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Potential role of veterinary flea products in widespread pesticide contamination of English rivers

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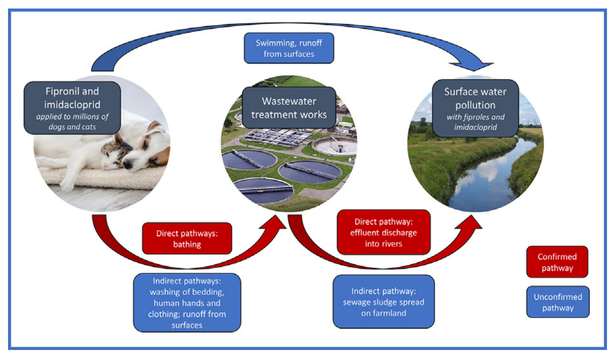
HIGHLIGHTS

- Environmental impact of pesticides used in veterinary flea treatments largely unknown
- Analysis of potential sources of fiprole and imidacloprid in English rivers
- Comparison of Environment Agency water monitoring data with reported toxicity limits
- Sewage works indicated as a possible route to rivers for fiproles and imidacloprid
- Veterinary flea products are a potential source of pollution and ecosystem harm

GRAPHICAL ABSTRACT



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ABSTRACT

Little is known about the environmental fate or impact of pesticides used to control companion animal parasites. Using data from the Environment Agency, we examined the occurrence of fipronil, fipronil metabolites and imidacloprid in 20 English rivers from 2016 to 2018, as indicators of the potential contamination of waterways from their use as ectoparasiticides on pets. Water samples were collected by the Environment Agency as part of their chemical surveillance programme and analysed using Liquid Chromatography Mass Spectrometry / Quadrupole-Time-of-Flight Mass spectrometry (LC/Q-TOF-MS) methods. A total of 3861 chemical analyses were examined, and the significance and potential sources of this contamination were assessed. Fipronil, fipronil sulfone, fipronil sulfide (collectively known as fiproles) and imidacloprid were detected in 98.6%, 96.5%, 68.7% and 65.9% of samples, respectively. Across the river sites sampled, the mean concentrations of fipronil (17 ng/l, range <0.3–980 ng/l), and fipronil sulfone (6.5 ng/l, range <0.2–39 ng/l) were 5.3 and 38.1 times their chronic toxicity limits of 3.2 and 0.17 ng/l, respectively. Imidacloprid had a mean concentration of 31.7 ng/l (range <1–360 ng/l), which was below its chronic toxicity limit of 35 ng/l, however seven out of 20 sites exceeded that limit. Chronic risk quotients indicate a high environmental risk to aquatic ecosystems from fiproles, and a moderate risk from imidacloprid. Sites immediately downstream of wastewater treatment works had the highest levels of fipronil and imidacloprid, supporting the hypothesis that potentially significant quantities of pesticides from veterinary flea products may be entering waterways via household drains. These findings suggest the need for a reevaluation of the environmental risks associated with the use of companion animal parasiticide products, and the risk assessments that these products undergo prior to regulatory approval.

Abbreviations: EA, Environment Agency; RQ, risk quotient; WL, watch list; WTW, wastewater treatment works.

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1. Introduction

Fipronil and imidacloprid are neurotoxic pesticides that are widely used in companion animal ectoparasite products in many parts of the world (Rust et al., 2018; Tyler et al., 2019). They have several properties which make them attractive pest control agents, including high toxicity towards a wide range of invertebrates (Pisa et al., 2014), high environmental persistence and water solubility (Simon-Delso et al., 2015). Unfortunately, these properties also increase the environmental hazards associated with their use.

Fipronil forms several transformation products, of which two of the most common (Tingle et al., 2003; Dyk et al., 2012) are fipronil sulfone, formed through oxidation in aerobic conditions, and fipronil sulfide, formed through reduction in anaerobic sediments or soils (Gunasekara et al., 2007). These metabolites are more persistent (PPDB, 2020; Table 1) and have higher toxicity to most invertebrates than fipronil (Weston and Lydy, 2014; Jinguji et al., 2018). Fipronil and its metabolites are hereafter referred to as fiproles (Sadaria et al., 2017). A summary of the environmental persistence and solubility of fiproles and imidacloprid is provided in Table 1. When compared to imidacloprid, fipronil has a relatively low solubility in water, however formulation with certain copolymers can be used to increase its solubility (Bonmatin et al., 2015).

Neonicotinoids (including imidacloprid) have a history of widespread agricultural use in the UK (FERA, 2020), however numerous studies have raised concerns about the environmental risks that agricultural use represents to non-target species (Woodcock et al., 2017; David et al., 2016; Whitehorn et al., 2012). In 2013 the European Union placed a moratorium on the use of imidacloprid, forbidding its use in flowering crops that attract pollinating insects (European Commission (2013a). Following a review of the scientific literature, the European Food Safety Authority concluded that most uses of neonicotinoids represent a risk to wild bees and honeybees (EFSA, 2018a, 2018b). As a result, the outdoor use of imidacloprid was banned by the European Commission in 2018 (European Commission, 2018a; European Commission, 2018b). Similarly, following a review which concluded that a high risk for bees could not be excluded, the agricultural use of fipronil was severely restricted in 2013 (European Commission, 2013b) and its approval for use as a plant protection product ended in 2017 (European Commission, 2019a).

In the UK, no agricultural use of imidacloprid is recorded after 2016 (FERA, 2020). Imidacloprid is also used on ornamental plants in greenhouses. The prevalence of this is not recorded, but a recent study found imidacloprid residues in 38% of potted plants on sale in garden centres in the UK (Lentola et al., 2017). Other licensed uses for imidacloprid include ant, cockroach and fly bait products (Health and Safety Executive, 2020).

Fipronil has a history of limited agricultural use in the UK, with no agricultural use recorded after 2015 (FERA, 2020). The only licensed use for fipronil in the UK, besides flea control products, is in ant and cockroach bait products, of which only one product (Antstop! Bait Station®, Evergreen Garden Care UK Ltd) is licensed for use by non-professionals (Health and Safety Executive, 2020). Residues of imidacloprid and fipronil may be present on imported food, however these amounts are likely to be very low (PRiF, 2018).

Table 1
Environmental persistence and solubility of fiproles and imidacloprid. DT50 = half-life degradation time, (−) = no data available (PPDB, 2020).

Compound	Aqueous photolysis DT50 at pH 7 (days)	Typical DT50 in aerobic soils (days)	Water-sediment DT50 (days)	Solubility in water at 20 °C and pH 7 (mg/l)
Fipronil	0.33	142	68	3.78
Fipronil sulfone	–	347	–	–
Fipronil sulfide	–	229	–	0.54
Imidacloprid	0.2	191	129	610

Despite being restricted for agricultural use, fipronil and imidacloprid are commonly used in topical veterinary ectoparasiticide products. Imidacloprid is primarily used to treat fleas, whilst fipronil has efficacy against both fleas and ticks (Maddison et al, 2008). In the United Kingdom there are 66 licensed veterinary products containing fipronil and 21 containing imidacloprid (VMD, 2020a), either alone or in combination with other parasiticides. These include spot-on solutions, topical sprays and collars impregnated with the active ingredient. Some products require veterinary prescriptions, whereas others can be bought without prescription from pet shops, supermarkets, pharmacies and online. A recent study investigating owner-reported flea treatment measures in cats found the spot-on products Frontline® (Boehringer Ingelheim Animal Health), containing fipronil, and Advocate® (Elanco Animal Health), containing imidacloprid and moxidectin, to be the flea products most commonly used in the UK (Tyler et al., 2019).

As set out by the VICH (International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicine Products) guidelines, the UK Veterinary Medicines Directorate (VMD) generally requires that extensive environmental safety testing be performed on parasiticides used on livestock and aquaculture. However, for companion animals no such testing is required. The assumption is made that there is less total amount of product used, and products used in these animals are usually individual treatments, therefore approval for use in non-food animals is likely to be associated with fewer environmental concerns (European Medicines Agency, 2000).

These assumptions warrant closer investigation. The 2019 PDSA Animal Wellbeing Report (PDSA, 2019) estimated that there are 9.9 million dogs and 10.9 million cats in the UK, and that approximately 80% of dogs and 82% of cats are treated for fleas (although the frequency of treatment was not determined in this report). Fipronil and imidacloprid-based spot-on products are licensed for monthly use, and routine prophylactic ectoparasite treatment is widely recommended by product manufacturers and veterinarians (ESCCAP, 2018; Bayer, 2020). Many owners receive year-round ectoparasite treatment for their pets through their veterinary practice via healthcare plans. Whilst annual sales data are unavailable, total sales data provided by the VMD show that 27,471 kg and 33,036 kg of fipronil and imidacloprid, respectively, have been sold as treatments against fleas since the products were first authorised as veterinary medicines in 1994 and 1997, respectively (VMD, 2020a, 2020b). Therefore, although it remains that these products are used as individual treatments of companion animals, their widespread use may result in usage volumes that are no longer considered to be insignificant, in terms of their potential release into the environment.

After spot-on application, imidacloprid and fipronil spread over the surface of the skin (Dyk et al., 2012), where they are incorporated into the superficial epidermis and sebaceous glands, and slowly released via hair follicles (Cochet et al., 1997; Chopade et al., 2010). From here they disperse widely throughout the household environment through pet hair, shed skin and direct transfer (Dyk et al., 2012; Craig et al., 2005). Imidacloprid has been demonstrated to persist and accumulate in the pet's environment, at levels sufficient to maintain a high level of flea larvicidal efficacy (Jacobs et al., 2001).

Little is known about the environmental fate and impact of pesticides used in pet parasiticide products, and it has long been assumed that veterinary medicinal products used in non-food animals are likely to be associated with fewer environmental concerns than those in food producing animals (Boxall et al., 2005; European Medicines Agency, 2000). However, there is growing concern that environmentally significant quantities of pesticides from spot-on flea products may be entering the environment. A report from Buglife - The Invertebrate Conservation Trust (Shardlow, 2017) found imidacloprid at levels exceeding published chronic toxicity limits (Morrissey et al., 2015) in a number of urban rivers in the UK. Veterinary flea spot-ons and collars were implicated as the most likely source. Bathing of pets treated with flea control products and washing of hands, pet bedding or other surfaces that come into contact with treated pets were identified as potential pathways for entry to sewers. Direct transfer into surface water where treated pets swim was identified as another potential pathway. Rainfall washoff from treated pets and shedding of hair and dander may lead to contamination of roads and other surfaced areas, resulting in surface runoff to wastewater treatment works (WTW) or directly into surface waters (DEFRA, 2002), although this pathway has not been described in the literature.

Teerlink et al. (2017) confirmed a pathway for fipronil spot-on products from households to wastewater influent by measuring the total mass of fiproles in rinsate from bathing treated dogs. This ranged from 3.6–230.6 mg per dog. Comparisons of mass loading calculated from California flea treatment sales data and wastewater monitoring results confirmed fipronil-containing spot-on products to be a potentially important source of fipronil to WTW in California (Teerlink et al., 2017).

Another study of fiprole and imidacloprid occurrences in Californian WTWs revealed ubiquity of both compounds, and persistence despite conventional wastewater treatment. Examination of potential pathways suggested that pet flea treatments may be the primary source of both pesticides (Sadaria et al., 2017).

In this paper, we examine the occurrence of fipronil, two of its main metabolites and imidacloprid in English rivers from 2016 to 2018, as indicators of the potential contamination of waterways from the use of these as parasiticides on pets. Given the findings of recent studies, we hypothesised that fipronil and imidacloprid used in veterinary flea products may be entering waterways via household drains in environmentally significant quantities and predicted that concentrations would be higher in locations immediately downstream of WTW. We also compared contamination levels with environmental toxicity limits to assess the likelihood that harm to aquatic ecosystems may be occurring due to the presence of these compounds.

2. Methodology

2.1. Data source

Imidacloprid and fiprole concentrations for English freshwater samples from 2016 to 2018, measured by Liquid Chromatography Mass Spectrometry / Quadrupole-Time-of-Flight Mass spectrometry (LC/Q-TOF-MS), were obtained from the Environment Agency (EA). Sampling was performed as part of an EA chemical surveillance programme supplementing Watch List (WL) surveillance under the Water Framework Directive (European Commission, 2000; European Commission, 2019b). This is a list of potential water pollutants that are monitored by EU member states to assess the risk they pose to the aquatic environment and determine if Environmental Quality Standards should be set for them. The first WL was published in 2015 and included five neonicotinoids, of which imidacloprid was one (Carvalho et al., 2015). Fiproles are not included in the official WL but are included in EA chemical surveillance monitoring. Analysis for fipronil sulfone and fipronil sulfide was performed from 2017 onwards.

Watch List site selection and sampling is required to follow the principles outlined in the Common Implementation Strategy for the Water Framework Directive guidance documents (European Commission, 2019b), namely including sites that are not atypical, with temporal and spatial sampling reflecting chemical use patterns. The WL comprises of a range of compounds including agricultural pesticides, human pharmaceuticals and industrial chemicals, and sampling sites were therefore selected to represent a range of land uses and catchments. Watercourses are seldom exclusively influenced by a single land use; however, sites were categorised based on broad land use categories obtained through Geographic Information System (GIS) mapping. The sample site network includes a variety of monitoring programmes, including the Catchment Sensitive Farming (CSF) network (Environment Agency, 2014), which forms the bulk of the regular monitoring data.

Sample sites for which all results, including non-detects, were available were included in the analysis. Specific sampling dates, beyond the year of sampling, were not available for all data and were therefore not included in the main analysis. A summary of available sampling dates and temporal sampling patterns is provided in Appendix A.

Based on EA categories, river sample site locations were classed as:

1) Sites <2 km downstream of WTW. Five sites, all of which were urban.
2) Sites >2 km downstream of WTW, or with no WTW impact. 15 sites, including 4 urban sites and 11 agricultural sites drawn from the CSF network.

The distance between sample sites and the nearest upstream WTW discharge point was measured using data gathered from the European Commission urban waste water website (European Commission, 2017) and open source map sharing website Map BBCode (MapBBCode Share, 2020). Table 2 provides a list of the 20 sample

Table 2
Sample sites with their respective location categories, distance from wastewater treatment works, latitude and longitude. WTW = wastewater treatment works, Agric. = Agricultural.

Sample site	Urban/Agric.	Distance from WTW (km)	Latitude, longitude
<2 km of WTW			
Arun downstream of WTW	Urban	0.9	51.0543, −0.3649
Old Forge Farm Somerhill Stream	Urban	1	51.1622, 0.2783
River Douglas at Wanes Blades Br.	Urban	0.9	53.6074, −2.7936
Sincil Dyke Washingborough	Urban	1.8	53.2257, −0.4804
Wyke Beck below Knostrop Works	Urban	0.03	53.7714, −1.4757
>2 km of WTW			
Arun tributary, Horsham	Agric.	No WTW impact	51.0490, −0.3158
Blyth at Bedlington Bridge	Urban	No WTW impact	55.1265, −1.5835
River Ancholme Cadney Bottom	Agric.	19	53.5130, −0.4920
River Ancholme Horkstow Bottom	Urban	11.8	53.6583, −0.5284
River Ivel Tempsford Depot Ft.Br.	Agric.	4.5	52.1658, −0.3040
River Nene Wansford Old Rd.Br.	Agric.	17.9	52.5791, −0.4150
River Ouse Roxton Lock	Agric.	3.4	52.1669, −0.3063
River Waveney Ellingham Mill	Agric.	3.4	52.4711, 1.4792
River Wensum Sweet Briar Rd.Br.	Agric.	22	52.6384, 1.2589
River Yare Trowse Mill	Urban	9.2	52.6126, 1.3116
River Eden at Sheepmount	Agric.	14.8	54.9053, −2.9522
River Irwell at Old Ringley Bridge	Urban	11.2	53.5439, −2.3587
River Ouse at Naburn Lock	Agric.	3.4	53.8938, −1.0986
River Ouse at Nether Poppleton	Agric.	11.2	53.9912, −1.1346
River Test at Longbridge	Agric.	3.3	50.9590, −1.4959

sites in this analysis with their respective location categories and distance from the nearest upstream WTW.

2.2. Sampling and chemical analysis undertaken by the EA

Samples were collected in new 1 l glass bottles and stored at 5 ± 3 °C. Chemical analysis was conducted at the EA's laboratory in Star Cross, UK, using a broad target based semi-quantitative screening method described in Moreau et al. (2019) and White et al. (2019). Solid phase extraction was conducted, using Waters Oasis HLB SPE cartridges with an automated extraction system. An isotopically labelled internal standard Carbutamide-d9 (CAS 1246820-50-7) was added to each of the pre-conditioned SPE cartridges to assess instrument performance. Target compounds have been analysed using a blank and a standard with a concentration of 0.1 μg/l, the response factor obtained is used to create a single point calibration curve. Estimate of concentration is based on quant ion response and response of the internal standard. Target compound identification is made by accurate mass, retention time and by isotope distribution patterns (ratio, mass, spacing). The combined results contribute to an overall match score. An internal AQC (analytic quality control) containing 9 target compounds was analysed with each sample batch, at a concentration of 0.01 μg/l. Prior to analysing the results all compounds that were detected in a laboratory blank were first screened for and removed from the results. Over 750 compounds were screened for using this method, including both WL substances and many which are not included on the WL. Detection limits for fipronil, fipronil sulfone, fipronil sulfide and imidacloprid were 0.3 ng/l, 0.2 ng/l, 0.2 ng/l and 1 ng/l, respectively. Further detail regarding the analytical method is provided in Appendix B.

2.3. Toxicity limits

Currently no environmental quality standards exist for imidacloprid, fipronil, fipronil sulfone or fipronil sulfide in British surface waters.

The acute and chronic toxicity limits for fiproles in this analysis (Table 3) are taken from Bower and Tjeerdema (2017). This report, from the Department of Environmental Toxicology at the University of California Davis, provides water quality criteria calculated using a combination of species sensitivity distribution (SSD) and safety factor methods, developed to evaluate acute and chronic endpoints when limited species data are available.

The acute and chronic toxicity limits for imidacloprid in our analysis (Table 3) are based on Morrissey et al. (2015). These values are based on SSD calculations, derived from a review of over 214 toxicity tests. Morrissey et al. (2015) cautioned that the application of further safety factors may still be necessary to ensure that no deleterious effects on ecosystems occur.

It is worth noting that the toxicity limits included in this analysis are higher than many limits that have been proposed or adopted elsewhere (ECHA, 2011, 2015; NORMAN Ecotoxicology Database, 2019). Further discussion regarding environmental thresholds is provided in Appendix C.

Knowledge gaps exist regarding the bioavailability of fiproles and imidacloprid in water. No studies were found that distinguish when these compounds are freely dissolved, sorbed to solids or sorbed to dissolved solids. Until there is more information available regarding these states, it has been recommended that toxicity limits are based on the total concentration of these compounds in water (Bower and Tjeerdema, 2017).

2.4. Data analysis

Summary statistics were calculated using R studio software (version 1.2.5042–1), and QGIS software (version 3.12) was used for geographic mapping of data. The dataset contains left-censored data, defined as values below the limit of detection (LOD; Zoffoli et al., 2013). Such values substituted with ½ LOD for statistical analysis. Further discussion of the method used to handle censored data is provided in Appendix D.

The chronic toxicity limits for fipronil sulfone and fipronil sulfide are below the limit of detection, meaning that all samples in which these compounds were detected exceeded the chronic toxicity threshold, but true exceedance rates are unknown. For this reason, the percentage of fipronil sulfone and fipronil sulfide samples, and fipronil sulfide sample sites exceeding their respective chronic toxicity thresholds are stated as being ≥the percentage exceeding the limit of detection - indicating the minimum estimate for these values.

Differences in contamination levels between location types were evaluated using the Wilcoxon rank sum test. Correlations were calculated using the Pearson method.

Risk quotients (RQ) are commonly used to quantitatively characterise the environmental risks of pesticides. A RQ is calculated by dividing the measured concentration by the toxicity limit – indicating the extent to which the toxicity limit has been exceeded. Therefore, the RQ is a ratio of exposure to effect (Peterson, 2006). RQs were included in this analysis to quantify the risks posed by each compound, and to compare the relative risks posed by different compounds. Acute RQs were calculated by dividing the mean concentration by the acute toxicity limit, and chronic RQs were calculated by dividing the mean concentration by the chronic toxicity limit. A commonly used risk ranking criteria for interpreting the

Table 3
Summary of detection rates, mean and median sample site values, toxicity limit exceedance rates, chronic risk quotients, toxicity limits and detection limits for fiproles and imidacloprid samples taken from English rivers from 2016 to 2018, analysed by LCMS. Summary statistics calculated using substitution of non-detects with ½ LOD (limit of detection). Where the limit of detection exceeds the toxicity limit, results are stated as greater or equal to (≥) the percentage exceeding the limit of detection – indicating the minimum estimate for this value. Toxicity limits for fiproles are taken from Bower and Tjeerdema (2017), toxicity limits for imidacloprid are from Morrissey et al. (2015).

	Fipronil	Fipronil sulfone	Fipronil sulfide	Imidacloprid
Dates of sampling	2016–2018	2017–2018	2017–2018	2016–2018
Number of samples	1322	607	607	1325
Number of sample sites	20	18	18	20
Sample detection rate	98.6%	96.5%	68.7%	65.9%
Minimum concentration (ng/l)	<0.3	<0.2	<0.2	<1
Maximum concentration (ng/l)	980	39	5.3	360
Mean concentration (ng/l)	17.0	6.5	0.78	31.7
Median concentration (ng/l)	7.0	4.5	0.38	11.5
Acute toxicity limit (ng/l)	20	1.3	0.62	200
Chronic toxicity limit (ng/l)	3.2	0.17	0.14	35
Samples exceeding acute toxicity limit	21.6%	91.9%	56.8%	0.3%
Samples exceeding chronic toxicity limit	82.5%	≥96.5%	≥68.7%	27.5%
Sample site means exceeding acute toxicity limit	30%	94.4%	44.4%	0%
Sample site means exceeding chronic toxicity limit	80%	100%	≥77.8%	35%
Acute risk quotient	0.85	5.0	1.3	0.16
Chronic risk quotient	5.3	38.1	5.6	0.90
Limit of detection (ng/l)	0.3	0.2	0.2	1

RQ was applied, namely "low risk" from 0.01 through 0.1; "medium risk" from 0.1 through 1, and "high risk" >1 (Zhao et al., 2010; Hernando et al., 2006).

3. Results

Table 3 provides a summary of results and detection limits for fiproles and imidacloprid.

3.1. Fipronil

Fipronil was detected in all 20 sites sampled between 2016 and 2018, and in 1303 out of 1322 individual samples. The highest levels of fipronil were found in sites <2 km downstream of WTW (Fig. 1), which had a mean concentration of 41.2 ng/l. This was significantly higher than sites >2 km downstream of WTW ($p = 0.0005$, one-sided Wilcoxon rank sum test), which had a mean concentration of 9.0 ng/l. Fipronil concentrations declined with distance from the nearest upstream WTW (Fig. 2), and were inversely correlated to the square root of the distance from that WTW (Pearson's $r = -0.63$, $p = 0.005$; Fig. 2). Sixteen out of twenty sites had mean concentrations that exceeded the chronic toxicity limit for fipronil, including all of the sites <2 km downstream of WTW, and six sites had mean concentrations that exceeded the acute toxicity limit (Fig. 3). The mean site concentration for fipronil of 17.0 ng/l (range <0.3–980 ng/l) was 5.3 times greater than its chronic toxicity limit. The corresponding chronic risk quotient indicates that fipronil poses a high risk to the aquatic environment. A summary of detection rates, number of samples taken, and results for each sample site is provided in the Appendix E.

3.2. Fipronil sulfone

Fipronil sulfone was detected in all 18 sites sampled between 2017 and 2018, and in 586 out of 607 individual samples. All sites tested for fipronil sulfone had mean values that exceeded the chronic toxicity limit, and 17 out of the 18 sample sites had mean values that exceeded the acute toxicity limit (Fig. 3). The mean concentration of fipronil sulfone in sites <2 km downstream of WTW (13.3 ng/l) was significantly higher than sites >2 km downstream of WTW (4.5 ng/l; $p = 0.006$, one-sided Wilcoxon rank sum test; Fig. 1). Fipronil sulfone concentrations declined with distance from the nearest upstream WTW and were inversely correlated to the square root of the distance from that WTW (Pearson's $r = -0.51$, $p = 0.04$; Fig. 2). The mean sample site concentration for fipronil sulfone was 6.5 ng/l (range < 0.2–39 ng/l). This resulted in a chronic risk quotient of 38.1, indicating a high risk to the aquatic environment.

3.3. Fipronil sulfide

Fipronil sulfide was detected in 16 out of 18 sites samples between 2017 and 2018, and in 417 out of 607 individual samples. The mean concentration of fipronil sulfide in sites <2 km downstream of WTW (0.99 ng/l) was marginally higher than sites >2 km downstream of WTW (0.72 ng/l; Fig. 1), however this was not statistically significant

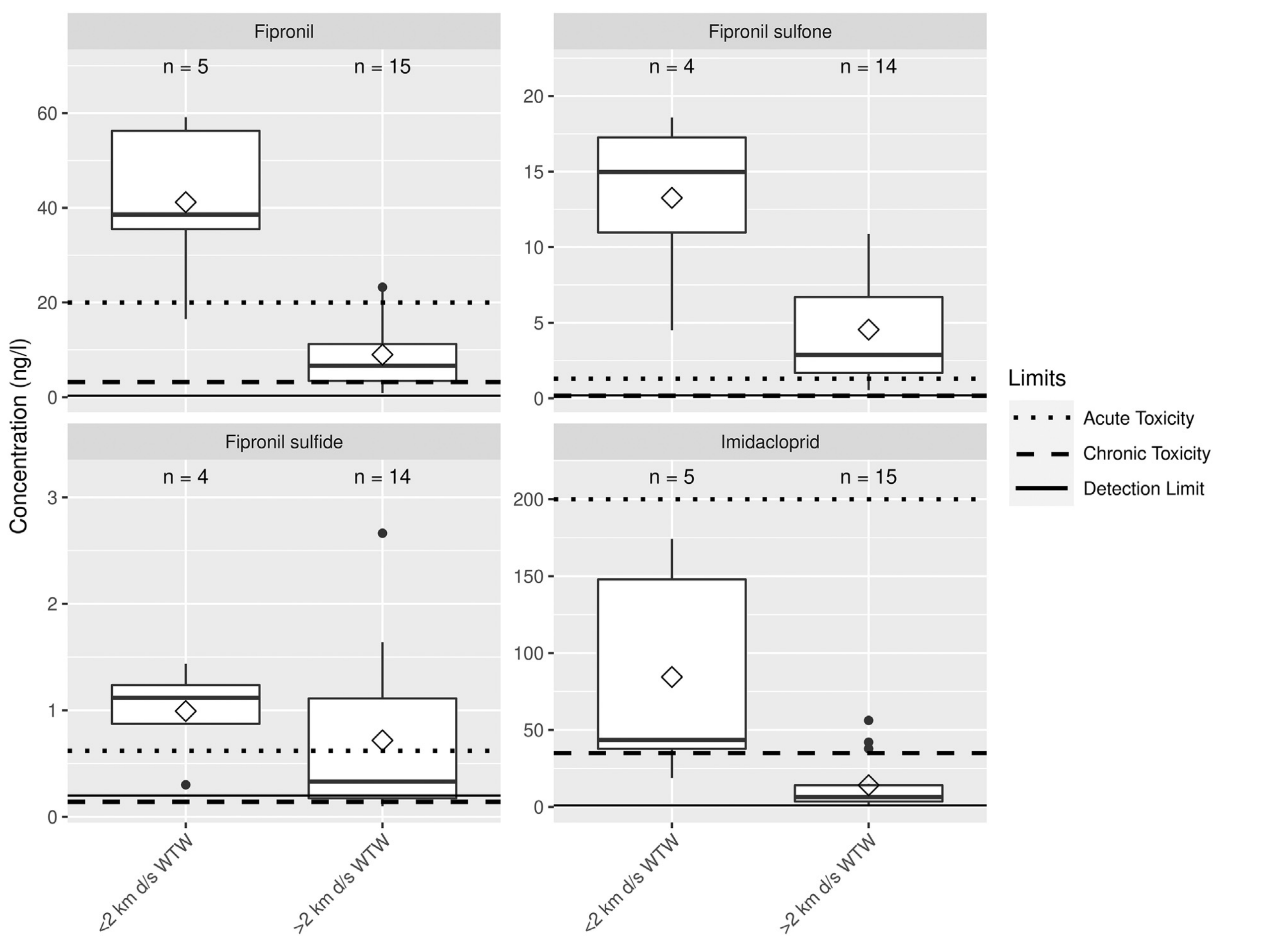

Fig. 1. Mean sample site concentrations of fipronil and imidacloprid (2016–2018), and fipronil sulfone and fipronil sulfide (2017–2018) in English rivers, measured by LCMS. <2 km d/s WTW = sites with 2 km downstream of wastewater treatment works, >2 km d/s WTW = sites further than 2 km downstream of wastewater treatment works or with no WTW impact. Acute and chronic toxicity limits and the limit of detection are shown. Dots indicate outliers, diamonds indicate mean values, n indicates the number of sample sites, lower and upper boundaries 25th and 75th percentiles (Q1 and Q3), respectively, line inside box median.

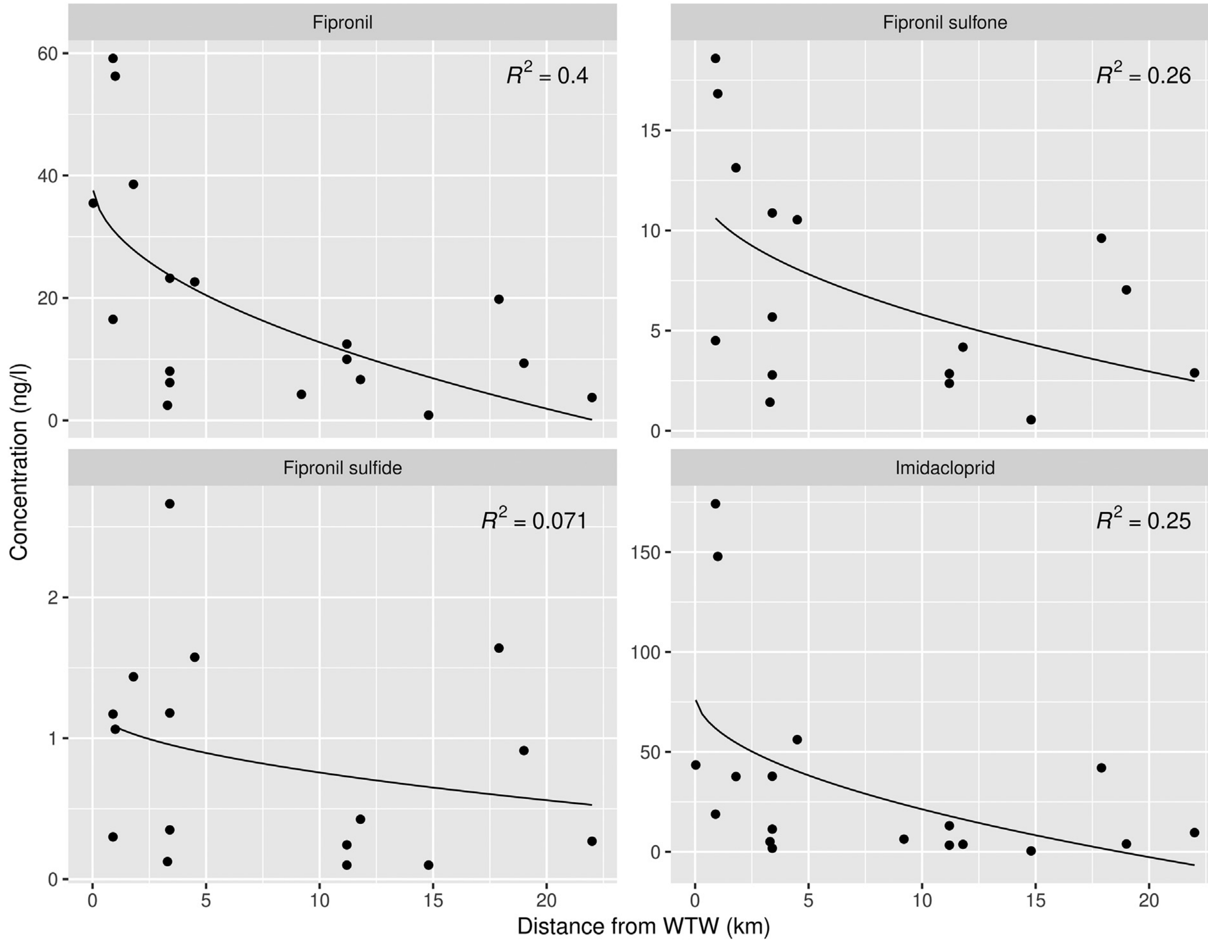

Fig. 2. Relationship between mean sample site concentrations and distance from WTW (wastewater treatment works) for fipronil and imidacloprid (2016–2018), and fipronil sulfone and fipronil sulfide (2017–2018) in English rivers from 2016 to 2018, measured by LCMS. Lines indicate linear least squares regression fits for concentration as a function of the square root of distance. Sites with no WTW impact excluded.

($p = 0.18$). Mean sample site concentrations for fipronil sulfide were not significantly correlated to the square root of the distance from WTW (Pearson's $r = -0.27$, $p = 0.3$; Fig. 2). The mean sample site concentration for fipronil sulfide of 0.78 ng/l (range < 0.2–5.3) resulted in a chronic risk quotient of 5.6, indicating a high risk to the aquatic environment.

3.4. Imidacloprid

Imidacloprid was detected in 19 out of 20 sites sampled between 2016 and 2018, and in 873 out of 1325 individual samples. Sites within 2 km of WTW had significantly higher concentrations of imidacloprid than sites >2 km downstream of WTW ($p = 0.003$, one-sided Wilcoxon rank sum test; Fig. 1), with a mean concentration of 84.4 ng/l versus 14.1 ng/l. Imidacloprid concentrations declined with distance from the nearest upstream WTW (Fig. 2), and were inversely correlated to the square root of the distance from that WTW (Pearson's $r = -0.50$, $p = 0.03$; Fig. 2). The mean sample site concentration for imidacloprid was 31.7 ng/l (range < 1–360 ng/l). This resulted in a chronic risk quotient of 0.91, indicating a moderate risk to the aquatic environment. However 7 out of twenty sites had a risk quotient greater than one, indicating that imidacloprid poses a high risk to the aquatic environment in these locations.

A strong correlation was observed between mean sample site concentrations of fipronil and imidacloprid (Pearson's $r = 0.92$, $p = 9.3\text{e-}09$; Fig. 4).

No significant differences were found between mean sample site concentrations from the nine urban and eleven agricultural sites for any compound ($p > 0.05$, two-sided Wilcoxon rank sum test).

4. Discussion

Fipronil and its toxic metabolites were found to be ubiquitous in English freshwater environments, with the majority of measurements occurring at concentrations that exceed environmental toxicity limits. Of particular concern was the metabolite fipronil sulfone, which had a mean concentration of 6.5 ng/l, 38.1 times higher than the chronic toxicity limit recommended by Bower and Tjeerdema (2017).

Approval for agricultural use of fipronil ended in 2017 (European Commission, 2019a), with no recorded UK use after 2015, when less than one kg was used (FERA, 2020). The only licensed use of fipronil in the UK - besides pet flea products - is in ant and cockroach control products, of which only one product (Antstop! Bait Station®, Evergreen Garden Care UK Ltd) is licensed for use by non-professionals. Whilst annual sales data for these products are unavailable, studies confirming the widespread use of fipronil spot-on flea products in UK cats and dogs (Tyler et al., 2019; Cooper et al., 2020) and the relatively low incidence of household pest ant infestations (Cornwell, 1978) implicate flea control products as the primary source of pollution.

The highest concentrations of fipronil were recorded in urban sites immediately downstream of WTW, consistent with a household source of contamination. Given the current licensed uses of fipronil, and the findings of previous studies demonstrating the dissemination and accumulation of spot-on products in the household environment (Dyk et al., 2012; Jacobs et al., 2001) and confirming a pathway for these products from households to waterways via WTW (Teerlink et al., 2017), the results here support the hypothesis

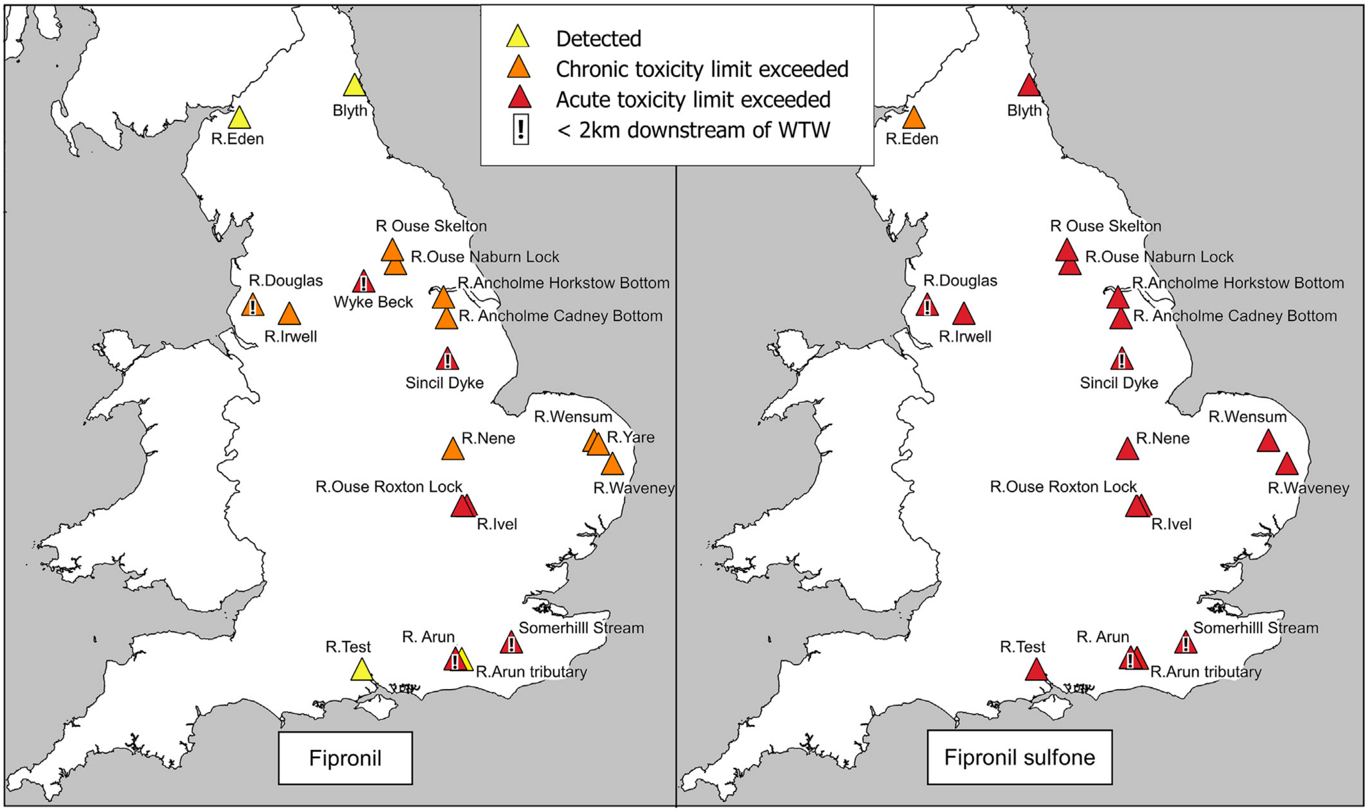

Fig. 3. Map of England indicating the locations and mean values of river sample sites tested by LCMS for fipronil (2016–2018) and fipronil sulfone (2017–2018). Toxicity limit exceedances and detections are indicated. Fipronil and fipronil sulfone were detected in all rivers tested. Fipronil: LOD (limit of detection) = 0.3 ng/l, chronic toxicity limit = 3.2 ng/l, acute toxicity limit = 20 ng/l. Fipronil sulfone: LOD = 0.2 ng/l, chronic toxicity limit = 0.17 ng/l, acute toxicity limit = 1.3 ng/l. WTW = wastewater treatment works.

that significant quantities of pesticides used in spot on flea products may be passing to waterways via households drains.

Like fipronil, imidacloprid is widely used in pet flea treatments, but is also the active ingredient in a larger and more diverse array of pest control products including ant, cockroach and fly baits. The highest

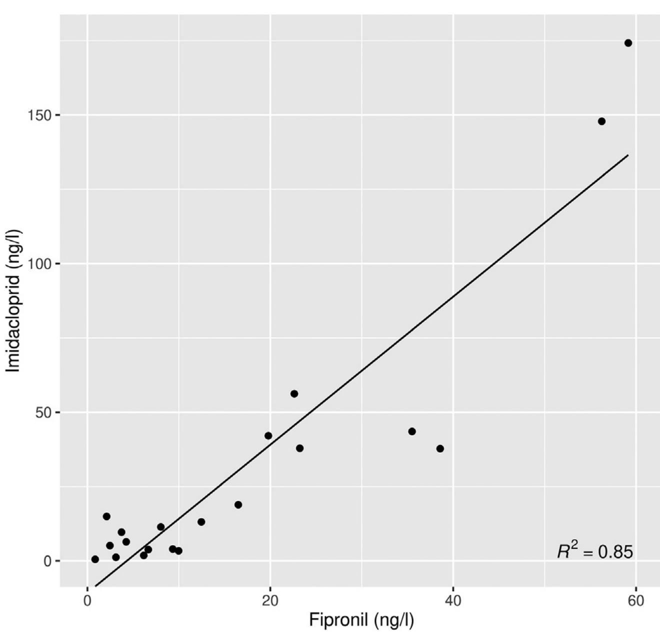

Fig. 4. Relationship between mean sample site concentrations for imidacloprid and fipronil in English rivers from 2016 to 2018, measured by LCMS. Line indicates least squares regression fit.

concentrations of imidacloprid were found in rivers immediately downstream of WTW, suggesting that transfer from households may be occurring and implicating flea products as a potentially significant source.

Imidacloprid had a higher mean concentration but lower detection rate than fiproles. The higher limit of detection and more rapid degradation by aqueous photolysis (Table 1) for imidacloprid may be factors in these findings. Whilst highly potent, imidacloprid is less toxic than fiproles to most aquatic insect species and has higher acute and chronic toxicity limits (Morrissey et al., 2015; Bower and Tjeerdema, 2017). This resulted in a lower chronic RQ (0.91) for imidacloprid, indicating a moderate overall risk to the aquatic environment. However, seven out of 20 sample sites were found to have a chronic RQ greater than 1, indicating a high environmental risk in these locations.

Prior to its ban in 2018, imidacloprid has a history of more widespread outdoor agricultural use than fipronil, and that agricultural use might be a potential source of freshwater contamination in the present study. However, no agricultural use of imidacloprid is recorded in the UK in 2017 and 2018, and only 186 kg of agricultural use was recorded in 2016 (FERA, 2020). This corresponds to 744,000 monthly applications of Advocate® or an equivalent spot-on flea product to a 15 kg dog, containing 250 mg of imidacloprid. Given reported cat and dog populations in the UK (PDSA, 2019), and prevalence of Advocate® (or equivalent flea product) use (Tyler et al., 2019; Cooper et al., 2020), it is likely that imidacloprid applications to dogs and cats greatly exceeded the weight used in agriculture in 2016. Regarding historical agricultural use, studies have suggested that leaching of neonicotinoids from soils occurs primarily immediately after application, before sorption to soil occurs, particularly if there is heavy rainfall (Goulson, 2013). Gupta et al. (2008) recovered up to 79% of applied thiamethoxam in leachate from a single simulated rainfall event, suggesting that historical agricultural use of neonicotinoids may not to be a significant contributor to the pollution of surface waters.

The strong correlation between fipronil (with negligible agricultural use) and imidacloprid concentrations across various sample sites (Fig. 4) supports the conclusion that the primary source of imidacloprid is likely to be non-agricultural, and suggests a common pathway/source for these pesticides, such as their use in flea control products. Similarly, a routine investigation of monitoring data by the Environment Agency found that both fipronil and imidacloprid levels showed similar patterns of correlation, being higher in more densely populated catchments than more rural ones. This suggests that these two pesticides may have similar, mainly urban sources and that these should be investigated further (pers. comm. Antony Williamson, 2020).

Significant knowledge gaps remain. The data presented above are correlative and may be confounded by other effects. River flow rate and river volume, origin of wastewater, wastewater volume load, treatment process, pH, rainfall, temperature and solar radiation could further explain variations in the levels of pollution between different sites and location types (Deblonde et al., 2011) and the role of surface runoff requires further elucidation (Cryder et al., 2019). Further, more detailed, hydrological studies are necessary to confirm the point sources of this pollution.

Annual non-agricultural usage data for fipronil and imidacloprid is needed to confirm the extent of possible pollution from different uses. Bathing of treated pets has been confirmed as a potentially important pathway for topical veterinary flea products to WTW (Teerlink et al., 2017) however further research is required to investigate pathways to terrestrial surfaces and additional pathways to waterways for flea products. The latter include pets directly entering waterbodies and washing of pet bedding, hands or other surfaces that come into contact with treated pets, as well as runoff from closed surfaces. More information is also needed on potential exposure pathways for other potential sources of pollution such as ant, cockroach and fly baits. Whilst the data presented above are not sufficient to definitively attribute pollution levels directly to veterinary flea products, the results reported here support the hypothesis that potentially environmentally harmful quantities of fipronil and imidacloprid used in veterinary flea treatments may be entering freshwater systems via household drains.

Current proposed ecotoxicity thresholds may change as more data becomes available, and more information is required regarding the bioavailability of these compounds in the water environment. Additionally, there is limited ecotoxicity data for fiproles and further research in this area is recommended. It is likely that the additive toxicity of total fiprole concentrations (Thuyet et al., 2012) and synergistic effects with other pesticides (Pisa et al., 2014; Vijver and Van Den Brink, 2014) will need to be taken into account in order to provide adequate environmental protection. Additionally, no data is available for levels of fiproles and imidacloprid in sediment, however the accumulation of fipronil in sediment has been reported (Demcheck et al., 2004). Baird et al. (2013) concluded that exposure to fiproles through sediment presents the greatest threat to aquatic organisms, suggesting this to be another area in which research is needed. The potential occurrence of these compounds in WTW sewage sludge is also a concern, as around 78% of treated sewage sludge is spread on agricultural land in the UK (Biosolids Assurance Scheme, 2020).

Further research on the environmental risks associated with the use of companion animal parasiticides is recommended. Many pesticides besides fipronil and imidacloprid are currently available for use in topical flea products, including macrocyclic lactones such as selamectin and moxidectin, and isoxazolines such as fluralaner. Little is known about the environmental impact of these products. It is possible that orally administered isoxazolines such as afoxalaner, lotilaner or oral formulations of fluralaner may pose a lower risk of environmental harm. Orally administered fluralaner is primarily excreted unchanged in the faeces, as opposed to being widely disseminated in hair and skin (European Medicines Agency, 2013). However, concerns still exist, as fluralaner is extremely persistent in the environment, with a DT50 in soil of up to 989 days and has a high toxic potency for non-target invertebrates (European Medicines Agency, 2017). Furthermore, a minor portion of fluralaner administered orally to dogs is excreted in the urine and is quantifiable in hair and skin (European Medicines Agency, 2013). Research on the comparative environmental toxicity, toxic load, degradation products, excretion routes and environmental fate of companion animal parasiticide products is recommended, in order to inform evidence-based treatment decisions that minimise environmental harm whilst delivering treatment efficacy.

5. Conclusion

Fiprole and imidacloprid contamination is common in English rivers, often at levels well above safety thresholds and thus likely to impact aquatic ecosystems. The results reported here support the hypothesis that environmentally significant quantities of fipronil and imidacloprid used in veterinary flea treatments may be entering freshwater systems.

Further research is needed in several areas. These findings suggest the need for a reevaluation of the environmental risks associated with the use of companion animal parasiticide products, and the environmental impact assessments that these products undergo prior to regulatory approval. Reappraisal of current ectoparasite management protocols may also be warranted. A more judicious and risk-based approach, in which serious consideration is given to both the risks posed by parasites and the environmental risks associated with the use of these products, is likely to be necessary if environmental harm is to be avoided. If appropriate, the introduction of stricter prescription-only regulations, and updated guidance for veterinary surgeons and sellers of flea and tick treatments on the provision of these products for pet owners, in which environmental risks are made explicit, should be considered. Annual sales data for veterinary antimicrobials in the UK are recorded and published by the VMD, and it is recommended that the same be done for veterinary parasiticides. Additionally, alternative non-ecotoxic parasite control measures should be investigated and developed.

At a European level, inclusion of these insecticides in the Water Framework Directive's priority list, with the introduction of formal environmental quality standards and the aim of reducing or eliminating pollution, should also be considered.

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CRediT authorship contribution statement

Rosemary Perkins: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Martin Whitehead:** Formal analysis, Validation, Writing - review & editing. **Wayne Civil:** Investigation, Writing - review & editing. **Dave Goulson:** Formal analysis, Supervision, Writing - review & editing.

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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