards following deindustrialisation alongside improved regulation to reduce insanitary pollution (Council Directive 91/271/EEC; Vaughan and Ormerod, 2012; Whelan et al., 2022). More recent assessments suggest that these improving trends have since slowed, stalled or even reversed, raising questions about the factors responsible (Haase et al., 2023; Pharaoh et al., 2023, 2024). While there may be intrinsic limits on the rates of biological recovery from pollution, other candidate explanations include the effects of legacy contaminants (Windsor et al., 2019a), climate change (Moss et al., 2011; Charlton et al., 2018), poorly performing wastewater infrastructure (Perry et al., 2024) and new or 'emerging' contaminants such as microplastics, pesticides and pharmaceuticals which might have biological effects even at low concentrations (Kumar et al., 2023; Lambert and Wagner, 2017). Among the latter group, attention has turned recently to several chemicals for which uses are now dominated by ectoparasite control in domestic pets (= companion animals) or livestock. Our specific focus in this paper is on three such compounds: imidacloprid and fipronil, currently implicated in environmental release through their use as pet flea-treatments (Perkins et al., 2021a); and diazinon, a compound currently in wide use in sheep-dips and in some anti-parasitic products for cattle (Sharpe et al., 2006;

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* Corresponding author.
E-mail address: ormerod@cardiff.ac.uk (S.J. Ormerod).

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Azzouz et al., 2021).

As systemic insecticides, fipronil (phenylpyrazole) and imidacloprid (neonicotinoid) have a range of uses globally in agriculture, horticulture and forestry, and together are the most widely used insecticides worldwide (Simon-Delso et al., 2015). Rather than targeting specific insects, however, they have potent neurotoxic effects on a wide range of invertebrates so that collateral impacts are possible once they reach the wider environment (Al-Badran et al., 2019; Domigo-Echaburu et al., 2021). These non-target effects on pollinators (vanEngelsdorp et al., 2009; Whitehorn et al., 2012; Laycock et al., 2014) led the European Commission to remove the use of imidacloprid in outdoor agriculture in 2018 (European Commission, 2018a; European Commission, 2018b). In the UK, fipronil and imidacloprid are respectively banned in outdoor agriculture or restricted to emergency use (eg FERA, 2020). Nevertheless, both are used widely and often prophylactically to control ectoparasites such as fleas (Siphonaptera) and ticks (typically *Ixodes* spp.) on domestic dogs, cats, rabbits and ferrets (Perkins and Goulson, 2023). In recent years, in excess of 3.5 million doses of products containing these two chemicals were sold annually mostly for the UK's 23 million dogs and cats, equivalent to a mass exceeding 6000 kg (Wells and Collins, 2022).

Linked to their widespread use, field data have revealed that chemicals associated with pet flea-treatments are widely detectable in English rivers (Perkins et al., 2021a; Richardson et al., 2022; Robinson et al., 2023). Perkins et al. (2021a), for example, recorded fipronil or its metabolites in over 95 % of over 1300 samples from 20 English rivers and imidacloprid from around two thirds, with both compounds exceeding thresholds for chronic toxicity on multiple occasions. Although these data on occurrence alone are insufficient to implicate veterinary sources, patterns of increasing concentration in urban wastewater or near to outfalls suggest that down-the-drain pathways and sewer networks are important routes of entry into surface waters (Sadaria et al., 2016; Perkins et al., 2021a). This is consistent with patterns of pet ownership for in the UK for dogs (29 % of adults own at least 1 dog) and cats (24 % of adults own at least 1 cat) which is dominantly urban (PDSA, 2023). Moreover, experimental investigations to assess emissions from treated dogs or their owners illustrate that releases to sewers are likely via washing of household pets following pesticide treatment, handwashing by owners and washing of pet beds (Diepens et al., 2023; Perkins et al., 2024). These concerns in the UK have been sufficient to prompt action on the part of the UK government who recently launched a plan to address the presence of chemicals from pet flea and tick treatments in UK waterways (https://www.gov.uk/government/publications/cross-government-pharmaceuticals-in-the-environment-group-roadmap).

Some uncertainties, nevertheless, remain. For example, some of the available data on environmental occurrence pre-date regulatory controls on agricultural use (Taylor et al., 2021) while there may still be residues from previous applications or possible release from imported goods (eg Bennett & Weeks, 2021). The suggestion that pet flea-treatment applications might not be sufficient to explain the observed patterns of imidacloprid in wastewaters has been mostly answered (Anthe et al., 2020; Perkins et al., 2021b), but in some urban locations elevated concentrations can occur independently from obvious wastewater outfalls (Anthe et al., 2020). Possible explanations in these circumstances include combined sewer overflows, through which untreated wastewaters by-pass treatment (Perry et al., 2024; Ramage et al., 2025), or household sewers that are misconnected to surface drains rather than foul sewer networks (Ellis and Butler, 2015; Revitt and Ellis, 2016). The possibility that imidacloprid or fipronil could reach surface waters through this route requires targeted sampling at appropriate spatio-temporal scales, for example in relation to land use or changing discharge, and ideally would be supported by indicators of anthropogenic wastewater contamination such as caffeine (Buerge et al., 2003).

Among other ectoparasiticides, the organophosphate insecticide, diazinon (=dimpylate), is licensed for use in the UK in some pet flea-treatment products, for example as treated, wearable collars, and for this reason warranted inclusion in our study. In contrast to imidacloprid and fipronil, however, its dominant current uses in the UK are in agricultural livestock such as cattle and more widely as to control a range of ectoparasites on sheep by dipping or spraying. Diazinon was developed in the 1950s as the organochlorine insecticide DDT was phased out (Gómez-Canela et al., 2017; Sergi, 2019). It has broad-spectrum efficacy, medium-to low-toxicity and low bioaccumulation properties (Wu et al., 2021) but occurs widely in groundwaters and surface water where it can pose significant risks to non-target species, including aquatic organisms (Cao et al., 2018). Locally elevated levels exceeding chronic toxicity thresholds for invertebrates have sometimes been reported (Poyntz-Wright et al., 2024). Current interest is heightened in Wales because of an ongoing initiative to eradicate sheep scab (= the mite *Psoroptes ovis*) (Paton et al., 2022). Diazinon is a key treatment for this parasite, but its use could lead to surface water contamination following disposal (see https://businesswales.gov.wales/farmingconnect/news-and-events/technical-articles/ectoparasites-sheep-sheep-scab). For all these reasons, we included diazinon in our study to assess likely contrasts in its geographical distribution relative to imidacloprid and fipronil.

Given current interest in the prevalence and environmental effects of parasiticides (Perkins et al., 2021a; Wells and Collins, 2022), in this study we aimed to extend understanding of i) pathways through which imidacloprid, fipronil and diazinon reach and move through aquatic systems; ii) variations in concentrations between urban and rural freshwater environments and iii) the effects of varying river flow (i.e. discharge). Specifically, we collected samples over three years (2021–2023) from 62 locations on nine Welsh rivers to test the hypotheses that.

1) Concentrations of imidacloprid, fipronil and fipronil sulfone would be greater in urban than in rural river systems reflecting wastewater sources from larger WWTPs (Wastewater Treatment Plants), combined sewer overflows or misconnected sewers.
2) Concentrations of diazinon would be greater along rural river systems linked to livestock rearing
3) Patterns of occurrence would be reflected in contrasting relationships with indicators of wastewater. Specifically, concentrations of imidacloprid and fipronil should increase i) with caffeine concentration, as a widely recognised indicator of wastewater contamination (Hillebrand et al., 2012; Spence, 2015) and ii) where inputs from wastewater sources were greatest. Diazinon should show no such influences.
4) Parasiticide concentrations would fluctuate with increasing river flow, reflecting either dilution or mobilisation.
5) Pollution-sensitive invertebrates (shown by tolerance metrics) and densities of three locally widespread fish species (Brown Trout *Salmo trutta;* Stone Loach *Barbatula barbatula* and Bullhead *Cottus gobio*) would decline with increasing ectoparasiticide concentrations where predicted no-effect concentrations (PNEC) were exceeded.

Our justification for hypothesis 5 was based on expectations from laboratory toxicity data (ECHA, 2011, 2015; NORMAN, 2025).

We focussed specifically on rivers in mid and south Wales where published data on ectoparasiticides are scarce, where a gradient from rural to urban conditions was available, and where our previous work has assessed legacy pollutants (Windsor et al., 2019a, 2019b; 2020) and other emerging contaminants (Windsor et al., 2019c; D'Souza et al., 2020).

2. Methods

2.1. Study area

Wales occupies a maritime, temperate location in western Britain with annual mean temperature of 9.5–11 °C and mean annual rainfall of

~1385 mm increasing to 2000–3000 mm at higher elevation in three upland massifs. A radial array of rivers mostly drains a combination of mixed, pastoral or livestock farming which covers 80 % of Wales and currently supports more than 10 million sheep and a million cattle (Welsh Government, 2025). Woodlands cover around 15 %, while urban land covers around 5 % mostly in the south, particularly around the formerly industrial south Wales valleys and the capital city of Cardiff. Population densities increase from 0 to 200 people/km^2 in rural Wales to over 900 people/km^2 in Cardiff, while around a third of households have a least one pet dog, 20 % at least one cat, and 2 % a rabbit (PDSA, 2023).

On the basis of these land use patterns, we selected three groups of rivers for sampling in mid and south Wales (Fig. S1a) that were, respectively: i) urban rivers influenced by combined sewer overflows (CSOs) and WWTPs (Taff: 11 sites; Ely: 5 sites; Clun: 3 sites); ii) small urban streams unaffected by discharge from CSOs or WWTPs but potentially at risk from sewer misconnections (Roath Brook: 7 sites; Whitchurch Brook: 5 sites; Nant Glandulais: 4 sites) (Fig. S1b; Table S1) and iii) rural rivers, dominantly draining agricultural land (dominantly livestock farming) with smaller urban settlements and smaller WWTPs (Wye: 11 sites; Usk: 6 sites; Tywi: 10 sites) (Fig. S1c; Table S1).

The urban rivers Clun, Ely and Taff have catchments of 155–526 km^2 that drain the formerly industrial coalfields of South Wales and were grossly polluted by a combination of sewage and colliery-related pollutants up until the 1970s (Scullion & Edwards, 1980; Murphy & Edwards, 1982). All three have since recovered biologically, consistent with other urban rivers in England and Wales (Vaughan & Ormerod, 2012; Pharaoh et al., 2023), but previous data show them to be contaminated by pharmaceutical compounds linked to wastewater sources, for example at Cilfynydd, Coslech and Rhiwsaeson WWTPs (Kasprzyk-Hordern et al., 2008, 2009). The smaller urban rivers (Roath Brook, Whitchurch Brook and Nant Glandulais) drain catchments of < 5–10 km^2 incorporating parts of the city of Cardiff as well as surrounding rural areas that allow an assessment of changes in chemical quality as they enter the city. None receives drainage from WWTPs or CSOs, but the streams receive surface drains which are potential routes for misconnected household waste from domestic appliances or toilets (Ellis and Butler, 2015; Revitt and Ellis, 2016). The rural rivers Wye, Tywi and Usk drain catchments of 515-4,258 km^2 formed from combinations of the Cambrian Mountains and Bannau Brycheiniog, and all three rivers are Special Areas of Conservation notified initially under the EU Habitats Directive (92/43/EEC). We did not sample downstream of the larger settlements in the catchments of these otherwise rural rivers (Hereford, Carmarthen and Newport), though there are smaller WWTPs (see Table S1; Fig. S1).

2.2. *Sampling sites and sample collection*

We collected a total of 140 samples over the nine river systems during the months of July and August 2021–2023 to coincide with periods of greatest risk of ectoparasites as well as the onset of sheep-dipping (Table S1). Logistical and financial constraints meant that studies in 2021 and 2022 were focussed on the Taff (urban river; n = 11 sites) and Roath Brook (small urban stream; n = 7 sites). Work in 2023 involved sampling at a sub-set of these sites while also expanding coverage to include a further 44 sites across the additional seven urban and rural rivers. The resulting coverage allowed an assessment of downstream changes in all rivers (3–11 sites per river), variations across river systems, and variations through time at the most frequently sampled sites. Although our study was not designed to investigate flow conditions, some effects could be appraised opportunistically. We used a flow-gauged location on the River Taff (Natural Resources Wales (NRW, 2023)/National Flow Archive Pontypridd: ST079897) as an index of varying runoff conditions. This was the nearest location to repeatedly sampled sites with daily data on flow volume.

At each site and on each occasion, unfiltered samples were collected into standard, pre-prepared, sterilised, 1L glass bottles. Immediately after collection, bottles were sealed with PTFE-lined plastic caps, stored in opaque containers and, on the same day of collection, temporarily stored at 4 ± 1 °C in a cold room prior to transport for chemical analysis within days of collection.

2.3. *Chemical analysis*

Samples were processed and analysed at the laboratories of Natural Resources Wales Analytical Services (NRWAS) in Swansea, accredited to international industry standards through ISO/IEC 17025:2017 by the UKAS, the UK accreditation body. The overall procedure involved solid-phase extraction followed by Ultra High-Performance Liquid Chromatography (UHPLC) (Taylor et al., 2022; Robinson et al., 2023) focussed on Imidacloprid, Fipronil, its metabolite Fipronil Sulfone (2021 and 2022 only), Diazinon and caffeine – used as a putative indicator of wastewater from human sources.

Solid-phase extraction was performed using sorbent disks in conjunction with a vacuum manifold. Oasis HLB-L SPE disks were selected for their high efficiency in concentrating trace levels of organic contaminants in the environment (below 0.01 μg/L) (Jeong et al., 2017). Prior to sample extraction, each disk was conditioned with 20 mL of high-purity methanol, followed by 20 mL of deionized, ultra-high-purity water. Water samples (500 mL each) were then passed through the disks under vacuum at a controlled flow rate of 10 mL/min or less. After extraction, the disks were dried in a clean room for 24 h to ensure complete removal of residual water and effective isolation of the target organic contaminants.

To elute organic contaminants from the HLB-LSPE disks, 40 mL of methanol solvent was used in increments of 10 mL at a controlled flow rate of 10 mL/min or lower. Subsequently, 250 μL of deionized water was added to the extracted solution which was then concentrated using a ‘Genevac SP Scientific: Rocket’ vacuum evaporator, resulting in a final solution volume of 1 mL (yielding a concentration factor of 500:1 for each sample). This extract was transferred into sealed 2 mL sample vials and stored in a freezer at −18 °C until UHPLC analysis.

Analysis of contaminants in the extracted samples was carried out using UHPLC coupled with high-resolution quadrupole time-of-flight mass spectrometry (HRMS-QTOF). The separation of compounds was performed using a Dionex Ultimate 3000 UHPLC system, comprising of a degasser, binary pump, autosampler, and heated column compartment. This system was interfaced to a ‘Mass Spectrometer Bruker Impact II QTOF’, featuring an electrospray ionization (ESI) source for accurate quantification of contaminant compounds.

Analyte recovery during the procedure was assessed by spiking 500 ml of river water with each compound at 25 ng/L, in all cases returning recovery in excess of 91 % (Fipronil 91.1 %; Imidacloprid 94.6 %, Diazinon 96.3 %). All solvents were of LCMS (liquid Chromatography Mass Spectrometry) grade quality and all sample batches included procedural blanks in each of the three sampling years. None contained the target compounds above detection limits.

2.3.1. *Quantification of contaminant concentrations*

We used Bruker Target Analysis for Screening and Quantification (‘TASQ®, 2021b’ software) for the quantification and identification of target compounds based on criteria including mass accuracy, isotopic distribution, retention time, and diagnostic MS/MS fragmentation patterns. Ion chromatograms from each sample were compared with specific limits for the target compounds in the combined Bruker database (merging ‘PesticideScreener™ 2.1’ and ‘ToxScreener™ 2.1’ databases), including ±7 ppm for mass accuracy, an isotopic fit of <1000 (milliSigma), and ±0.5 min for retention time limit.

Positive identification of each target compound required detection of its molecular adduct, associated isotopes, and at least one characteristic compound fragment ion. For quantification, reference standards with known contaminant concentrations were used to ensure analytical accuracy. Specifically, calibration standards were analysed alongside the field samples at concentrations of 0.05, 0.10, 0.25, 0.50, 1.0 and 2.0ug/L. Given the sample concentration factor during processing of X500, these equate to sample concentrations of 0.1, 0.2, 0.5, 1.0, 2.0 and 4.0 ng/L. This allowed a quantification limit for all measured contaminants of 0.1 ng/L, with concentrations below this threshold considered to be below detection (ie < LD).

As well as the target parasiticides and caffeine, the screening process detected and quantified between around 150 other pharmaceutical agents and pesticides, with many occurring at more than 5 % of sites, but these are not reported in the current paper.

2.4. Invertebrate communities and fish populations

Following initial assessments of chemical patterns, we assessed communities of aquatic invertebrates and fish populations at six morphologically similar sites in the Roath Brook system which had contrasting concentrations of Fipronil and Imidacloprid within a 2 km distance (L1, L2 and RB0 v RB1, RB2 and RB3). We collected invertebrates in autumn 2023 using kick-samples of 3-min duration with a standardised hand net (1 mm mesh), preserving samples on-site in 70 % ethanol before return to the laboratory and identification mostly to species. This is a quality-assured procedure which collects around 70 % of the taxa present at any given site on small streams and is robust enough to distinguish between communities at different locations (Bradley & Ormerod, 2002). Fish densities per square metre were estimated in July 2023 over a 25–30 m reach at each site using standardised, timed, single-pass electro-fishing procedures with backpack equipment at 180v DC and a single anode. This single pass method allows population estimates that are representative of those from more intensive, multiple-pass methods (Reid et al., 2009; Matson et al., 2018).

2.5. Data analysis

Data were analysed using R version 4.2.2 (R Core Team, 2025; Rstudio Team, 2025) and the fit of all models was checked using plots of model residuals. After examining the frequency of occurrence and relative sources of variability for each chemical, we tested each of the hypotheses as follows:

Hypotheses 1 and 2, that imidacloprid, fipronil and diazinon occurrence would differ between urban and rural rivers (see study area), were tested using binomial Generalized Estimating Equations (GEE) models with log link function using the geepack package (Halekoh et al., 2006). GEE are considered more appropriate than mixed-effects models for data with many small clusters of observations, such as along rivers (Vaughan et al., 2007). Detection (presence = 1, absence = 0) was aggregated at the site level across all sampling years, removing temporal autocorrelation. 'River' was used as the clustering factor to account for potential spatial autocorrelation among sites on the same river. Due to the irregular sample spacing across river networks, we used a general 'exchangeable' correlation structure in the GEE models'. This approach assumes a constant within-site correlation, which provides a practical, robust solution given the irregularity of the sampling design. The odds ratios reported indicate how much more (or less) likely a compound is to be detected in urban rivers compared to rural ones. Predicted detection probabilities for both rural and urban sites were calculated by converting the model's log-odds coefficients back to probabilities. We used the same binomial GEE approach to assess the presence/absence of caffeine between urban and rural sites.

Hypothesis 3 was tested first by examining relationships between the concentration of each chemical and caffeine using Pearson correlation tests. Next, to assess relationships with the likely cumulative risk of wastewater input at all our sites, we again used GEE models – this time with Gaussian error distribution and log link function. Parasiticide concentrations were modelled using explanatory variables reflecting i) wastewater spillage duration from all CSOs upstream of each sampling points and ii) the population served by WWTPs upstream of each site as an index of sewered population and likely treated sewage input to each location. McFadden's Pseudo-R^2 values were calculated to estimate the variance explained by the GEE models. Additionally, we produced boxplots of the models' residuals for each river and compound to identify outliers or unusual distributions, for example large positive outliers which might indicate misconnected sewers. To parameterise these models, we first took the coordinates and names of WWTPs and CSOs from the Catchment Based Approach Data Hub (2024) (https://data.catchmentbasedapproach.org/) along with the sewage spillage durations for each location for all three sampling years. Population estimates for each WWTP 'sewershed' (= wastewater catchment) followed the method described by Wilde et al. (2022), using mid-2020 ONS (Office for National Statistics, 2024) data for Lower Layer Super Output Areas (LSOAs). Populations were allocated to built-up areas within each LSOA excluding fluctuations from commuting, migration, or tourism. Additional details on WWTP size and treatment methods were sourced from Welsh Water's Bioresources Market Information 2022–23 (Welsh Water, 2024 https://corporate.dwrcymru.com/en/library/bioresources-trading-documents). For WWTPs not listed in this dataset, size was estimated using population figures derived from Wilde's method. Consistent with Welsh Water's classification, any site serving 2000 people or fewer was categorised as "small." This compiled dataset was used to produce the maps shown in Figure S1 using QGIS version 3.34.1 (QGIS Development Team, 2024).

These analyses were supported by an additional mapping analysis to show changes in concentrations among sites within rivers. To enable comparison across parasiticides with differing concentration ranges, we standardised concentrations for each pesticide relative to the overall mean for all samples using z-scores prior to plotting.

Temporal variation in concentration (Hypothesis 4) was assessed across the eight sites from two rivers which were sampled in multiple years (Taff and Roath Brook, sites RB1–4 & T7–10). We used linear mixed-effects models (GLMM), fitted with R's lme4 package (Bates et al., 2015), to analyse variations in parasiticide concentrations across years and between rivers, treating year and river as categorical, fixed effects, with sampling 'Site' included as a random effect. Compound concentrations were log transformed. We also examined the relative magnitude of spatial versus temporal variation in diazinon, fipronil, and imidacloprid concentrations between 2021 and 2023 using a variance components model (VCM). This model, as described by Goldstein (2003), was a GLMM featuring only an intercept term and site as a random effect. The intraclass correlation coefficient, derived by dividing the among-site variance by total variance (among site + within site), signified the proportion of unexplained variation attributed to differences between sites rather than within them (Vaughan and Ormerod, 2012). Separately for each site with multiple samples, we used Pearson correlations to assess any relationships between concentrations of fipronil or imidacloprid on each sampling occasion and flow volume as measured in the Taff at Pontypridd.

To assess any relationships between densities of individual fish species and parasiticides at the six sites surveyed in Roath Brook (Hypothesis 5), we used General Linear Modules with Gamma error distributions and log link functions to examine patterns among the widespread species in relation to average Fipronil and Imidacloprid concentrations at each site (Brown Trout *Salmo trutta*; Stone Loach *Barbatula barbatula* and Bullhead *Cottus gobio*). For invertebrates, we calculated a general index of pollution impact, the WHPT Average Score per Taxon (= WHPT ASPT, initially after Walley & Hawkes, 1996) in which invertebrate communities are scored according to the averaged, observed tolerances of their constituent families to water quality variation. We regressed WHPT ASPT against mean concentrations Fipronil and Imidacloprid and also examined individual families for any trends with changing water quality.

3. Results

3.1. General patterns

Fipronil, imidacloprid and caffeine occurred respectively in 44 %, 77 % and 71 % of 140 available samples in each case with a marked urban association (Table 1). The odds of detecting fipronil were 25.7 times greater in urban than rural sites (i.e. odds ratio (OR) = 25.7, p = 0.002; Table 1, Table S2) reflecting estimated probabilities of occurrence respectively of 50 % and 4 %. Its metabolite, fipronil sulfone, occurred in 46 % of 84 samples collected from the Taff and Roath Brook in 2021 and 2022 with a near-identical pattern of occurrence to fipronil. Concentrations averaged 43 % of those of Fipronil (±23 % SD, range 5–89 %) which they reflected closely ($y = 0.22x + 0.51$, $r^2 = 0.57$, $n = 39$) and we did not consider it further. The odds of detecting imidacloprid were 8.1 times greater in urban sites compared to rural sites (OR = 8.1, p = 0.0003), with respective probabilities of occurrence of 85 % and 41 %, while odds of detecting caffeine were 8.7 times greater in urban compared to rural sites (OR = 8.7, $p < 0.0001$) with probabilities of occurrence of 78 % and 30 %, respectively. In contrast, the odds of detecting diazinon in urban sites were much lower compared to rural rivers (OR = 0.17, p = 0.0005), occurring in 28 % of all samples, with probabilities of occurrence of 25 % (urban) and 67 % (rural).

Turning to concentrations, variance component models showed that imidacloprid (78 %) varied mostly among sites, fipronil varied roughly equally within and among sites (52.3 %), and diazinon (64.5 %) varied marginally more within-sites than among sites (Tables 1 and 2). For fipronil, there was some variance among study years (2022: p = <0.001; 2023: $p < 0.0001$ relative to 2021) while diazinon concentrations varied among both years ($p < 0.0001$) and river systems (p = 0.04). In contrast, imidacloprid concentrations differed significantly between river systems (p = 0.049), but not years (Table 2). These patterns of within or between-site variability were supported across all three study years from the two most intensively sampled systems – the Taff and Roath Brook (n = 5–7 samples per site): imidacloprid (CV = 86 % ± 56 %, n = 17 sites) was less variable within sites than fipronil (mean coefficient of variation (CV) = 135 % ± 52 %, n = 15 sites) and diazinon (CV = 157 % ± 52 %, n = 5 sites). We first consider the spatial patterns responsible for these variations and then address temporal changes.

3.2. Spatial patterns among and within rivers

Individual concentrations of imidacloprid (12–36.7 ng/L) and fipronil (1.6–16.6 ng/L) were elevated in the three urban rivers (Taff, Clun, Ely), but were greatest of all in the small, urban Roath Brook (imidacloprid 74.6 ng/L; fipronil 34.7 ng/L; Fig. 1). Downstream trends were also apparent in the most contaminated rivers, particularly for imidacloprid, which increased either below entry points from WWTPs (Taff, Ely) or, in the case of the smaller Roath Brook, where the stream entered Cardiff's urban area (Fig. 1). Otherwise, concentrations for fipronil and imidacloprid were generally lower at the 27 rural locations and the remaining two small urban streams (Table 1). For diazinon, spatial patterns were characterised by patchiness, with locally elevated concentrations in two of the rural rivers (Wye and Tywi), but also in the Clun and upper reaches of the Ely (Table 1; Fig. 1).

3.3. Relationship to wastewater sources

For caffeine, around 70 % of all 140 samples had concentrations exceeding 20 ng/L and around 50 % exceeded 50 ng/L with values greatest in the urban Taff, Clun, Ely and Roath Brook (Table 1). Across the 117 samples where at least one of the contaminants was detectable, concentrations of both imidacloprid (r = 0.61) and fipronil (r = 0.45; Fig. 2) were highly significantly correlated with caffeine, but diazinon showed no such relationship (r = - 0.04). Consistent with this apparent link to wastewater sources, 23.5 % and 27.8 % of the variance in concentrations of imidacloprid and fipronil, respectively, were explained by discharge from WWTPs of increasing size – expressed as the population they served. Concentrations of diazinon were linked statistically to WWTPs, but apparent effects were smaller (see Wald Statistics, Table 3). There was no such positive effect from the durations of wastewater spills from WWTPs or CSOs (all $p \leq 0.001$; Table 3) for any of imidacloprid, fipronil or diazinon concentration, and in fact these relationships were significantly negative implying declining rather than increasing concentrations when spills were most frequent.

Notwithstanding the above significant effects, variations in imidacloprid and fipronil concentrations were not fully explained by known wastewater sources (ie WWTPs and CSOs) as shown by model residuals (Fig. 3). Specifically, residuals for imidacloprid and fipronil were markedly elevated in the Roath Brook system, while caffeine residuals were elevated here and in the Afon Clun. When Roath Brook was removed from the GEE models, the variance explained by WWTPs increased to 75.9 % for imidacloprid and 90.1 % for fipronil. For caffeine, variance in concentrations explained by WWTPs increased when Roath Brook was excluded reflecting elevated concentrations in this stream. Residuals for diazinon concentrations – i.e. values beyond those explained by wastewater – were greatest in the Wye, Ely and Clun (Fig. 3).

3.4. Within-site variability and variations with flow

Sampling events during the study covered over 80 % of typical variations in flow volume as judged from flow gauges on the Afon Taff (National Flow Archive Station 57005 - Taff at Pontypridd). On the Taff itself, these ranged from prolonged drought in July 2022 (flow volume = 2.8 m^3/s) to stormflow on July 14/15th 2023 (flow volume = 22 m^3/s). There was some evidence that interannual differences reflected these flow patterns, and for example diazinon concentrations increased during

Table 1

The occurrences and range of estimated concentrations of diazinon, fipronil, imidacloprid and caffeine in 140 spot samples collected from nine Welsh rivers (2021–2023) split into three groups (see methods, Fig. S1 and Table S1). Percentages of samples exceeding predicted no-effect concentrations (PNECs) are also given as well as the odds of detecting each chemical in urban versus rural sites (see Table S2 and text). LD indicates values below detection limits.

River type	Diazinon[c]	Fipronil[b]	Imidacloprid[a]	Caffeine
Urban rivers (n = 64)				
Percentage > LD	31 %	53 %	78 %	74 %
Percentage > PNEC	9 %	5–45 %	23–39 %	
Taff ng/L	LD – 15.0	LD – 16.6	LD – 36.7	LD – 1189
Ely ng/L	5.2–94.4	1.4–3.2	4.8–16.8	108–267
Clun ng/L	20.3–28.9	LD – 1.6	10.6–12.2	143–357
Small urban streams (n = 48)				
Percentage > LD	3 %	52 %	96 %	90 %
Percentage > PNEC	0 %	4–48 %	21–42 %	
Roath Brook ng/L	LD	LD – 34.7	0.8–74.6	LD – 1231
Whitchurch Brook ng/L	LD	LD	0.6–1.4	LD – 21
Nant Glandulais ng/L	LD – 0.4	LD	LD – 3.6	14–116
Rural rivers (n = 28)				
Percentage > LD	64 %	7 %	43 %	32 %
Percentage > PNEC	14 %	0–4 %	0–4 %	
Wye ng/L	LD –174.0	0–0.2	LD – 2.2	LD – 35
Usk ng/L	LD – 1.5	LD	LD – 3.2	LD – 91
Tywi ng/L	LD – 28.6	LD	LD – 2.6	LD – 68
All samples (n = 140)				
Percentage > LD	28 %	44 %	77 %	71 %
Percentage > PNEC	7 %	4–36 %	18–31 %	
Odds ratio (urban:rural)	0.17	25.7	8.1	8.6

Notes.

[a] Also known as Confidor, Premise 75, Admire.

[b] Fluocyanobenpyrazole, Taurus, Termidor

[c] Dimpylate, Diazinone, Oleodiazinon

Table 2
Longitudinal regressions for diazinon, fipronil, and imidacloprid concentrations in the Taff vs Roath Brook across 3 years (2021–2023), showing estimated coefficients, their standard errors and statistical significance from the mixed-effects models. (n = 96 samples from 18 sites).

	Diazinon				Fipronil				Imidacloprid			
Parameter	Estimate	SE	*t-value*	*P*	Estimate	SE	*t-value*	*P*	Estimate	SE	*t-value*	*P*
Intercept	−10.687	0.833	−12.832	<0.0001	−0.073	1.737	−0.042	0.967	2.374	1.501	1.582	0.131
Year 2022 (vs 2021)	2.294	0.516	4.449	<0.0001	−2.653	0.744	−3.567	<0.001	−0.515	0.504	−1.022	0.310
Year 2023 (vs 2021)	0.512	0.753	0.681	0.498	−6.426	1.085	−5.921	<0.0001	−1.186	0.735	−1.613	0.111
River Taff (vs Roath Brook)	2.086	0.933	2.236	0.04	−2.691	2.093	−1.286	0.217	−3.955	1.851	−2.136	0.049

low flow in summer 2022 ($p < 0.0001$) while fipronil concentrations declined ($p = 0.0006$) (Table 2). However, at sites in the Taff and Roath Brook with repeated samples (n = 6 pr 7 per site), fipronil (mean $r = -0.35 \pm 0.04$ SD after z transformation; n = 8 sites) and imidacloprid (mean $r = -0.39 \pm 0.04$ SD n = 8 sites) tended to decline with increasing flow in the Taff, but in none of the 16 possible instances (i.e 8 sites x 2 chemicals) was the relationship significant because concentrations varied even between base-flow samples. Multiple diazinon data were available from only three sites in the Taff, and there was no significant relationship with flow on the day of sampling.

3.5. Fish populations and invertebrates

Densities of Brown Trout (0.08 m^{-2} $\pm$ 0.06 SD), Stone Loach (0.19 m^{-2} $\pm$ 0.18 SD) and Bullhead (0.53 m^{-2} $\pm$ 0.49 SD) were relatively low across the six surveyed sites in Roath Brook. For Stone Loach and Bullhead, values overlapped among putatively clean and more contaminated locations so that neither declined significantly with increasing parasiticide concentrations (all $p > 0.1$). Increasing mean concentrations of imidacloprid explained 45 % of the variance in the densities of Brown Trout, although the relationship was not statistically significant ($p > 0.1$).

For invertebrates, WHPT ASPT values calculated from 30 families present at the six surveyed sites declined significantly with increasing concentrations of Imidacloprid ($r^2 = 0.75$, $p < 0.01$, Fig. 4) but not fipronil. This effect reflected reductions of over 90 % in the abundances of Heptageniidae (mostly *Rhithrogena semicolorata*) and Limnephilidae (mostly *Potamophylax* spp.) at sites affected by contaminated surface drains while both Ephemeridae (*Ephemera danica*) and Baetidae (mostly *Baetis rhodani* agg.) declined by over 30 %.

4. Discussion

In addition to growing public interest and scientific questions about the environmental occurrence of chemicals used in veterinary parasiticides (Wells & Collins, 2022; Holdsworth & Fisher, 2025), the recent publication of a UK government 'roadmap' has brought further attention to their sources and effects in UK rivers and streams (Defra, 2025: https://www.gov.uk/government/publications/cross-government-pharmaceuticals-in-the-environment-group-roadmap). The roadmap calls for education and the development of regulatory standards, evidence on the pathways through which flea or tick treatments reach freshwater ecosystems and improved understanding of any environmental impacts. Our targeted survey, focussed for the first time on Welsh rivers, was aligned with these needs: we aimed to assess likely sources of imidacloprid, fipronil and diazinon, compare their occurrence in rural and urban rivers, and appraise potential ecological effects. The resulting data revealed imidacloprid, fipronil and diazinon respectively in 77 %, 44 % and 28 % of samples. More importantly, the results illustrated important spatio-temporal patterns as well as illustrating a previously overlooked route for imidacloprid and fipronil contamination. Consistent with Hypothesis 1, imidacloprid and fipronil were associated significantly with urban rivers, where concentrations increased downstream of discharges from WWTPs or suspected sewer misconnections. Links with wastewater were supported not only by statistical modelling, but also by positive relationships with caffeine as an indicator of the presence of human wastewater (Hypothesis 3 and Buerge et al., 2003). In contrast, in support of Hypothesis 2, diazinon occurred dominantly but patchily in rural rivers characterised by sheep rearing such as the upper Ely, Wye and Tywi catchments. Support for Hypothesis 4, predicting variations with flow, was weak based on the interannual and event-related patterns observed. Finally, there was some qualified support for Hypothesis 5 in that changes in invertebrate composition were related statistically to concentrations of imidacloprid. We discuss these results more fully in the paragraphs that follow, firstly with respect to imidacloprid and fipronil before considering diazinon. We also outline caveats and potential confounds that should be borne in mind when interpreting our data.

4.1. Occurrence of fipronil and imidacloprid

Comparisons between the occurrence or concentrations of ectoparasiticides in our study and previous work are complicated by varying sampling methods, different survey designs and contrasting patterns of regulation, especially for fipronil and imidacloprid. Comparisons with data collected in North America are particularly difficult because legal uses of insecticides differ from those in the UK and Europe (Sadaria et al., 2016; Miller et al., 2024). With respect to sampling methods, data from integrative, passive samplers (eg 'chemcatchers') are more likely to detect contaminants present in the dissolved phase whereas spot samples like ours provide a measure of total concentration from dissolved contaminants as well as those bound to suspended particulate matter (eg Taylor et al., 2021). The magnitude of these effects will also differ between substances such as fipronil and diazinon, which have low water solubility and greater affinity for particulate sorption, and imidacloprid, which is more water-soluble (Hayasaka et al., 2012).

Differences in sampling methods and assessments made under changing regulatory circumstances also affect comparisons between studies within the UK. For example, imidacloprid occurred in 77–88 % and Fipronil in 6–10 % of samples collected from a southern English chalk stream during 2017–2018, but these data were from integrative 'chemcatcher' samplers deployed shortly after controls were imposed from 2018 onwards on imidacloprid in outdoor agriculture (Taylor et al., 2021). Perkins et al. (2021a) appraised regulatory data collected from 20 English rivers, detecting fipronil and imidacloprid respectively in 98.6 and 65.9 % of a large array of spot samples and at mean concentrations, again respectively, of 17 ng/L (range 0.3–980) and 31.7 ng/L (range 1–360). These data were also collected shortly after the 2017-18 period of tightened agricultural regulation but the authors argued that pet-flea treatments were likely major sources on the basis of i) prior reductions in recorded uses of these chemicals in agriculture; ii) large recorded doses for domestic pets; iii) the most elevated concentrations being linked to WWTPs rather than agriculture, hence domestic sewage and iv) limited likelihood of other licensed domestic uses such as indoor ant or cockroach control. More recent assessments in 2021-22 by Robinson et al. (2023) using chemcatchers illustrated how imidacloprid occurred throughout the year in the English Rivers Test and Itchen, exceeding the upper estimated PNEC of 13 ng/L in the River Test throughout the year. Using field methods similar to ours, Ramage et al. (2025) detected imidacloprid in almost all of 38 samples collected in

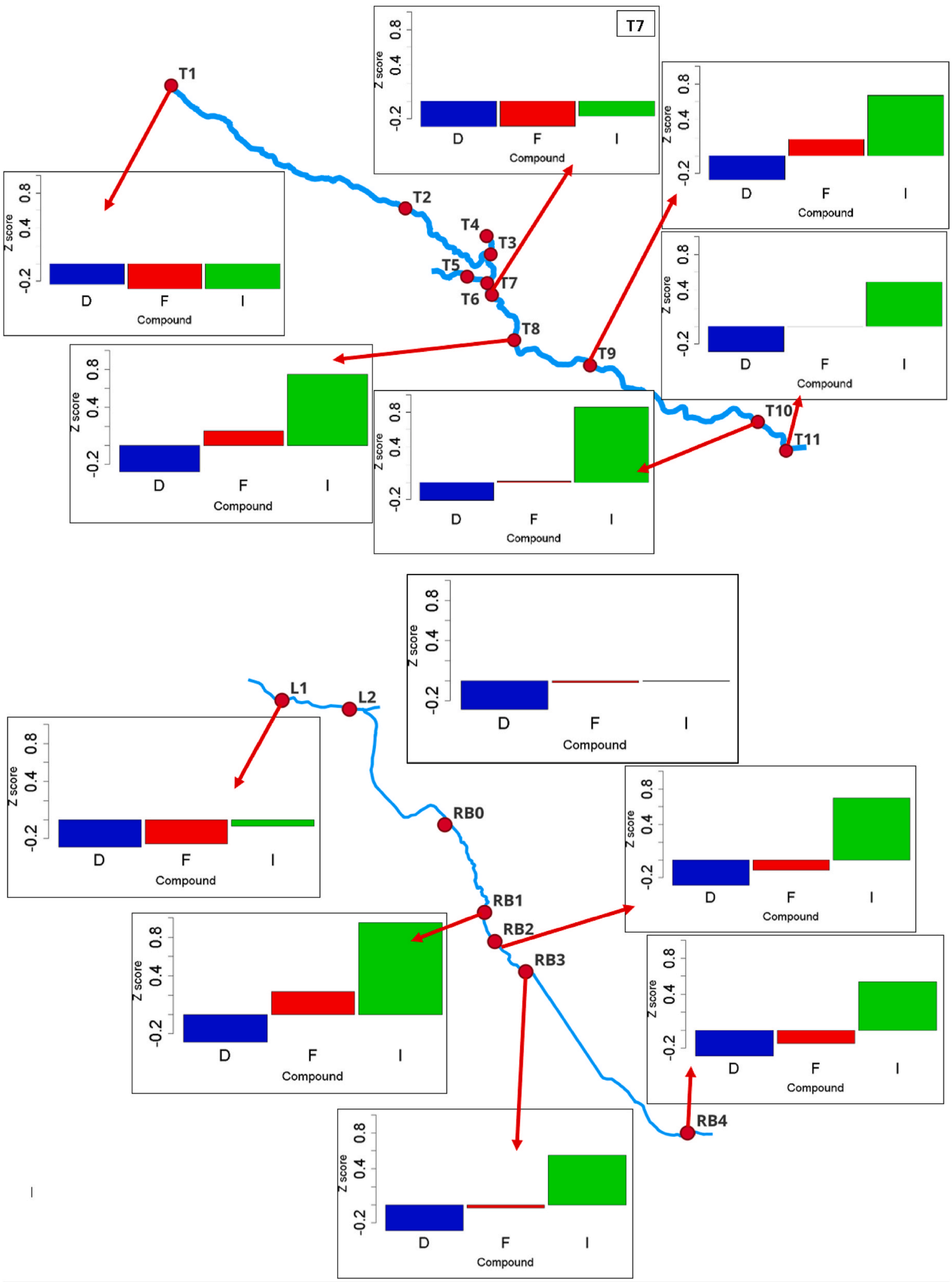

Fig. 1. Z-scores of downstream changes in the concentrations of diazinon (blue), imidacloprid (red) and fipronil (green) on the Taff (T), Roath Brook (L and RB) and Wye (W) during 2021–2023. Contains OS data © Crown copyright and database right (2024). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2021 from the Somerset River Tone and Norfolk River Wensum respectively at mean concentrations of 25.8 ng/L (max. 97.1) and 15.2 ng/L (max. 34). Potentially reflecting the methods used, fipronil was not detected in water samples by Ramage et al. (2025), though it occurred widely in sediments at concentrations that exceeded benchmarks for toxicity. Sedimentary concentrations were not measured during our

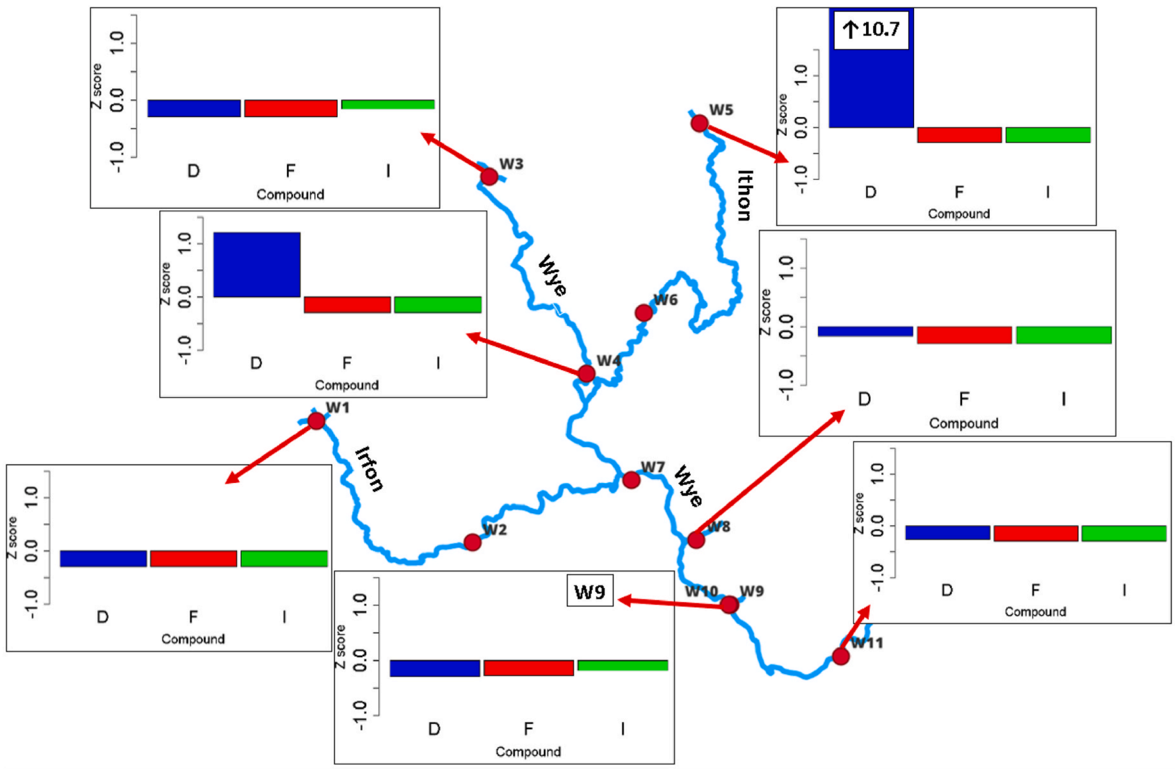

Fig. 1. (*continued*).

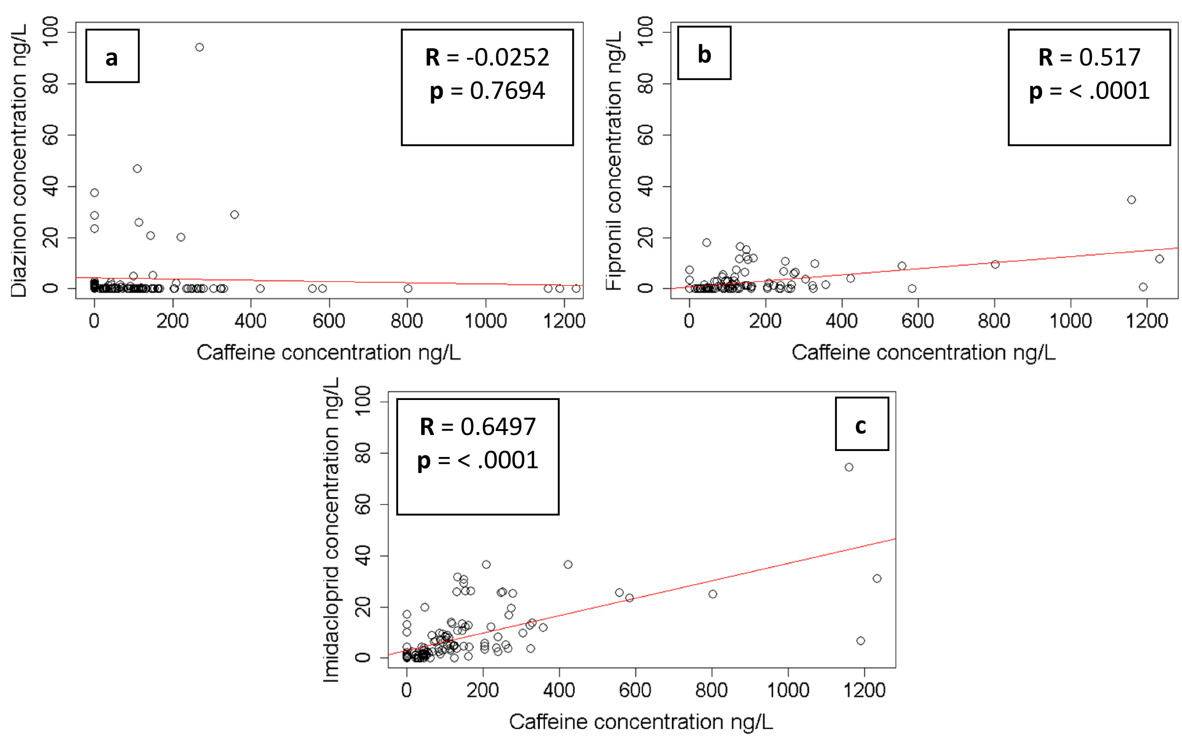

Fig. 2. Relationship between parasiticide compound concentrations and caffeine in all samples collected from nine Welsh rivers (2021–2023) where at least one contaminant was present. Pearson's correlation r values included on the figure, and points represent individual samples.

work and are seldom appraised by others despite their likely importance to the exposure of benthic organisms. Otherwise, both imidacloprid and fipronil were generally as widespread and at similar concentrations in Welsh rivers to previous UK assessments for all except maxima recorded by Perkins et al. (2021a) that were over 10 times higher.

4.2. *Wastewater sources*

Against this background of widespread occurrence, our data extend insights into variations in the concentrations of imidacloprid and fipronil between rural and urban settings, particularly with respect to

Table 3
Results of generalized estimating equation (GEE) models (Gaussian error distribution, log link) assessing the influence of wastewater-related predictors on compound concentrations (Diazinon, fipronil, imidacloprid, and caffeine). Analyses were performed both including and excluding data from Roath Brook (RB) sites.

Parameter	Diazinon				Fipronil				Imidacloprid				Caffeine			
i) Including Roath Brook (n = 62 sites)																
	Estimate	SE	Wald	P	Estimate	SE	Wald	P	Estimate	SE	Wald	P	Estimate	SE	Wald	P
Intercept	1.228	0.7756	2.5	0.113	−0.0279	0.36	0.01	0.9382	1.39	0.206	45.8	<0.0001	4.322	0.2398	324.7	<0.0001
WWTP Spill Duration	−0.0009	0.0003	10.6	0.001	−0.0001	0.00001	105.3	<0.0001	−0.00005	0.00001	28.8	<0.0001	−0.000002	0.00001	0.02	0.88
CSO Spill Duration	−0.0075	0.0023	10.9	0.001	−0.0001	0.00001	9.6	0.0019	−0.00002	0.00001	8.1	0.0044	−0.000023	0.00003	0.62	0.43
WWTP Population Served	0.0002	0.0001	13.8	0.001	0.0001	0.000003	75.2	<0.0001	0.00002	0.000002	102.2	<0.0001	0.000009	0.000005	3.7	0.05
ii) Excluding Roath Brook (n = 55 sites)																
	Estimate	SE	Wald	P	Estimate	SE	Wald	P	Estimate	SE	Wald	P	Estimate	SE	Wald	P
Intercept	2.14	0.599	12.7	0.001	−1.27	0.284	20.1	<0.0001	0.8	0.198	16.4	<0.0001	3.46	0.22	247.6	<0.0001
WWTP Spill Duration	−0.0004	0.0002	7.6	0.006	−0.0001	0.00001	106.5	<0.0001	−0.00005	0.00001	28.6	<0.0001	−0.000003	0.00002	0.04	0.85
CSO Spill Duration	−0.0011	0.0005	4.3	0.037	−0.0001	0.00001	10.5	0.0012	−0.00002	0.000005	13.4	0.00025	0.000017	0.00001	2.3	0.13
WWTP Population Served	0.0001	0.0001	15.6	<0.0001	0.0001	0.000002	234.6	<0.0001	0.00002	0.000001	209.7	<0.0001	0.000009	0.000002	15.63	<0.00001

wastewater sources. The spatial patterns detected in Welsh rivers support observations in England of increased concentrations linked to wastewater. For example, Perkins et al. (2021a) recorded elevated concentrations of both imidacloprid (mean 84.4 ng/L) and fipronil (mean 41.2 ng/L) within 2 km of wastewater outfalls while Robinson et al. (2023) detected elevated concentrations of imidacloprid (to 24 ng/L) in the River Test downstream of WWTP discharges. In our case, clear evidence that fipronil and imidacloprid were linked to wastewater came from i) patterns downstream from wastewater treatment works (e.g. Afon Taff Fig. 1); ii) marked intercorrelation with concentrations of caffeine as a recognised wastewater indicator (Fig. 2); and iii) modelling results showing how WWTPs of increasing size could account for significant variance in the concentrations of caffeine, imidacloprid and fipronil (Table 3). Additional insights from the latter modelling exercise suggested that spillages from combined sewer overflows (CSOs) were not important in increasing concentrations, instead tending to reduce concentrations potentially through dilution. Nor did we find systematic relationships between ectoparasiticides and flow conditions. The CSO pathway has been much debated as a source of untreated sewage and associated chemicals reaching British rivers – but fuller assessment requires data on volumes of wastewater discharged and local dilution as well as spill frequency (Perry et al., 2024). Potentially more importantly, model residuals and model iterations revealed anomalous chemical patterns in the small urban stream, Roath Brook, which receives no discharges from either treated wastewater or CSOs. Not only did this stream have the highest recorded concentrations of caffeine, imidacloprid and fipronil (Table 1), but concentrations increased at the point where Roath Brook reached sub-urban Cardiff (Fig. 1). Other compounds, including a wide range of human pharmaceuticals and chemicals linked to recreational drugs, also increased at this point (unpublished data). These patterns are consistent with long-suspected sewer misconnections in this catchment – in which household appliances such as dishwashers, showers and toilets are mistakenly plumbed into surface drains rather than foul sewers. In the UK, estimates suggest that 1–5 % of properties could be misconnected in this way at rates sufficient to affect surface water quality. In some locations misconnections could affect as many as 20–30 % of properties, for example where drainage from household extensions has been carried out incorrectly (Ellis and Butler, 2015; Revitt and Ellis, 2016). While we believe ours to be the first data to show flea-treatment chemicals reaching rivers in this way, parallel results from two other small city streams (Whitchurch Brook and Nant Glandulais) showed less marked effects likely reflecting local variation in misconnection rates. While there is evidence for dogs shedding fipronil and imidacloprid directly into small, standing waters (eg Yoder et al. 2024), overall our data are consistent with previous assessments in showing that various 'down the drain' routes are important for imidacloprid and fipronil reaching British rivers. These patterns, in turn, reflect well-evidenced pathways linked not only to their use in pet flea-treatments but also their release into domestic wastewaters as the result of washing pets, pet-bedding, or owners' hands after handling flea-treatment chemicals (Teerlink et al., 2017; Diepens et al., 2023; Perkins et al., 2024).

4.3. Toxicity and potential biological effects

Appraising the ecotoxicity of neonicotinoids and fiproles in real freshwater ecosystems is challenging because of the need to extrapolate from laboratory toxicity tests with limited exposure periods, artificial conditions and generic test organisms. By contrast, under field circumstances toxicants might have different bioavailability, sensitivity varies among species or life stages, body mass might affect dose rates, exposure is sometimes chronic, and other stressors are present (Hayasaka et al., 2012; Morrissey et al., 2015; Wells & Collins, 2022; Nagloo et al., 2024; Hermann et al., 2025). Against this background, predicted no-effect concentrations (PNECs) for freshwater organisms for regulatory purposes in Europe are suggested to fall in the range of 0.77–12.1 ng/L for

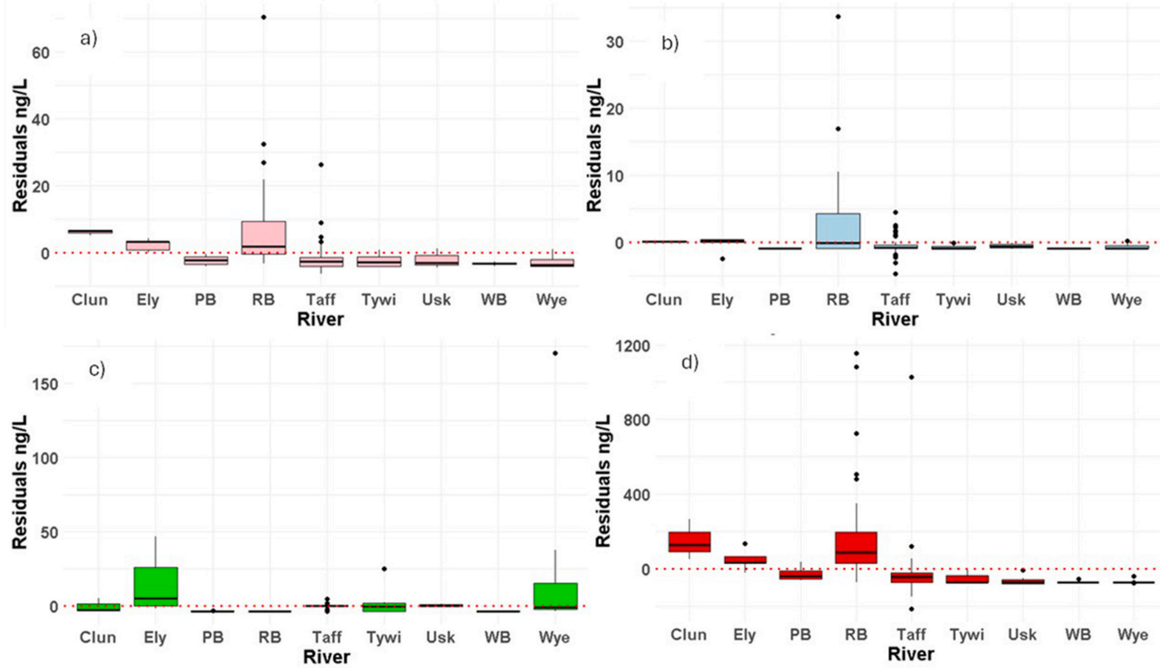

Fig. 3. Residuals from GEE models, displayed by river, for (a) imidacloprid, (b) fipronil, (c) diazinon, and (d) caffeine from samples collected across all nine rivers (2021–2023) (see methods, Fig. S1 and S1). GEE models (Gaussian, log link) included WWTP spillage duration, CSO spillage duration, and WWTP population as predictors. Predicted values were on the log scale, and residuals were calculated on the original concentration scale. Red dotted line = 0. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

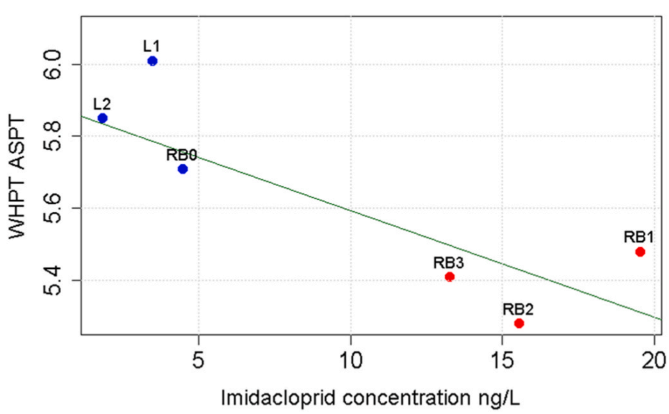

Fig. 4. Changes in average score per taxon (WHPT ASPT: Walley & Hawkes, 1996) with increasing imidacloprid concentration in Roath Brook (2022). Blue points = sites uninfluenced by wastewater input. Red points = sites influenced by contaminated surface drains. WHPT scores reflect increasing sensitivity to pollution such that higher scoring taxa are more sensitive. ASPT values are the mean WHPT values averaged across the taxa present in any sample. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

fipronil and 6.8–13 ng/L for imidacloprid (ECHA, 2011, 2015; NORMAN, 2025). In our urban rivers, peak concentrations exceeded the most stringent of these values by 2-21X for fipronil and 2.8-5X for imidacloprid, exceeding the lowest PNECs in 39–45 % of urban samples (Table 1). Specifically in Roath Brook, the lowest PNECs were exceeded by 45X for fipronil and 11X for imidacloprid, and in its most urban reaches PNECs were exceeded in over two thirds of 27 samples available. In these same reaches, detectable reductions in WHPT ASPT scores correlated strongly with imidacloprid concentrations and reflected reduced numbers of the ephemeropterans *Rhithrogena semicolorata*, *Ephemera danica* and *Baetis rhodani* agg. alongside lower numbers of *Potamophylax* trichopterans. Although we caution strongly that these patterns were correlative and occurred where imidacloprid and fipronil co-occurred in a cocktail of other wastewater pollutants, current understanding of imidacloprid toxicity supports the possibility that our observations could reflect causal processes. As a neonicotinoid, imidacloprid toxicity to insects involves interference with neuro-transmission, specifically by binding to acetylcholine receptors in the post-synaptic region of nerve cells that leads to neuronal over-excitation. As this binding process is irreversible, toxic effects are time dependent such that prolonged exposure is potentially damaging to aquatic insects even at very low concentration (Tennekes and Sanchez-Bayo, 2011; Beggs et al., 2025). As a time-dependent-toxicant (TDT), empirical data for imidacloprid show that no-effect concentrations fall rapidly with prolonged exposure – especially for sensitive taxa such as Ephemeroptera and Trichoptera (Morrissey et al., 2015; Neelamraju et al., 2025). This is consistent with the reductions observed among these taxa in Roath Brook where exposure to imidacloprid was almost certainly chronic. Such time-dependent effects have led to calls for further tightening of regulatory limits (Sánchez-Bayo and Tennekes, 2020; Stehle et al., 2023).

4.4. Diazinon distribution and likely sources

Patterns of occurrence for diazinon contrasted markedly with those for fipronil and imidacloprid in occurring significantly more frequently in rural than urban locations and having no relationship with caffeine and only weak links to wastewater sources. Concentrations, instead, were markedly elevated in the sheep-rearing areas of the Wye (to 174 ng/L), the upper reaches of the Ely (to 94.4 ng/L), Clun (to 28.9 ng/L) and Tywi (28.6 ng/L). This is consistent with the uses of diazinon in sheep-dips where treated animals or subsequent dip disposal to land provide a potential pathway for the contamination of streams and rivers. Despite our surveys overlapping only partially with the major sheep-dipping period of August–October, the peak concentrations we detected locally were around 3-17X higher than the predicted no effect concentration (PNEC) of 10 ng/L for aquatic organisms (Lepper et al., 2007). Similar locally elevated concentrations have been detected also by Wales' environmental regulator, Natural Resources Wales, using combinations of spot and passive samplers, and have led to increased controls on sheep-dip disposal (see https://naturalresources.wales/about-us/news-and-blogs/news/nrw-changes-waste-sheep-dip-disposal-methods-for-cleaner-rivers/). Previous studies of Welsh rivers have provided circumstantial evidence of impacts on river invertebrates from spent sheep-dip (Jones et al., 2017), and we advocate wider and more systematic assessments of current diazinon disposal and effects in British rivers to improve assessments of environmental risk assessments.

4.5. Caveats and cautions

As with many field studies, several caveats should be borne in mind when considering our data. First, our study was aimed at summer conditions so that seasonal variations in the occurrence or concentrations of the chemicals studied could not be appraised. While the surveys covered an estimated ~80 % of likely variations in flow volume over three years in the rivers sampled repeatedly, more extensive seasonal or event-related sampling is likely to provide further insight. Second, logistical constraints meant some variability in the numbers of samples collected per site which could have particular significance for assessing concentrations that varied within sites (eg fipronil) more than between sites (imidacloprid). The extent of sample replication across sites and rivers means, however, that any resulting errors are likely to have been small. Third, as noted above, while some of the ecological patterns detected are consistent with ecotoxicological effects, we cannot categorically ascribe these to cause and effect because ectoparasiticides co-occurred with other contaminants.

5. Conclusions and implications

Notwithstanding the above caveats, these data extend previous observations from English rivers to show how fipronil and imidacloprid occur widely in Welsh rivers. As well as revealing a previously overlooked wastewater pathway through which these chemicals reach streams and rivers, important and novel aspects of our data were that.

i) Frequent and considerable exceedance above PNECs in Welsh urban settings were linked to wastewater treatment, misconnected sewers and elevated caffeine concentrations rather than current or past agricultural fipronil or imidacloprid uses. This is particularly valuable new evidence given that previous assessments were made shortly after imidacloprid was mostly withdrawn from agricultural uses. In the absence of other well-evidenced urban sources, extensive uses of pet flea-treatments containing these substances are the most plausible origin (Perkins et al., 2021a, 2024);
ii) Occurrences and concentrations of imidacloprid and fipronil were linked to release from increasingly large WWTPs but, in contrast to some predictions, were not consistent with episodic spillage from combined sewer overflows (Perry et al., 2024).
iii) Although the observed changes in communities of aquatic organisms could not be tied explicitly to ectoparasiticides, sensitive organisms declined in locations where PNECs for were exceeded for the time-dependent-toxicant, imidacloprid. This pattern is consistent with the possibility that emerging contaminants such as flea-treatments could constrain the recovery of urban streams from traditional insanitary pollution (Pharaoh et al., 2023, 2024).
iv) There are potential risks from substantial diazinon exceedances above PNECs in sheep-rearing areas, supporting the need to reevaluate and regulate disposal practices for sheep dip.

Overall, our data support calls for fuller assessments of the environmental occurrence, effects and management of all three of the ectoparasiticides we studied.

CRediT authorship contribution statement

Molly Hadley: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Laura Rodwell:** Writing – review & editing, Investigation, Conceptualization. **Matt Stewart:** Writing – review & editing, Methodology, Conceptualization. **Daniel Crowther:** Writing – review & editing, Investigation, Conceptualization. **Jasper Linley-Adams:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Sian Craig:** Writing – review & editing, Methodology, Investigation. **Suzanne Thomas:** Writing – review & editing, Methodology. **Anthony Gravell:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis. **Isabelle Durance:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Ian P. Vaughan:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **S.J. Ormerod:** Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Funding acquisition, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2026.127713.

Data availability

Data will be made available on request.

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