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Pet dogs transfer veterinary medicines to the environment

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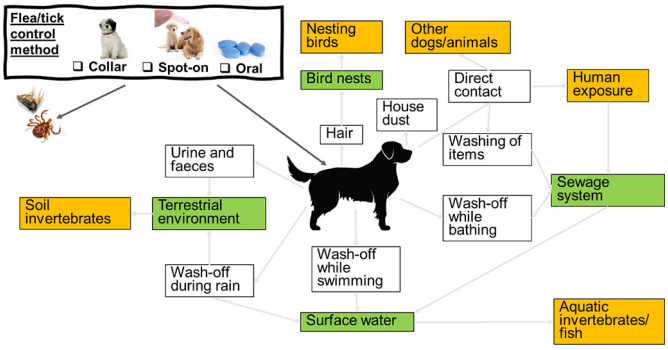
HIGHLIGHTS

- Worldwide dog population increases and therewith use of veterinary medicines.
- Explore potential transfer of veterinary flea products from dogs to the environment
- Afoxolaner, fluralaner, fipronil and imidacloprid detected in hair and urine samples.
- Fluralaner and imidacloprid detected in swimming water.
- Dogs transfer anti-flea and tick products to their environment causing potential risk.

GRAPHICAL ABSTRACT



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ABSTRACT

Worldwide, the number of pet dogs increases yearly, and as a result so does the use of veterinary medicines for flea and tick control. We investigated the potential transfer of veterinary flea products from dogs to the environment in a 'proof of principle' experiment. For this purpose, samples of hair, urine, and water after swimming were investigated. Nine dogs were recruited for this study, eight of which had been recently treated with an ectoparasiticide product. Hair and urine samples were tested for afoxolaner, fluralaner, fipronil and imidacloprid. Interestingly, contamination with ectoparasiticides was frequently demonstrated in samples from dogs untreated with these particular substances, suggesting widespread secondary transfer.

In addition, hair retrieved from a bird's nest contained fipronil, fluralaner and imidacloprid, indicating a potential pathway for the exposure of juvenile birds. Three of the dogs also participated in a swimming experiment. One had been treated with oral fluralaner, whilst the remaining two had received other compounds not included in our study. However, in all three dogs, both fluralaner and imidacloprid were detected in hair samples. Fluralaner concentrations in the swimming water exceeded Dutch water quality standards, indicating a potential risk to the aquatic environment. Imidacloprid levels increased after each swimming dog, but did not breach Dutch water quality standard levels. These findings all call for improvements in the current risk assessment and management for veterinary medicines, by including companion animals and their exposure pathways into ecosystems.

1. Introduction

There is ongoing discussion and growing concern about the environmental impact of, and approaches used in the regulation of companion animal pharmaceutics (Anthe et al., 2020; Bennett and Weeks, 2021; Domingo-Echaburu et al., 2021; Little and Boxall, 2020; Murphy and Wright, 2020; Perkins et al., 2021a, 2021b; Wells and Collins, 2022). In contrast to pharmaceuticals for farm livestock, no data on companion animal drug fate or effects on non-target organisms is required by the European Medicines Agency for authorization, because environmental risk is assumed to be low by default (Domingo-Echaburu et al., 2021; Perkins et al., 2021a). Nevertheless, as the number of pet dogs annually increases worldwide, so does the use of veterinary medicines. The total

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number of dogs was estimated in 2018 to be 471 million worldwide (Statista, 2020a), with 77 million in the USA (AVMA, 2019) and 85 million in China (Statista, 2022). In Europe there were 89 million dogs in 2020 (Statista, 2021). In China, the estimated number in 2022 is around 136 million (Statista, 2020b), a 60 % increase in just four years.

Fleas and ticks are a persistent issue with pet dogs and consequently dogs are commonly treated with veterinary flea control products, often on a prophylactic basis. Commonly used product application methods are oral, spot-on and collars, containing a wide variety of active ingredients (AI) either acting on the nervous system of the adult flea or working as a growth regulator affecting development of immature phases (Peribáñez et al., 2018). For example: collars may contain AIs such as deltamethrin, flumethrine, imidacloprid, diazinon or dimpylate; spot-on products may contain fipronil, imidacloprid, permethrin or piryproxyfen; and products administered by oral application include fluralaner, sarolaner, afoxolaner or spinosad.

Active ingredients from collars and spot-on applications are transported through the hair coat and along the outermost layer of the epidermis (*Stratum corneum*) over the skin surface (i.e. cutaneous distribution). They may not spread uniformly over the dog's body, and may be lost post-treatment influenced by several factors influencing dog coat structure and physiology such as shedding of skin, grooming behavior and environmental aspects e.g. sunlight, bathing and swimming (Pfister and Armstrong, 2016). Active ingredients from oral application, however, spread relatively rapidly to all body parts via the dog's blood circulation (i.e. systemic distribution). Systemically distributed AIs can also be applied on the skin surface or can be injected. Cutaneous products can also work as repellents, reducing the likelihood of fleas and ticks feeding on the dog, while systemic products only affect the parasites when they are actually feeding.

The use of veterinary flea products has been linked to both environmental as well as human exposure and effects (Cochran et al., 2015; Davis et al., 2008; Muilerman and Manthingh, n.d.; Perkins et al., 2021a; Teerlink et al., 2017). These anti-flea and tick products may end up in the indoor and outdoor environment, through direct and indirect pathways such as urine, shed hair, bathing, swimming and rain events. For example, fipronil could be discharged into waste water directly by bathing dogs, but could potentially also come from indirect sources such as indoor dust, secondary transfer to humans and the washing of clothes and materials that came into contact with dogs (Teerlink et al., 2017).

Previous studies have measured a wide range of organic chemicals in dog hairs and urine (Forster et al., 2014; González-Gómez et al., 2018). Dog hairs and urine have been used as an indicator for organic pesticide exposure and as a sentinels for human exposure, as pets and humans often share the same habitat in which secondary transfer can occur (Forster et al., 2014; González-Gómez et al., 2018). This secondary transfer has been demonstrated for a range of AIs from flea and tick products (Bigelow Dyk and RI, 2012; Boone et al., 2001; Chambers et al., 2007; Cochran et al., 2015; Craig et al., 2008; Davis et al., 2008; Jennings et al., 2002). Secondary transfer between dog hair used as a nesting material was also proposed to explain juvenile mortality in the great tit (*Parus major*) (Guldemond et al., 2019); in this study, 26 pesticides were found in the birds, including fipronil and imidacloprid. Direct contact between dog hair and bird skin was thought to be the exposure pathway for some of the chemicals, but empirical evidence for this theory was lacking.

Although several potentially important pathways of antiflea and tick products to the environment have been identified, such as bathing of treated pets and secondary transfer between humans and dogs, significant knowledge gaps remain, which include the additional pathways to waterways by pets directly entering waterbodies, the effects of using hairs as nesting material, urine and feces as a pathway to the terrestrial environment, and the extent to which each pathway contributes (Bennett and Weeks, 2021; Domingo-Echaburu et al., 2021; Perkins et al., 2021a; Wells and Collins, 2022).

The aim of this study was to explore, as a proof of principle, the potential transfer of veterinary flea products from dogs to their environment via their hair and urine. A small survey among dog owners was held to get an indicative idea of veterinary flea products used and swimming behavior of the dog. A subset of these dog owners then voluntarily collected hair and urine samples for chemical analyses. Additionally, water was analyzed from a swimming experiment in which dogs were allowed to swim briefly in a pool. This study focused on four AIs: afoxolaner, fluralaner, fipronil and imidacloprid. The well-studied AIs fipronil and imidacloprid were chosen to represent two chemical classes, phenylpyrazole and neonicotinoid, from an older generation of cutaneous distributed ectoparasiticides. In comparison, the much less studied AIs afoxolaner and fluralaner were chosen to represent one chemical class, isoxazoline, from a newer generation of systemic distributed ectoparasiticides. Additionally, these chemicals were identified as the four most commonly used companion animal antiparasitics in the UK, yet toxicity data for afoxolaner and fluralaner are scare (Wells and Collins, 2022).

To our knowledge this is the first study to determine the leaching of veterinary flea products by swimming dogs. Results of this exploratory study may stimulate future research to fill the important knowledge gaps currently existing, and therewith contribute to the debate to improve the risk assessment for companion animal pharmaceutics and the management of veterinary flea products used for the increasing pet population worldwide.

2. Material and methods

2.1. Survey

A survey was distributed within the Environmental Science group of Wageningen University and Research Centre, The Netherlands, reaching approximate 350 people. The survey consisted of questions about how many dogs an owner had, the sex and breed of the dog(s), what type of veterinary flea products the owner used on the dog(s), application methods for the product(s) used, knowledge of the owner about the products, and the swimming behavior of the dog(s). In the last question, dog owners could indicate whether they were willing to participate further in the research by collecting hair and/or urine samples and taking part in the swimming experiment. All dog owners participating in the follow-up experiments and sampling provided full consent after being informed of the study's objectives. A check with the Animal Experimental Committee (DEC) revealed that all activities involving dogs were not considered to be animal tests and consequently no formal approval was required for the swimming experiment and sampling.

Response on the survey was approximately 8 % (27 responded of approx. 350); not all recipients of the survey were dog owners, lowering the number of possible respondents. The response was, however, considered to be too low to be scientifically sound, so the outcomes of the survey are only shown in the supporting information (SI) for indicative purposes only.

2.2. Chemical concentrations in hair, urine and nesting material

Hair samples were collected from nine dogs and urine samples from six dogs. Samples were collected by the dog owners themselves and subsequently sent or brought to the laboratory.

Methods for hair collection were not standardized among dog owners. Hairs were either taken from a brush, or hair was cut from the tail or chest. Additionally, hairs were obtained from a nest of the great tit (*Parus major*) located in a residential area in Wageningen, The Netherlands. This nest originated from a nest box abandoned prematurely by the parent birds (as one died and the other gave up) and came to the authors by chance. The remaining chicks were rescued and found their way back into the wild once big enough. The chicks were not part of this investigation.

2.3. Swimming experiment

Three dogs participated in the swimming experiment in an artificial pool. The plastic swimming pool (1.94 × 2.95 m) contained approx. 2.6 m^3 of uncontaminated groundwater from the Sinderhoeve, Renkum, The Netherlands. The water was not replaced during the experiment, thus

facilitating a potential accumulation of AIs in the water phase. The first dog was an English springer spaniel (male, 18.2 kg), the second a German longhaired pointer (female, 29.0 kg) and the third an Sprocker spaniel (male, 18.6 kg). The dogs were not forced to stay longer in the pool than they wanted, and swimming times comprised 1.32 min, 3.10 min and 5.48 min, for the first, second and third dog, respectively. At the start, a water sample was taken as a background control. Sequentially, after each dog had left the pool, a 400 mL water sample was taken in a high density polyethylene plastic bottle with low sorption capacity for our chemicals and immediately frozen at < -20 °C until further analysis.

2.4. Chemical analyses

All hair, urine and water samples were analyzed for four chemicals: afoxolaner, fluralaner, fipronil and imidacloprid. Afoxolaner and fluralaner are systemic isoxziles (Table S1). Fipronil is a cutaneous phenylpyrazole and imidacloprid a cutaneous neonicotinoid. The four compounds were extracted and analyzed by reversed-phase liquid chromatography-tandem mass spectrometry (LC-MS/MS). For more details on materials and methods see supporting information (SI).

2.4.1. Stock standard solutions

Stock standard solutions of fluralaner, fipronil and imidacloprid were prepared by dissolving reference material in acetone. Afoxolaner stock solution was prepared by dissolving reference material in acetonitrile. All stock solutions with concentrations between 150 and 350 μg/mL were subsequently diluted with the same solvents to prepare working standard solutions with concentrations ranging between 1 and 4 μg/mL. All stock solutions were stored in a freezer (< -10 °C).

2.4.2. Extraction and analysis of water samples

For each sample, a solid phase extraction (SPE) column, Oasis @ HLB 3 cc (60 mg) from Waters was preconditioned with methanol (3 mL) and rinsed twice with Milli-Q water (3 mL) before being loaded with the water sample. After that, the SPE cartridge was rinsed again with 6 mL Milli-Q water and dried under vacuum for about 3 min. The target analytes were eluted with $2 * 0.5$ mL of acetonitrile. The eluate was brought up to 4 mL with Milli-Q water before the analysis. All samples were extracted twice (in duplicate).

To check the recovery of the four compounds through the extraction procedure, blank water samples (five) were extracted and measured in the same way as the experimentally derived water samples. The calibration standards with concentrations ranging from 0.5 to 100 ng/mL were freshly prepared prior analysis by diluting appropriate individual stock standard solution with acetonitrile/MilliQ-water: 25v/75v, directly in GC vials, using a dilutor Hamilton 600.

The analyses were performed on an Agilent 1260 Infinity liquid chromatograph coupled with an 6460 Triple quad mass spectrometer (Agilent Technologies, USA) and equipped with Agilent jet stream electrospray ionization source (AJS-ESI). Injected samples were quantified by peak area with reference to the respective external standards calibration curve measured in the same sample sequence. Agilent MassHunter software (version 10.1) was used for both data acquisition as quantification of the results. For recovery limit of detection (LOD) and limit of quantification (LOQ) see SI Table S2.

2.4.3. Sample preparation and extraction procedure of hair and urine

After cutting the hair of one sample in small fragments of 2–4 mm, about 200 mg of subsample and 5.0 mL ACN:MilliQwater (1:1 v:v) were added to a polypropylene (PP) tube for extraction. After homogenization using a vortex mixer and shaking for about 2 h at 175 rot/min on a shaking device, the samples were incubated overnight at 32 °C under agitation. Extraction was subsequently completed by shaking again for about 2 h at 175 rot/min and 15 min sonication. Then 1 g $MgSO_4$ and 0.25 g NaAc were added to each tube, samples were vigorously shaken by hand and vortexed for 1 min. After that, the tubes were cooled to room temperature by using cold water, shaken again for 5 min and centrifuged for 10 min at 4000 rot/min.

Urine samples (1 mL) were added to a polypropylene (PP) centrifuge tube and extracted with 2.0 mL ACN:MilliQwater (1:1 v:v). After homogenization by using a vortex mixer, the samples were sonificated for 15 min. Then 1 g MgSO4 and 0.25 g NaAc were added to each tube, samples were vigorously shaken by hand and vortexed for 1 min. After that, the tubes were cooled to room temperature by using cold water and centrifuged for 8 min at 4000 rot/min.

2.4.4. Clean-up and analysis of hair and urine

Supernatant (2 mL) was transferred in a 15 mL centrifuge tube packed with 500 mg *Z*-Sep + sorbent (Supelco Supel QuE Z-Sep+) and vortexed for 1 min and centrifuged for 5 min (hair) or 10 min (urine) at 4000 rot/min. After diluting the cleaned-up extract with MilliQ water (250 μl extract +750 μl water) samples were analyzed in the same way as the water samples. To check the recovery of the compounds through the whole procedure for urine, three blank MilliQ water samples were extracted and measured in the same way as the urine samples.

3. Results and discussion

3.1. Chemical concentrations in hair and urine

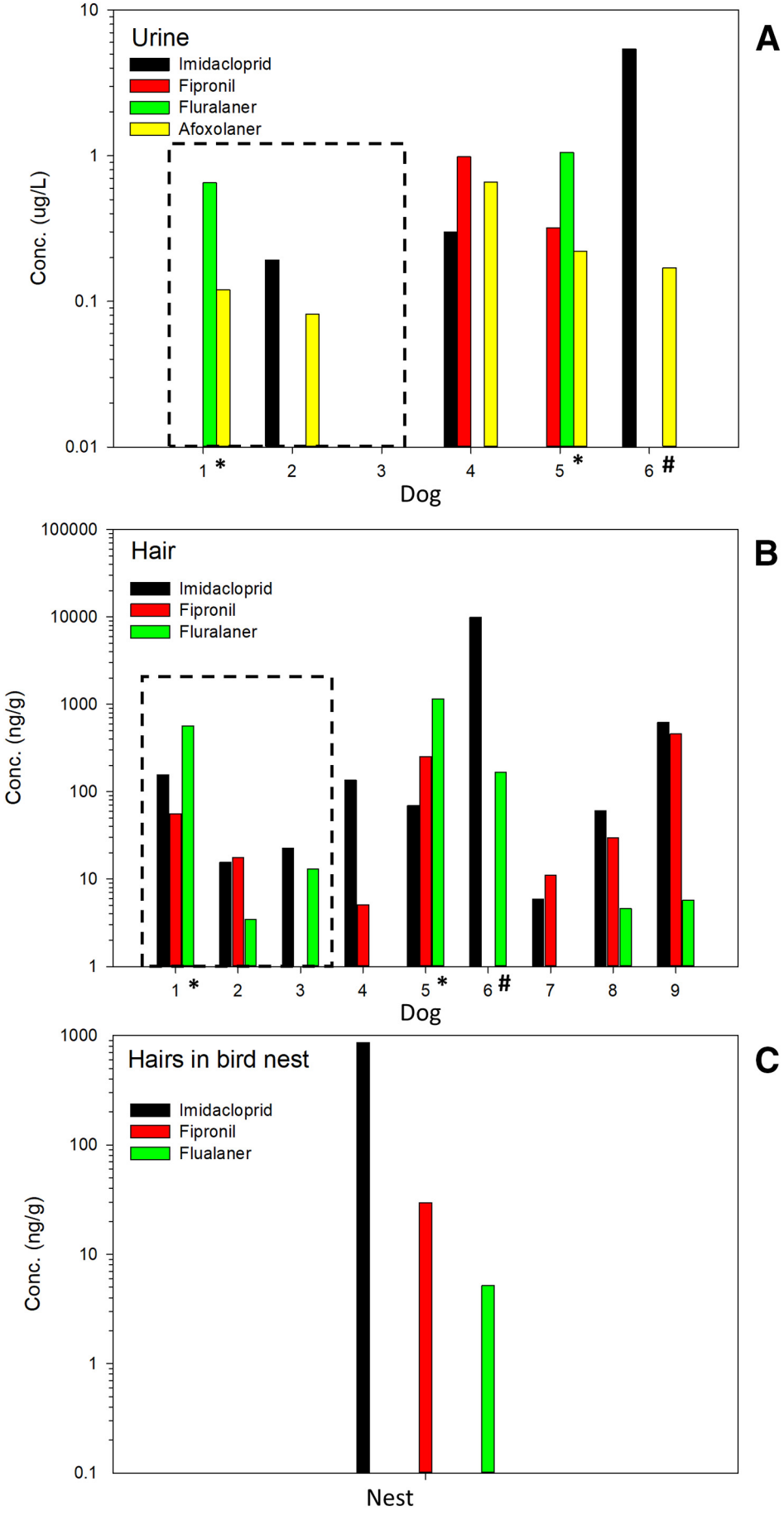
Concentrations of fluralaner, fipronil and imidacloprid were more often above the detection limit in hair than in urine samples, whereas afoxolaner was only detected in urine (Fig. 1, Table 1). The highest concentrations found in dog hairs were 460 ng/g for fipronil, 1156 ng/g for fluralaner, and 9841 ng/g for imidacloprid (Fig. 1B, Table 1). One, to a maximum of three of the analyzed chemicals were found in a urine sample with the highest concentrations of 0.66 μg/L for afoxolaner, 0.98 μg/L for fipronil, 1.06 μg/L for fluralaner and 5.40 μg/L for imidacloprid (Fig. 1A, Table 1).

Of the nine dogs that provided urine and/or hair samples, two had been treated with the active ingredient fluralaner (dog 1 and 5) and one with imidacloprid/flumethrine (dog 6). The other dogs had been treated with active ingredients not measured in this study, mainly sarolaner (4 out of 9 dogs) or had not been treated at all (dog 9) (Fig. 1, Table 1). The highest imidacloprid concentrations in urine and hair samples were measured in dog 6, which had been treated with an imidacloprid/flumethrine Seresto collar. Similarly, the highest concentrations of fluralaner were found in the dogs (1 and 5) treated with fluralaner Bravecto. Dogs were, however, additionally found to be contaminated with one or more chemicals that they were not directly treated with. For example, imidacloprid was found in 100 % of the hair samples, yet 8 out of the 9 dogs (89 %) had not been actively treated with this compound. Likewise, fipronil was found in 100 % of the dog hairs and afoxolaner in almost all urine samples yet none of the dogs had been treated with these ingredients. This indicates a secondary source of these chemicals such as contact transfer from other animals.

There are existing data for afoxolaner and fluralaner concentrations in plasma samples whereas there is little to no information available for hair and urine, so here we present a first dataset (Fig. 1, Table 1). To our knowledge, only one study has tested the urine of dogs treated once with afoxolaner in a soft chewable oral formulation (Nexgard®), where all concentrations were below the detection limit (<1.25 ng/mL; Letendre et al., 2014). Here, we demonstrate convincingly that this chemical is present in urine, potentially because of our lower LOQ (0.14 ng/m urine).

For fipronil, data on dog urine were not available in the literature so no direct comparison is possible. However, rat and human urine studies have shown that fipronil was not detected, but that its metabolites fipronil sulfone and hydroxy-fipronil were instead a good indicator (Cravedi et al., 2013; McMahen et al., 2015; Shi et al., 2021; Vasylieva et al., 2017). This could explain our low number of measured concentrations above the detection limit (2 out of 6).

Fipronil concentrations in hair brushed from dogs were found to be between 52,000–610,0000 ng/g ($n = 9$) 24 h after application of "Frontline"


A
Urine
Imidacloprid
Fipronil
Fluralaner
Afoxolaner
Conc. (ug/L)
10
1
0.1
0.01
1 *
2
3
4
5 *
6 #
Dog
B
Hair
Imidacloprid
Fipronil
Fluralaner
Conc. (ng/g)
100000
10000
1000
100
10
1
1 *
2
3
4
5 *
6 #
7
8
9
Dog
C
Hairs in bird nest
Imidacloprid
Fipronil
Flualaner
Conc. (ng/g)
1000
100
10
1
0.1
Nest

and decreased to 190–250,000 ng/g four weeks post application (Bigelow Dyk and RI, 2012). Fipronil has also been found in dog hair at a concentration of 18,500 ng/g ($n = 1$) (Muilerman and Manthingh, n.d.). In comparison, fipronil (+sulfone) was found in human hairs in the range of 110–310 ng/g in 3 of 21 samples (14.3 %) (Muilerman and Manthingh, n.d.). Also in 98 % of samples of the hair of French children, fipronil was detected at concentrations as high as 114 ng/g (Iglesias-González et al., 2020).

These concentrations in human hair were in the range of our findings. However, dog hair concentrations were only approaching our range four weeks after application. As none of the dogs in this study, like humans, had been actively treated with fipronil, post-treatment level values were not expected. Hair clippings showed a time-dependent distribution of fipronil over the dog, with higher concentrations on the neck and back (Bigelow Dyk and RI, 2012). Higher concentrations were also found in hair samples taken from brushes after brushing, compared to hair cut at a certain location of the body. These results call for standardized hair sampling methods, both in terms of collection method (brush vs cutting) and body location to reduce variability within and across samples. In this study hair, collection was done by the dog owners themselves, which might have introduced unquantifiable variation between the samples.

Imidacloprid was among the most frequently detected compounds in dog urine, even after a four week controlled diet (Forster et al., 2014). Unfortunately, concentrations were not given in that study, making further comparison impossible. However, imidacloprid was also detected in human urine (Harada et al., 2016; Wang et al., 2020, 2015) with concentrations ranging between 0.46 and 142 μg/L in Wuhan, China (Wang et al., 2020). This range overlaps with concentrations in dogs urine in this study. As dog and humans share similar environments and thus similar exposure pathways, concentration ranges may be used to predict exposure from one group to the other.

Concentration of imidacloprid in dogs hair was followed over time after a single imidacloprid application of spot-on Advantage®. Transferable residue concentrations, taken by stroking with cotton gloves, decreased over the course of five weeks from $254 * 10^3$ ng/g to non-detectable levels (Craig et al., 2008). Although direct hair measurements were not available in the study of Craig and co-workers, this gives an good indication of temporal patterns and concentrations in dogs' hair. The maximal concentration in hair found in our study (9841 ng/g in dog 6) corresponded with concentrations on gloves after 3 days to one week post treatment. This dog (6) had been treated with imidacloprid by a Seresto collar instead of spot-on. A collar provides constant chronic exposure, while spot-on is a multi-dose exposure with a maximal peak at time of the application of the product and concentration sequentially decreasing over time till next application.

3.2. Secondary transfer to dogs

All four analyzed chemicals were detected in hair and/or urine of dogs untreated with those particular active ingredients (Fig. 1, Table 1), indicating another source of contamination. Similarly, imidacloprid was found in urine samples of untreated dogs fed with a controlled diet for one month (Forster et al., 2014). Imidacloprid was not detected in the food, and its half-life is <48 h, indicating that acute dietary intake was not the main uptake route. As another example, imidacloprid was detected in untreated rabbit hair ranging from 630 to 1610 ng/g measured over a period of 6 months (Kavvalakis et al., 2013). Currently, imidacloprid and fipronil are banned from outdoor agricultural use by the European Commission, but are approved for flea and tick control treatment, and afoxolaner and fluralaner are specially designed and approved for flea and tick control treatment, with limited other uses. As these four chemicals have a limited use beyond flea and tick control treatment products, and food intake was not indicated as a major uptake route in dogs, secondary transfer by direct dog-to-dog contact may be a major pathway for these chemicals e.g. when playing, or indirectly via urine and feces (Westgarth et al., 2009, 2008). In addition to dog-to-dog transfer, pets of other species kept in the same home can also be a source. Examples for these were the two homes in which cats were treated with imidacloprid while the dogs were treated with sarolaner, but the dogs still had detectable imidacloprid residues in hair and/or urine (Table 1, Table S4).

The hypothesis of secondary transfer by direct dog-to-dog contact is supported by several studies showing a significant transfer of administered chemicals from dogs onto cotton gloves and other items. This route is, for example, further highlighted by a study of Davis et al. (2008), which indicated that dogs treated with tetrachlorvinphos collars showed a significant transfer of the chemical onto cotton gloves used to pet the dog and cotton tee shirts worn by children who were in contact with the dog. Residues decreased over time after treatment, but were still measurable at the end of the study at 112 days. Similar patterns were also observed for fipronil (Bigelow Dyk and RI, 2012; Cochran et al., 2015; Jennings et al., 2002), imidacloprid (Craig et al., 2008) and chlorpyrifos (Boone et al., 2001; Chambers et al., 2007). For example, after Frontline application, 0.56 to 11 % of fipronil was transferred to cotton glovers (Bigelow Dyk and RI, 2012). Exposure concentration and duration differ among chemicals, which may be attributed to sample analyses, composition of dog fur, and dose and application time (Davis et al., 2008). These results also indicate a secondary transfer route from dogs to humans which share similar environments and are in close contact, as discussed earlier.

Unexpectedly in our study, however, afoxolaner was not detected in hair, while it was found in urine of untreated dogs. This is not in line with the dog hair transfer route discussed earlier. Afoxolaner is a small hydrophobic unionized molecule expected to cross cell membranes freely, has a low body clearance rate (Letendre et al., 2014) and a high hydrophobicity ($\log k_{ow} \sim 5.5$). The chemical is by design systematic and thus these characteristics could explain this. Additionally, the major uptake pathway for this chemical could be through oral uptake e.g. by licking or coprophagia instead of direct fur contact.

3.2.1. Transfer to birds

Dog owners may comb their dogs outdoors and removed hairs may be left behind, and in addition, dogs also shed hair naturally as they are active in the environment. This presents a transfer route to other compartments of the environment. In the terrestrial environment, the exposure pathway from dog hair used in birds' nests to juvenile birds was suggested by Guldemond et al. (2019). Our measurements showed that hair found in the bird's nest that we collected had concentrations of 30 ng/g for fipronil, 5.18 ng/g for fluralaner and 864 ng/g for imidacloprid (Fig. 1C). Fipronil (22 ng/g), imidacloprid (60 ng/g) and five other chemicals (seven in total) were also found in hairs from another great tit bird's nest (Guldemond et al., 2019). Moreover, in both natural and agricultural areas, fipronil (average range 5.8–23 ng/g), fluralaner (average range 8–450 ng/g) and imidacloprid (average range 9.1–100 ng/g) were found in dead juvenile birds in the Netherlands (Guldemond et al., 2019). These authors hypothesized that mortality of juvenile great tits could be linked to the contaminated hairs found in the nests. It must be mentioned, however, that this study only analyzed dead chicks, so living chicks could possibly contain equal amounts. Thus only the transfer of these contaminants to chicks could be established and actual causality with mortality cannot be established based on these results. In the Netherlands, fluralaner is permitted for use in flea and tick control products for pet dogs and cats, and may also be used in the poultry industry (Annex to Regulation (EU) No 37/2010). Whereas Guldemond et al. (2019) did not found fluralaner in their two sampled bird nests, our study did. Our findings strengthen the hypothesis that hairs in birds' nests are a potential pathway for exposure of chicks. However, currently, no other data are available to further investigate this link. To close the data gap between exposure and any effect on chicks, additional research is required.

Fig. 1. Chemical concentration (ng/L) of afoxolaner, fipronil, fluralaner and imidacloprid in: dog urine (**A**); hairs taken directly from a dog (**B**); and hairs found in a bird nest (**C**). * indicate dogs that were treated with the active ingredient fluralaner (dog 1 and 5) and # with imidacloprid (dog 6). Note that the first three dogs (see dotted line box) participated in the swimming experiment in that specific order.

Table 1
Details of dogs and chemical concentrations of afoxolaner, fluralaner, fipronil and imidacloprid in hair (all), urine (dogs 1 to 6) and water after swimming of each dog (dogs 1 to 3).

							Afoxolaner[4]		Fluralaner			Fipronil[4]		Imidacloprid		
Nr.	Breed	Sex	Weight (kg)	Anti-flea and tick product	Active ingredient	Pet cat's active ingredient[3]	Hair (ng/g)	Urine (ug/L)	Hair (ng/g)	Urine (ug/L)	Water (ng/L)	Hair (ng/g)	Urine (ug/L)	Hair (ng/g)	Urine (ug/L)	Water (ng/L)
1	English springer spaniel[1]	M	18.2	Bravecto tablets (500 mg)	Fluralaner	Fluralaner	BDL	0.12	***569.65***[5]	***0.65***	***1.071***	55.83	<0.5	155.99	<0.02	0.144
2	German longhaired pointer[1]	F	29	Vectra 3D dog	Dinotefuran/ pyriproxyfen/ permethrin	Yes but not treated	BDL	0.08	3.44	<0.08	0.924	17.64	<0.4	15.48	0.19	0.216
3	Sprocker spaniel[1]	M	18.6	Simparica once per month	Sarolaner	–	BDL	<0.14	12.95	<0.08	0.909	19.50[6]	<0.6	22.43	<0.02	0.248
4	Jack Russell terrier	F	7.5	Unknown brand (15 mg)	Sarolaner	Imidacloprid/flumethrine	BDL	0.66	0.90[6]	<0.08	–	5.08	0.98	*136.17*	*0.30*	–
5	Dutch Smoushond	M	17	Bravecto tablets	Fluralaner	–	BDL	0.22	**1156.51**	**1.06**	–	253.40	0.32	68.80	<0.02	–
6	Old German herding dog	F	30	Seresto collar	Imidacloprid/flumethrine	–	BDL	0.17	166.13	<0.08	–	9.00[6]	<0.3	**9841.46**	**5.40**	–
7	Short haired dachshund	M	13.8	Unknown brand 40 mg once per month	Sarolaner	Natural products	BDL	–	1.31[6]	–	–	11.02	–	5.90	–	–
8	Border collie X Australian shepherd	M	32	Easecto 1 tablet per month	Sarolaner	Imidacloprid/flumethrine	BDL	–	4.59	–	–	29.64	–	*60.08*	–	–
9	English cocker spaniel	F	13.5	Not treated by owner but dog had been in a boarding kennel for 1 week before sample was taken	–	–	BDL	–	5.73	–	–	460.21	–	621.35	–	–
10	Unknown dog hairs from bird nest[2]	–	–	–	–	–	BDL	–	5.18	–	–	29.53	–	863.51	–	–

[1] Dog 1, 2 and 3 participated in the swimming experiment in this order.
[2] The unknown dog hairs were taken from a great tit bird nest from an urban area in Wageningen, The Netherlands.
[3] The only other pets in the household were cats, and some were treated with anti-flea and tick products.
[4] Concentrations for afoxolaner and fipronil in water were below detection limit (BDL) and for afoxolaner in hair.
[5] **Bold** values indicate that dog has been treated with this active ingredient, *cursive* values indicated that cat has been treated.
[6] Concentration is indicative.

Additionally, increasing public awareness could reduce the potential risk of dog hair in the environment. Currently, some dog owners deliberately leave dog hair in gardens, parks and natural areas for birds to build nests, even though general warnings are given not to leave dog hair, or only leave untreated dog hair out of doors. However, our results indicate that even hair from non-treated dogs can contain chemicals e.g. due to secondary transfer. Therefore, a precautionary option would be dispose of any dog hair resulting from grooming into waste containers by default. With additional data, the general public could be reliably informed how to dispose of dog hair properly and increase their awareness on the potential consequences. In a short follow up survey among the owners of the participating dogs, some stated that by taking part in this study their awareness had been raised and they were now more careful to dispose of dog hair in appropriate waste streams (Table S4).

3.2.2. Transfer to the aquatic environment

To determine transfer from dogs to the aquatic environment, a swimming experiment was performed. Two of the four measured chemicals, fluralaner and imidacloprid, were detected in the water after the first dog had swum (Fig. 2, Table 1). Fluralaner decreased, whilst imidacloprid increased after each dog swam in the water. Maximal concentration in water was 1.07 ng/L for fluralaner and 0.25 ng/L for imidacloprid, respectively. Hair and urine samples showed that concentrations for fluralaner and imidacloprid were highest in dog 1 then in dog 3 and lowest in dog 2 (Fig. 1, Table 1). This explains the sequential increase in imidacloprid concentrations in the water, as each dog added an additional chemical load, and imidacloprid has a low hydrophobicity (e.g., is very soluble in water; $\log k_{ow}$ 0.57). Even though each dog added an additional load of fluralaner to the water, fluralaner water concentrations did not increase. This might be explained by the high hydrophobicity ($\log k_{ow} \sim 5$), resulting in a fugacity gradient towards organic matter and thus a tendency to adsorb to the dogs. Note that only dog 1 had been actively treated with fluralaner and none of the dogs had been treated with imidacloprid, indicating a tertiary exposure pathway i.e. from dog-to-dog and then to the environment.

To our knowledge, fluralaner has not been analyzed during any sampling campaigns in surface or effluent water, so there are no data to compare with our findings. However, water concentrations during our whole experiment were above Dutch acceptable surface water limits (0.47 ng/L) (Lahr et al., 2019), indicating a potential risk for the aquatic environment. At present, no environmental risk assessment of fluralaner has been made for Dutch surface waters as environmental concentrations are not available (Lahr et al., 2019).

In Dutch surface waters, imidacloprid has been detected in 19 % of samples and in 90 % of in- and effluent samples from waste water treatment plants, with all values above annual average environmental quality standards (AA-EQS; 8.3 ng/L) (Lahr et al., 2019). In UK surface water, imidacloprid has been found in 66 % of the samples, at a maximal concentration of 360 ng/L (Perkins et al., 2021a). It is unclear how much of these results are contributed by flea and tick control products. Our values are low compared to the maximal values found in surface water and below the AA-EQS. This can be explained by a higher number of dogs and multiple sources that may contribute to a total higher concentration in a natural environment.

Although fipronil was measured in the hair of all three dogs in our study, it was not found at detectable levels in the swimming water, which may be explained by the low water solubility ($\log k_{ow}$ 4) in combination with the relative low chemical concentration on dog 1. The contrast with fluralaner, which was detected in the water despite its low water solubility, may be explained by the 10× higher chemical concentration on dog 1. In Dutch surface waters, fipronil was measured in 1.7 % of the samples and in 43 % of effluent samples from waste water treatment plants, with all values above acceptable limits (0.07 ng/L) (Lahr et al., 2019). In Northern California, USA, wastewater effluent concentrations (range 14–45 ng/L) were above the USEPA chronic aquatic benchmark of 11 ng/L (Teerlink et al., 2017). In English surface water, fipronil has been detected in 99 % of samples with a maximal concentration of 980 ng/L which is well above the acute (20 ng/L) and chronic (3.2 ng/L) toxicity limit (Perkins et al., 2021a). A direct pathway from dogs to wastewater treatment plant was confirmed by Teerlink et al. (2017), which showed that 2 days post-application, 21 % and at 28 days 4 % of the applied dose could be detected in wastewater used to wash dogs. Despite the use of fipronil for other purposes, it is likely that veterinary medicines are an important contributing source to waste and surface water (Perkins et al., 2021a). Afoxolaner was not measured in the swimming water in our experiment. To our knowledge, no data are available on surface water or effluent measurements, however, it may be possible to find detectable concentrations in the aquatic environment.

3.3. Implications and risks for the environment

This study provided proof of principle that dogs transfer anti-flea and tick pesticides into the environment through different direct and indirect pathways, and therewith pose a potential risk to both terrestrial and aquatic ecosystems.

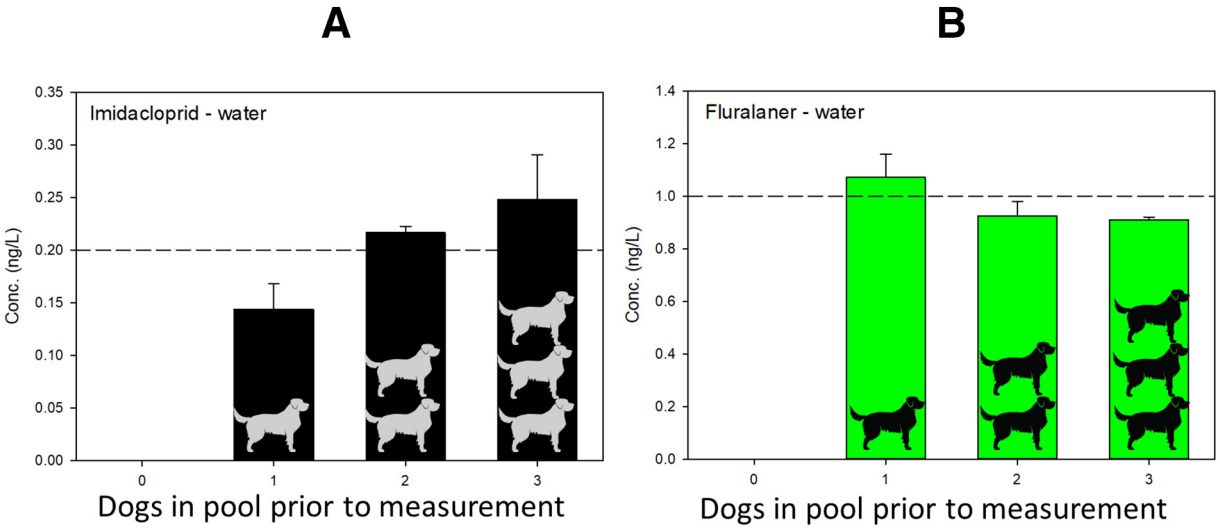


Fig. 2. Chemical concentration in water (ng/L) of imidacloprid (**A**) and fluralaner (**B**) before (0) and after each dog swam in the water. Dotted line indicates limit of quantification and error bars represent standard deviation between duplicate measurements. Note that only the first dog was actively treated with fluralaner, and none of the dogs with imidacloprid.

Currently, environmental risk is by default assumed to be low by the European Medicines Agency (Domingo-Echaburu et al., 2021; Perkins et al., 2021a). Due to the data gaps, however, a proper environmental risk assessment is hard to make. However, the available data and findings in our research indicate that the potential risk to the environment should not be neglected and further investigation is required.

Estimated use of fipronil and imidacloprid for dogs and cats is between 500 kg and 3000 kg of active ingredient per year in the Netherlands. Lahr et al. (2019) demonstrated that when 0.01 % to 0.3 % of this fipronil and 1.15 to 6 % of this imidacloprid enters the waste water treatments plants, this could lead to concentrations in surface water above acceptable limit in the Netherlands. Similarly, if 25 % of treated dogs were to be washed within 7 days post-treatment and this water were to enter the wastewater treatments plants, a load equivalent to the total fipronil load of the San Francisco Bay Area would be reached (Teerlink et al., 2017). These calculations only take into account the chemicals entering the natural water systems through the waste water plant, and do not take into account additional pathways such as leaching while swimming, wash-off during rain, urine or feces, thereby underestimating the potential environmental risks. For example, the average dog urine production is 20–100 mL/kg/day (Yadav et al., 2020). Dogs in our study weighed on average 19.2 (7.5–30) kg. Taking an average urine volume of 60 mL/kg/day and the average chemical concentrations measured in urine (Table 1) this would lead to a daily load of 0.29 μg afoxolaner, 0.49 μg fluralaner, 0.75 μg fipronil and 2.26 μg imidacloprid from just one dog of 19.2 kg. In addition, Wells and Collins (2022) corroborated our finding that dogs can leach AIs during swimming. A scenario for one dog of 40 kg predicted that selamectin leaching leached during swimming resulted in a risk ratio of 0.8, where below 1 is considered acceptable. However, this only considered leaching from a single dog (Wells and Collins, 2022), making the outcome of a scenario of a small pond at a popular site visited by many swimming dogs at potentially high risk of serious environmental contamination.

The real magnitude of environmental impact from dog parasiticide treatments depends on many variables such as environmental characteristics, dog behavior, population density, human behavior and environmental awareness. Currently data about pet dog population structure and density is scare. In the UK, dog and cat populations are denser in urban than in rural areas, with densities exceeding 2500 animals/km^2 in large city centers (Aegerter et al., 2017). A high overall dog network connectivity increases the chance of secondary transfer and thus environmental impact. Common areas such as parks and recreation spaces have been found to have a high degree of connectivity (Westgarth et al., 2009). Indeed, our additional inquiry indicated that all of the participating dogs in our study had frequent contact with other dogs during their outdoor walks (Table S4). This is in agreement with the estimate that most dogs interact with one to five other dogs and three to five persons outside the household per day, with higher interaction on weekend days, with maximal interactions with 15 or more (Westgarth et al., 2008). Other places for potential interaction seem, however, to be low. In the UK, most dogs never visit a training class (93 %), boarding kennel (67 %) or grooming parlor (67 %) (Westgarth et al., 2008). In our study too, none of the dogs involved spend time in a boarding kennel apart from dog 9, twice a year for one or more weeks. However, even this dog, which was not treated by the owners, showed high levels of fipronil and imidacloprid, suggesting that boarding kennels are potential hot spots for secondary transfer and this may also apply to training classes, dog walking services and other places where dogs are kept together and have high interactions. Moreover, secondary transfer may occur in homes where multiple pets are kept. In our study, five households also kept pet cats, of which four out of five were treated with antiflea and tick products (Table 1, Table S4). Similarly, a study in Spain showed that about 54 % of dogs coexist with other pets in a household, but in Spain dogs cohabit more often with other dogs (30 %) than with cats (10 %) (Peribáñez et al., 2018).

Besides additional research to fill the current data gaps, educating and increasing the awareness of this issue with dog owners, together with enforcement is recommended (Bennett and Weeks, 2021; Murphy and Wright, 2020; Wells and Collins, 2022), especially with the growing number of pet dogs worldwide. Human behavior, for example in product choice and use is also important. Some owners use products recommended and provided by veterinarians, while for others the choice may often be random. Dog characteristics, geographical and cultural components may also influence the choice to apply a product and the type of product preferred. The age of dogs influences the products used, with higher treatment after 4 months (Peribáñez et al., 2018). After choosing a product, the likelihood that owners keep using the same product depends on the efficacy and ease of use of the product, as higher efficacy increases customer satisfaction and product loyalty (Peribáñez et al., 2018). Inaccurate treatment in terms of product type, calendar compliance, correct application and management after treatment, together with the knowledge gap about the parasite life cycle may lead to a failure to control fleas, and therefore possible product change (Peribáñez et al., 2018). Moreover, dog owners may not be familiar with the prescriptions of the product used, or read it only once and depend on their memory for future dosing, i.e. 12 weeks later (Wells and Collins, 2022). They may therefore use the product inappropriately or ignore warnings about swimming and bathing of treated dogs. For example, spot-on products warn dog owners to keep treated dogs away from waterways for a specified period after treatment, and collar products instruct owners to remove the collar before swimming.

In conclusion, our research has demonstrated that dogs can transfer anti-flea and tick pesticides into the environment, posing a potential risk to both terrestrial and aquatic ecosystems. Although the results of this study contribute to filling some of the current knowledge gaps, many questions about the exposure and effects of anti-flea and tick products still remain, calling for more detailed research. Moreover, better education and increasing awareness among dog owners could contribute to the correct use of anti-flea and tick products and decrease additional exposure to the environment. The results of this study call for improvements in the current risk assessment and management of veterinary medicines by including companion animals and their exposure pathways.

CRediT authorship contribution statement

ND contributed by conceptualization, data analyses, literature review, visualization, writing, DB by conceptualization, methodology, experimental investigation, data analyses, literature review, writing, LB by methodology, chemical analyses and interpretation, writing and IR by funding acquisition, conceptualization, methodology, experimental investigation, data analyses, visualization, writing.

Data availability

Data has been made available in SI

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.scitotenv.2022.159550.

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