Cecilia Testa, Severyn Salis, Nicola Rubattu, Paola Roncada, Roberto Miniero & Gianfranco Brambilla (2019) Occurrence of Fipronil in residential house dust in the presence and absence of pets: a hint for a comprehensive toxicological assessment, Journal of Environmental Science and Health, Part B, 54:6, 441-448

The final published version is available online at:
http://dx.doi.org/10.1080/03601234.2019.1607133

Occurrence of Fipronil in residential house dust in presence and absence of pets: a hint for a comprehensive toxicological assessment

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Abstract:

The presence of the insecticide Fipronil and its main products of toxicological relevance, namely, Sulfone and Desulfinyl, was assessed in 161 residential house dust samples in the absence (N = 101) and presence (N = 60) of cats and dogs in Italy. High-resolution mass spectrometry analysis revealed a significant difference ($p < 0.001$) in the dust contamination in the presence of pets (median: 467 vs 24 ng/g dry weight), even if the highest value was found in the absence of pets (82,069 vs 67,799 ng/g dry weight). Fipronil intake estimates from dust in toddlers, computed according to US-EPA and EU-ECHA guidelines, ranged from 333 – 556 and from 20–34 ng/kg per day for acute and chronic scenario, respectively. Dust seemed not able itself to lead to Fipronil overexposure with respect to acute and chronic toxicity health-based guidance values. Kittens were potentially overexposed to Fipronil under both acute (26,076 ng/kg per day) and chronic (1,633 ng/kg per day) scenarios. The mild symptomatology associated with acute intoxication could possibly determine case

underreporting within pharmacosurveillance schemes. Its administration was estimated in 7.3–9.7 tons per year. Such a range suggests its prudent use under strict veterinary control to prevent pest resistance and ecotoxicological outcomes.

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KEYWORDS: Fipronil, pets, house dust, intake, use, assessment

Introduction

Fipronil (F) (CAS no. 120068-37-3) is a broad-spectrum phenylpyrazole insecticide recently put into the spot of the public opinion for two main reasons: a) the impact it has on bees due to its use for seed and soil treatments against soil-dwelling arthropods and early-season leaf-feeding and sucking insect pests, together with neonicotinoids, [1, 2] and b) its unauthorized use as veterinary drug in poultry farms in the European Union that lead to the presence of residues in poultry products of potential concern for children. [3, 4] Fipronil blocks the GABA A-gated chloride channels in the central nervous system, thus provoking the pest death from a prolonged and uncontrolled neuronal stimulation. After its application, F degrades under sunlight into Fipronil Desulfinyl, which has been described as being 9-10 times more active when inhibiting the mammalian chloride channel than the insect chloride channel, possibly through the formation of Fipronil Sulfone. [5, 6] The urban use encompasses the control of ectoparasites as a veterinary medicine product in anti-flea/tick/mosquito lotions; sprays for topical use on pet hair; and to fight turf beetles, cockroaches, fleas, termites, thrips, black vine weevil and other insects as a biocidal product for households, hardy ornamentals, and nonedible ornamentals. [7] Fipronil release in urban watersheds with its main active products (Desulfinyl and Sulfone) has been considered recently for its ecotoxicological impact on nontarget organisms, with particular attention paid to aquatic organisms due to its toxicological features and its wide and regular use. [8] Dust samples collected in Northern California around individual homes revealed the presence of Desulfinyl and Sulfone in 75.5% and 67.3% the of the 441 samples analyzed against the 52.4% of the parent compound, with geometric means at 71, 78, and 60 ng/g dust, respectively. [9] The analysis of run-off waters from urban civil wastewater treatment plants from this and other regions reported Fipronil concentrations between 14 and 45 ng/L, which are above the US-EPA chronic aquatic benchmark for Fipronil of 11 ng/L [10, 11] and the Dutch Environmental Quality Standards for surface waters of 0.7 ng/L. [12]

According to the National Health Authority authorization, in veterinary medicine, Fipronil can be sold without a prescription at the supermarket level as sprays and spot-on formulations at 0.05–0.10% (w/v) for dogs and cats. The posology ranges between 6.7–13.3 mg/kg pet per month with the claim of protecting animals and the indoor environment from pests. The veterinary use could be of relevance for the environmental release of Fipronil and the exposure of nontarget species for the following reasons: a) its regular use in a very large pet community in urban and urban/rural contexts (Figure 1); [13, 14] b) the handling of concentrated solutions by nonprofessional people, such as groomers and pet owners, and c) the indoor pollution in the presence of treated animals that could drive postapplication exposure in sensitive groups such as toddlers and kittens, via dust intake, as result of their mouthing and licking behavior and the fur petting of treated pets. [15, 16]

The first evidence of Fipronil occurrence at ppm level in indoor house dust and its association to the presence of pets was given by Mahler et al. [17] in 2009; 19 of 24 samples of indoor dust showed a total Fipronil concentration (the sum of the parent and main products) below 270 ng/g; in the remaining five samples, concentrations ranged from 1,320 to 14,200 ng/g. All three of the residences with a dog on which a flea-control product containing Fipronil was used were among the five residences with elevated Fipronil concentrations. More recently, Starr et al. [18] (2016), in a study about the Fipronil oral bioaccessibility from house dust, found Fipronil in the range 1–108,000 ng/g in 31 out of 37 samples, and Sulfone (21/37) (4–10,200 ng/g) and Desulfinyl (6/37) (4–1,900 ng/g) were also found. The reported oral bioavailability of Fipronil ranges between the 50 and 85% of the total ingested dose, [19] as matter of the presence of two fluoromethyl groups that may increase its absorption. [20] Considering the quite relevant pet population in Italy targeted by potential F treatments (**Figure 1**), exploration of the association between indoor contamination and the presence of pets for a more comprehensive toxicological assessment seemed worthwhile.

Materials and methods

Sampling design and analysis

During the spring-autumn period of 2016, a convenient sampling design was set up in the Bologna District, among people regularly attending the Veterinary Teaching Hospital of Bologna University, Italy. Residential house dust samples (N = 161) were drawn from vacuum cleaner bags, along with records about the presence of pets (cats and dogs), their body weight, and the availability of a private garden. From each vacuum bag (200 g of dust per bag on average), 10 individual samples of 1 g were drawn from different sites of the bag to form the composite sample. To avoid heterogeneity, the larger particles and sand deposits were removed, and the dust was sieved to collect the 0.2 mm portion.

Fipronil and its metabolites were supplied by Lab Service Analytica (Bologna, Italy), and ACN, Methanol (LiChrosolv, hypergrade for LC-MS), and other reagents and materials were purchased from Merck (Darmstadt, Germany). The analytical procedure adopted is one routinely used to determine Fipronil in food commodities, in agreement with the work of Guo et al. .[21] Briefly, a test portion of 0.4 grams of dust was weighed into a 15 mL centrifuge tube. A volume of 1 mL water and 9 mL acetonitrile was added to each sample, the mixture was sonicated for 5 mins, vigorously mixed for 10 min, and then centrifuged (4000 rpm, 5°C, 10 min). Then, 5 mL of extract was transferred to a new 15 mL centrifuge tube containing dispersive adsorbents (QuEChERS-based clean-up with 900 mg MgSO4, 150 mg C18, 150 mg PSA from Waters Italia, Milan, Italy) and centrifuged at 4000 rpm for 10 min at 5°C; finally 0.4 mL of extract was added with 0.6 mL Methanol and analyzed on UPLC-HRMS. The chromatographic analysis of Fipronil and its products was performed using an Ultimate 3000 UPLC system (Thermo Fisher Scientific, San Jose, CA, USA). Separation was carried out on an ACQUITY BEH C18 Analytical Column (100 x 2.1 mm, 1.7 µm) from Waters Corporation protected by a VanGuard Pre-Column (2.1 x 5 mm) (Waters Italia, Milan, Italy). The mobile phase consisted of (A) water and (B) methanol at a flow rate of 0.3 mL/min. The elution gradient was initially set to 10% B and ramped linearly to 50% B in 3 min; then, it was increased to 100% B in 5 min and held for 5 min; and finally, it was returned to the initial condition in 0.5 min and maintained for 2.5 min to allow column conditioning for the next injection. The injection volume was 5 µL. The UHPLC system was coupled to a Q-Exactive Orbitrap MS (Thermo Fisher Scientific, Bremen, Germany), which was operated with a heated electrospray interface (HESI) in negative electrospray ionization (ESI). The ion transfer capillary temperature, spray voltage, sheath gas flow rate, auxiliary gas flow rate and S-lens RF level were set to 325°C, 3.5 kV, 60, 30 and 55, respectively. The Q-Exactive MS was tuned and calibrated in positive and negative mode once a week using the calibration solutions, including caffeine, MRFA, and a mixture of Ultramark 1621 fluorinated phosphazine. The mass spectra were acquired with full MS mode at a resolution of 70,000 FWHM with 2.0 × 106 of Automatic Gain Control (AGC) target and 100 ms of maximum ion injection time. The analyses were performed without lock mass. Data were processed using XcaliburTM3.1.66.102.2.1 and Trace Finder 3.2 software (Thermo Fisher Scientific, Les Ulis, France) was used for identifying the untargeted Fipronil Desulfinyl according to the exact mass as identification criteria; confirmation of identity was achieved by automated matching of the given and additional criteria from the HRAM MS/MS Spectra Library.

Quality Control

Linearity was investigated by analysis of the calibration curves over the concentration ranges of 0.01–20 ng/g using least squares linear regressing of peak area versus concentration. Limits of Detection (LODs) were estimated analyzing three blank samples spiked at 0.01, 0.05 and 0.1 ng/g levels. They are defined as the minimum concentration at which the accurate mass error was <5ppm; LODs resulted in 0.01 ng/g for both Fipronil and Sulfone. The limits of quantification (LOQs) were estimated based on the lowest concentration measurable (0.05 ng/g) with precision expressed in terms of calculated Intra-day Repeatability (RSDr = 11.35 for Fipronil and 16.75 for Sulfone); Trueness was estimated with the recovery test at 10 ng/g (recovery = 101%) and 100 ng/g (recovery = 125%) levels. The results indicated the method was suitable for the quantification of the target analytes in considered matrix.

More detailed information is included in the supporting materials. Data for the dust were computed on a ng/g dry weight basis, as total Fipronil.

Data handling and statistics

Left censored data were computed adopting the medium bound (1/2 of the LOQ = 5 ng/g) approach. The differences between i) the groups of dust collected in the absence and presence of pets and ii) within the presence of pets group, the groups reporting the presence or absence of private garden in the households were evaluated by the U-Mann-Whitney nonparametric test (Statgraphics XVI, Statpoint Technologies, Inc) The outliers taken in considerations in both the evaluations were identified by the MAD/0.6745, which is an estimate based on the median absolute deviation (the median of the absolute differences between each data value and the sample median) (Statgraphics XVI, Statpoint Technologies, Inc, supplied by Adalta, Arezzo, Italy).

Fipronil use in veterinary medicine

The estimated use of Fipronil as an anti-ectoparasitic agent in dogs and cats accounted for the following data: a) the inventory of the number of cats (6,967,000) was derived from the 2018 report of the National Petfood Association, [13], and for the number of dogs (10,717,479), the 2018 official registry from the Italian Ministry of Health report on dogs was used, which reported more or less the equivalent of 1 dog to every 6 inhabitants in Italy (**Figure 1**), and b) the low range posology of 6.7 mg/kg bw per month for an average weight of adult cats (4.42 ± 1.55 kg bw, N = 352) and dogs (17.07 ± 12.34 kg bw, N = 1427) was determined from records made on the admission of cats and dogs to the Bologna University Veterinary Small Animals Clinic. We estimated an 8-month-long treatment for ticks from the spring to autumn, with an application rate once a month per pet. To discriminate when the amount of Fipronil in house dust could be associated with treated pets and not to other nonagricultural uses, appropriate cut-off values were identified in the median (50^{th} percentile, P50) + the Median Absolute Deviation (MAD) and in the 75^{th} percentile (P75) contamination recovered from the dataset of house dust in absence of pets (N = 101). The percentage of house dust samples collected in the presence of pets falling above such cut-off value was then applied to the overall pet population in Italy to achieve a reliable estimate of the F amount used on a yearly basis as a veterinary drug.

Intake assessment via dust

The intake assessment of dust was performed to account for the following consensus-based default values: a) average intake rate of 60 mg of dust per day, given as a central tendency for children 1 to 6 years old by the U.S. Environmental Protection Agency; [22] b) 100 mg per day referred to observational data in the European Children and adopted by the European Chemical Agency; [23] and c) the mean default body weight assigned to 2-old Italian toddlers of 12.2 kg. For kittens, a default 200 mg dust per day intake was assumed for an 8-week old kitten of a weight of 0.52 kg, as already reported. [24, 25] The desorption coefficient was assumed equal to 1, and the bioavailability posed at 85% according to evidence from *in vivo* oral exposure in laboratory animals. [19]

Risk characterization

The following Health-Based Guidance Values (HBGVs) were taken into account: an Acute Reference Dose (ARfD) of 3,000 from FAO [19] and of 9,000 ng/kg bw per day from EFSA [26] and the same Acceptable Daily Intake (ADI) of 200 ng/kg bw per day considered both by FAO and EFSA. We considered the maximum and the 95th percentile of dust contamination as total Fipronil for acute toxicity in the presence and absence of pets under a worst-case scenario. For chronic toxicity, the 75th percentile occurrence in dust seemed the most reliable indicator because that value was less biased than the mean value from the presence of outliers in the dataset considered. The risk characterization was expressed as the Margin of Safety (MOS, ratio between the HBGV for acute and chronic toxicity and the related estimated exposure).

Results

House dust contamination

Of the overall 161 samples of house dust, 101 belonged to households in the absence of pets, and of the remaining 60 samples collected in homes with pets present, 38 samples were from houses with a private garden. The Whiskers box-plots (including the outliers) **of Figure 2** show the data distributions of total Fipronil in dust in the presence and absence of pets, respectively, with net significant differences ($P<0.01$). The boxes are drawn extending from the lower quartile of the sample to the upper quartile. This is the interval covered by the middle 50% of the data values when sorted from smallest to largest. The whiskers are drawn from the edges of the box to the largest and smallest data values, where values were unusually far away from the box (points more than 1.5 times the interquartile range (box width) above or below the box are indicated by point symbols. The statistical descriptors on occurrence of each of the analytes, including the ones describing the box intervals, are detailed in **Table 1**. The significance of the Fipronil occurrence in dust referred to those households with pets only, in the presence (N = 38) or absence (N = 22) of a private garden fell between >0.5 and <0.1. Further details are provided in the supporting materials section.

Estimated use of Fipronil as veterinary medicine

The cut-off values (median + MAD and P75) from total Fipronil occurrence in the dust from homes without pets were computed in the range of 32.82 and 109.2 ng/g, which when applied to the house dust dataset in the presence of pets (N = 60), indicated F use as veterinary product in 62–82% of the considered dogs and cat population (**Figure 1**). The estimated amount of Fipronil used as a veterinary product per year, under the assumption of an 8-month treatment, at the lowest posology (6.7 mg/kg per month), resulted in the range of 7.292–9.656 tons per year, with a contribution from dogs up to the 86%. Further information is available in the Supporting Materials

Fipronil intake via dust

In **Figure 3**, the computed dust intake in toddlers under the acute – maximum value and 95th percentile (max and P95) –, and chronic (median and 75th percentile) scenarios relative to the total Fipronil house dust contamination in the presence and absence of pets is illustrated, whereas, in **Figure 4,** the assessment carried out in kittens is shown. The risk characterization for acute (worst-case scenario) and chronic exposure is reported in **Table 2** under an aggregate exposure scenario that accounts for the contribution from animal petting, [16] and for dietary intake of F residues in food commodities, [4] respectively, along with the related Margin of Safety.

Discussion

Uncertainties

From the analytical procedure, uncertainties can be restricted to the determination of Fipronil Desulfinyl as a result of the nontarget analysis. Due to an inappreciable difference in its chromatographic behavior and instrumental response with respect to matrix interferences and with respect to the differences in Fipronil and Sulfone, in agreement with Guo et al., [21] the postacquisition determination via TraceFinder software did not affect the reliability of the results. Notably, the Sulfinyl accounts for 10% of the total Fipronil, on average.

The contamination found in vacuum bags can be assumed to be an average of 1–2 months collection; therefore, uncertainty can be reasonably addressed toward higher intakes under the worst-case scenario, with acute toxicity for dust intakes typically occurring a few days after the Fipronil application.

The oral bioavailability of Fipronil has been assumed to be at 85%, accounting for the evidence from experimentally treated animals fed on Fipronil-contaminated diets, [19] An *in vitro* bioavailability study reports a lower bioavailability, with a maximum of 64% as a function of the organic carbon content in dust.[18] *In vivo* evidence is considered more robust than from *in vitro* experiments, as a matter representative of the overall digestive array. Therefore, the uncertainties show negligible influence toward lower intake estimates.

Desulfinyl, the primary environmental metabolite (photoproduct) of F, is reported to be 9–10 times more active than the parent compound at the chloride channel of the rat brain GABA receptorNotably, a provisional ADI (pADI) 0.00003 mg/kg bw for FD was proposed by the FAO in 1999 [19] against the consolidated ADI of 0.0002 mg/kg valid for Fipronil and Sulfone; [19, 26] Desulfinyl is not currently framed in the among residues to be monitored within food safety plans, even if it has been recommended for the estimation of long-term and short-term dietary intake from plant and animal commodities, [19] and from dust, according to the evidences from this and previous papers. [9, 17, 18] Regarding the estimated use of Fipronil as a veterinary product, the uncertainties can be considered quite high because the purchase of the drug is not traceable from veterinary prescriptions, and the administration of the drug is done by nonprofessionals. The consideration of an 8-month long treatment per year (winter season excluded) and the low posology chosen may suggest the real use could exceed this estimate in a local context.

Fipronil occurrence in residential house dust

This wide survey on 161 samples confirms the evidence from previous papers by Malher et al., [17] and Starr et al., [18] that an approximate range of 10,000–100,000 ng/g of total Fipronil contamination exists in residential house dust as a consequence of indoor application and/or veterinary use of Fipronil on pets. Because Starr et al. [18] reported a maximum level as sum of Fipronil, Sulfone, and Desulfinyl in house dust of 119,006 ng/g, our worst findings of 82,069 and 67,799 ng/g in the absence and presence of pets, respectively (**Figure 2, Table 1**), were not considered as outliers for an acute exposure scenario. However, in this work, even if the highest contamination recorded in dust was observed in households in the absence of pets, the presence of pets resulted in significantly greater total Fipronil contamination in the dust ($p<0.001$). These chronic exposure estimates, computed on 50^{th} and 75^{th} percentiles in the presence/absence of treated pets, are relevant (**Figures 3 and 4**). Treated pets, in households without a private garden (N = 22), could lead to a higher house dust contamination than that recorded in those households with a private garden (N = 38), even if the number of observations hamper the achievement of a full significance (P = 0.051; **Figure 5**). Within a risk orientation, the absence of a garden, and more generally, the more time spent indoor by treated pets, can be considered as a risk factor for exposure via dust intake, because of the release of cutaneous cell debris and hair in a confined environment. This risk suggests the need to further investigate Fipronil presence in the dust from residential flats.

Estimated amount of Fipronil in veterinary medicine

In the European Union, while sales for the agriculture use of pesticides can be recovered from official statistics, no information is available about the amounts sold for urban uses. In Italy, Fipronil is marketed in approximately 85 different commercial veterinary products, and access to the amount of each commercial product sold, which is recorded under The Periodic Safety Update Report of the European Union pharmacovigilance schemes, is restricted. On a national basis, our estimates indicate the Fipronil use in veterinary medicine is in the range of 7–10 times more than that use reported in agriculture before the implementation of restrictions in Italy due to the worldwide bee health concern. The Italian Environment Research Institute ISPRA in 2017 reported an average of 0.9 tons per year of Fipronil sold for agricultural use for the treatment of seeds in the timeframe 2009–2012, sufficient to trigger surface-water monitoring oriented toward risk prioritization due to the ecotoxicity of Fipronil and its products on aquatic biota, as a matter of its environmental persistence and bioaccumulative features. [27] Within this framework, Fipronil sourcing from pet treatments could be strictly monitored when administered under veterinary prescription, thus improving the efficacy of the pharmacovigilance and pharmacosurveillance activities; this approach would affect the potential onset of resistance as in the case of *Rhipicephalus sanguineus*—a three-host dog tick found worldwide and able to complete its' entire lifecycle indoors, [28] as well as affecting the toxicological effects on most vulnerable and sensitive nontarget organisms. [3]

Toxicological evidences in humans and pets

The quite large volumes of Fipronil used by nonprofessionals may be evident in the admission of children to antipoisoning centers. A surveillance study carried out in the United States from 2001 to

2007 [7] showed that of 103 Fipronil-related recorded admissions to first aid/ antipoisoning centers (Sentinel Event Notification System for Occupational Risks), the majority (76%) had exposure in a private residence, and 37% of the admissions involved the use of pet-care products. The French agency ANSES in its opinion on Fipronil in eggs [3] reported 1,104 accidental Fipronil-related admissions to the French Antipoisoning Center network in 2011–7. Children were involved in 30% of the reports, which showed a typical seasonality (higher incidence in May–September period), with low severity toxicity in 85% of the cases. In Italy, in the last report of the National Antipoisoning Centers [29] (data referred to 2014), 102 out of 166 cases of unknown insecticide intoxication involved children; in this group, Fipronil was identified in 11 out of 15 cases with mild symptoms.

From our data and estimates, the impact on toddlers' health from dust intake in terms of acute and chronic toxicity does not seem sufficient to cause potential overexposure (MOS in the range of 9–16 and 6–10, respectively), without the contemporary contribution of other aggregated sources (**Figure 3, Table 2**). Under the acute worst-case scenario, when the 95th percentile of the contamination values are considered, the dust intakes estimate a drop to 182-304 ng/kg bw per day in the presence of pets. However, the uncertainties related to the house dust sampling, which are not representative of recent postapplication contaminations, should be considered. For chronic exposures, the presence of Desulfinyl and its potential toxicity, along with a higher oral bioavailability of Fipronil and its products with respect to its usually exposure via the dermal route (1%), [19] may deserve more attention. The computed exposures increase the uncertainties related to the Fipronil posology and appropriate application on the pets from nonprofessional people, which has led to potential higher direct and indirect exposures relevant to both acute and chronic toxicity. Its environmental release and persistence from the nonagricultural use may cause future contamination of food commodities, such as root vegetables, eggs, meat, and milk from food production animals coming in contact with Fipronil contaminated grazing areas as result of irrigation with remediated waters and/or from the use of biosolids from civil WWTPs. [8, 30]

In the United Kingdom, veterinary pharmacovigilance reports on intoxication outbreaks in pets when Fipronil has been administered with food instead of as a pour-on. [31] These reports stress the relevance of the oral exposure as a determinant of potential intoxication, due to its increased bioavailability *via* such a route. With respect to kittens as nontarget organisms, dust intake itself can contribute to overexposure. Our estimates indicate that, under both an acute and chronic scenario, exceeding the HBGV (MOS = 0.1 – 0.3) is pertinent. (**Table 2**). For Fipronil, at present, sparse evidence exists on intoxication in kittens from the pharmacosurveillance system in place in the European Union. This can be attributed to the following factors: a) Fipronil acute intoxications usually do not lead to severe symptoms, and the reported inappetence, anorexia, vomiting, diarrhea in kittens could be easily confounded with the more frequent perinatal clinical outcomes, i.e., from roundworms present as a result of prenatal exposure; [32] b) the HBGVs for Fipronil account for a conservative uncertainty factor of 100 (EFSA, 2012); and c) underreporting may occur due to the improvable networking between vet practitioners and the veterinary antipoisoning centers. This last point could highlight again the relevance of risk-oriented stewardship programs from the Health Authorities to promote a One Health approach in the management of persistent pesticides in pet care.

Conclusions

The reported widespread use of Fipronil in urban and agriculture contexts, its persistence, and environmental release are supported by the evidence provided from our work, which focused on its use as a veterinary drug in Italy. The evidence suggests its inclusion among the so-called pseudo-persistent organic pollutants to receive priority monitoring in environmental and food matrices, as well as in pharmacovigilance and pharmacosurveillance schemes supported by records of veterinary prescriptions.

Authors declare no conflict of interest.

Acknowledgments

The work was partially supported by the Italian Ministry of Health grant no. RF-2009-1534860 “ENVI-FOOD.” The authors wish to thank Dr. Olga Tcheremenskaia for the support in the QSAR analysis of Fipronil and its products and Prof. Marco Pietra for the availability of body weight records of pets.

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Table 1. Statistical descriptors of the Fipronil (F), Sulfone (FS), and Desulfinyl (FD) occurrence (ng/g) in residential house dust in the absence (above) and in the presence (below) of pets.

PET -	F	FS	FD
min	5	5	5
P25	6	5	5
P50	11	5	5
mean	1075	230	156
P75	76	24	5
P95	1400	295	145
max	60219	13909	7941
N	101	101	101
LCD*	21	57	85

PET +	F	FS	FD
min	5	5	5
P25	43	20	5
P50	367	164	42
mean	4987	757	507
P75	3175	659	292
P95	31065	3648	3067
max	55904	6288	5607
N	60	60	60
LCD*	2	7	20

* Values below the LOQ of 10 ng/g indicated as Left Censored Data (LCD) and computed as Medium Bound (½ of the LOQ).

Table 2. Computed Total Fipronil exposure as ng/kg bw per day in toddlers and kittens via dust in the presence of pets, compared with that from animal petting and contaminated food.

Source	**Dust (intake)**		**Petting (dermal & intake)**	**Food (intake)**	**Aggregate MOS**
	US-EPA	EU ECHA			
Toddlers					
Acute	333	556	560	6,600	
(worst case)	*(9.0 FAO)*		*(5.4 FAO)*	*(0.5 FAO);*	*(0.4 FAO)*
		(16 EFSA)	*(16 EFSA)*	*(1.4 EFSA)*	*(1.2 EFSA)*
chronic	20	34	75	10	
	(10 EFSA)	*(5.9 EFSA)*	*(2.7 EFSA)*	*(20 EFSA)*	*(1.7 – 1.9 EFSA)*
Kittens					
Acute	26,076		na	na	*(0.1 FAO)*
(worst case)					*(0.3 EFSA)*
chronic	1,633		na	na	*(0.1 EFSA)*

na: not applicable; Between brackets and in *italics* —the Margin of Safety (MOS)—referred to the ArfDs of 3,000 and 9,000 ng/kg bw per day from FAO, [19] and EFSA, [26] respectively, and the ADI of 200 ng/kg bw per day from FAO and EFSA. Dust exposure in the presence of pets, petting, and alimentary intakes derived from Cochran et al., [16] and BfR. [4]

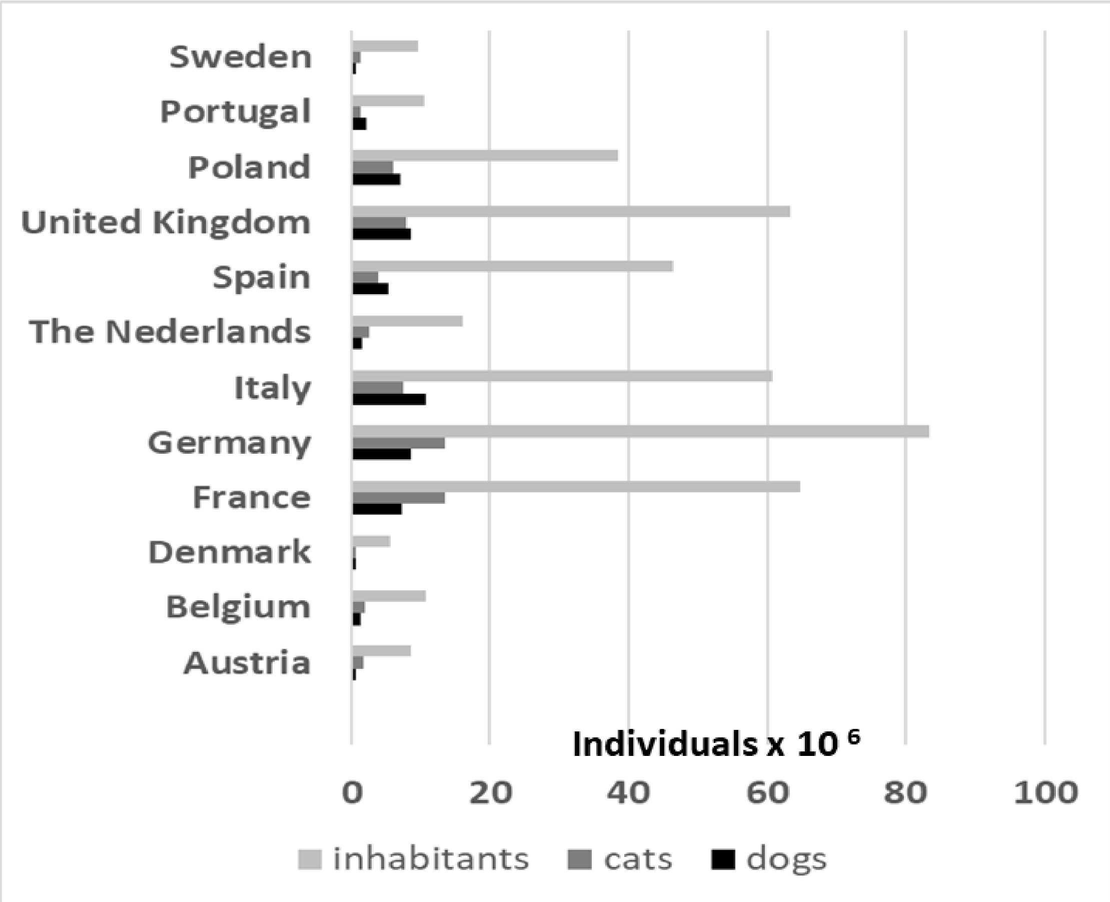
Sweden
Portugal
Poland
United Kingdom
Spain
The Nederlands
Italy
Germany
France
Denmark
Belgium
Austria
Individuals x 10 6
0
20
40
60
80
100
inhabitants
cats
dogs

Figure 1.

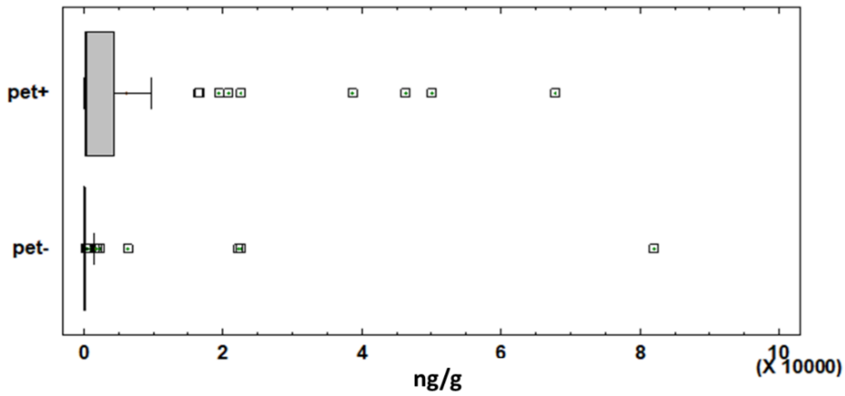

Figure 2.

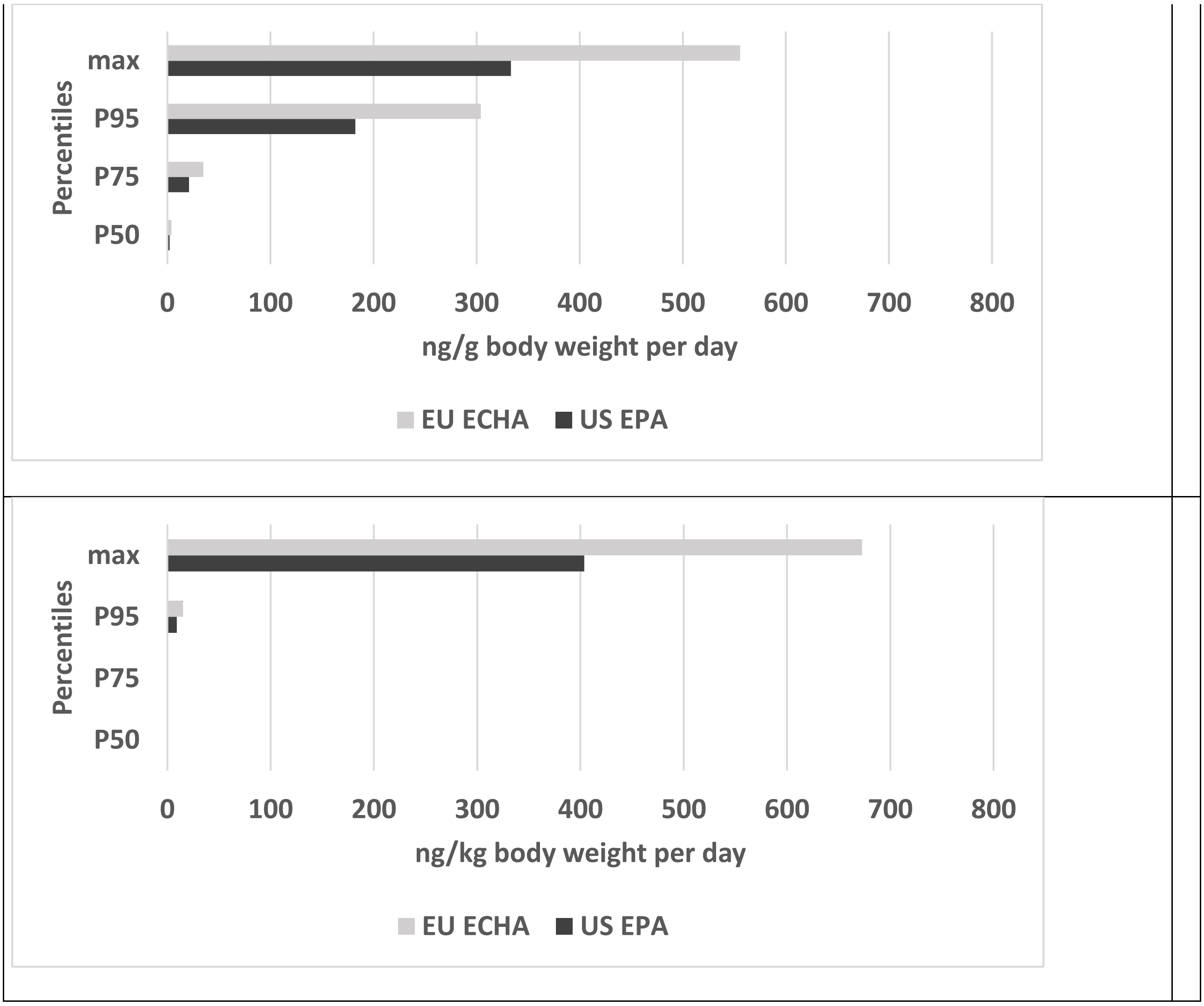

Figure 3

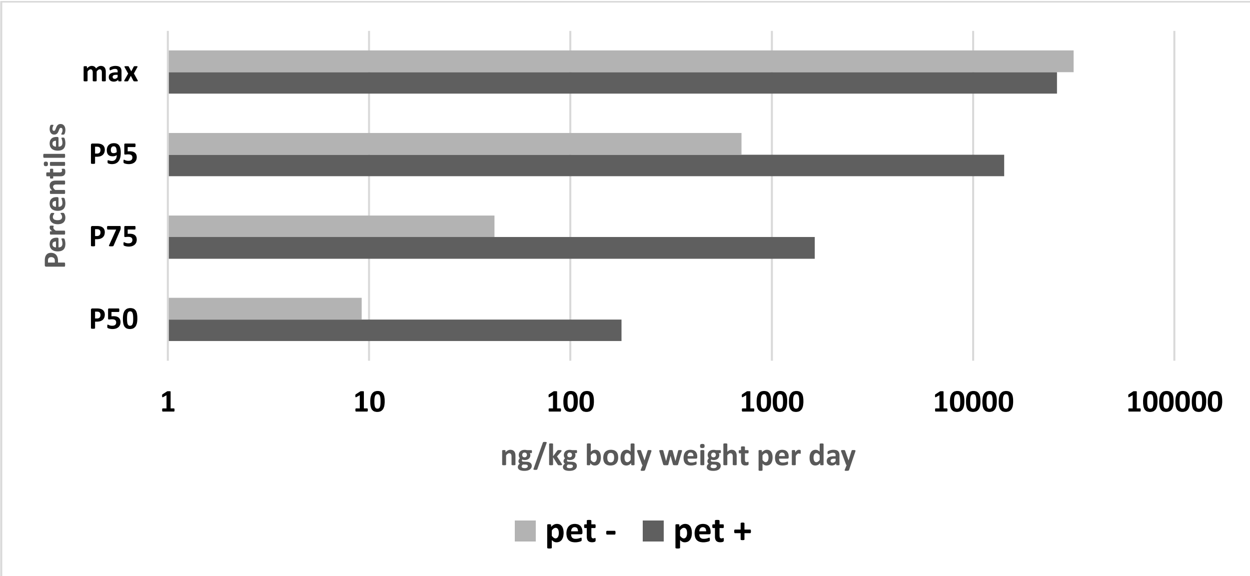

Figure 4.

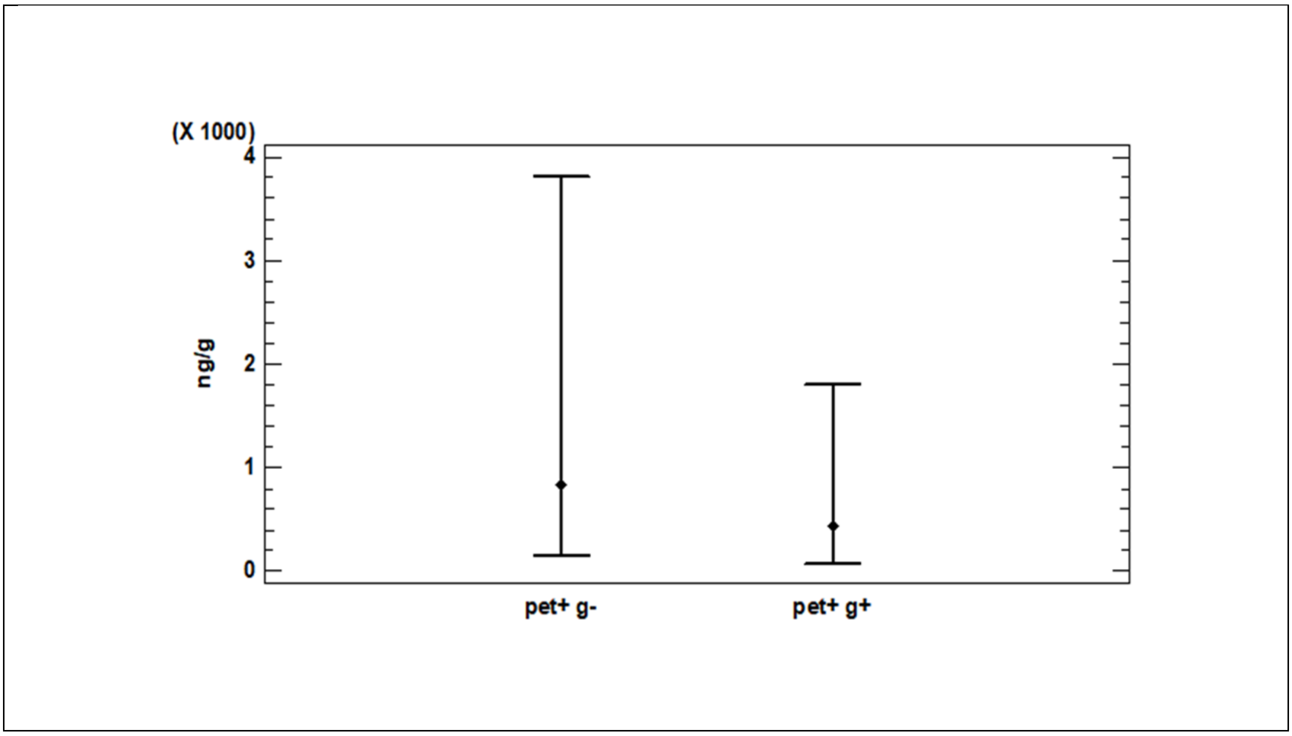

Figure 5

Figure captions

Figure 1. Canine and feline population in EU Countries (x 10^{6}), compared with demographic data, provided by the European Pet Food Industry (FEDIAF, 2017). Dog population in Italy from the national canine registry from the Ministry of Health (2018).

Figure 2. Whisker box-plot output of the total Fipronil house dust contamination (ng/g x 10,000) in the presence (pet +) and absence (pet -) of cats and dogs.

Figure 3. Intake assessment (ng/g bw per day) of total Fipronil in toddlers according to the US EPA and EU ECHA guidelines from the different occurrence percentiles in house dust in the presence (above) and in absence (below) of pets.

Figure 4. Acute and chronic intake assessment of total Fipronil (ng/g bw per day) in kittens from the different occurrence percentiles in house dust in the presence and in the absence of pets.

Figure 5. Comparison of Fipronil median contamination (ng/g) and of the related 95th percentile confidence intervals in dust in households: a) in presence of pets and of a private garden (pet+ g+) (N = 38); and b) in presence of pets and without a private garden (pet+ g-) (N = 22).

SUPPORTING MATERIALS

Occurrence of Fipronil in residential house dust in presence and absence of pets: a hint for a comprehensive toxicological assessment

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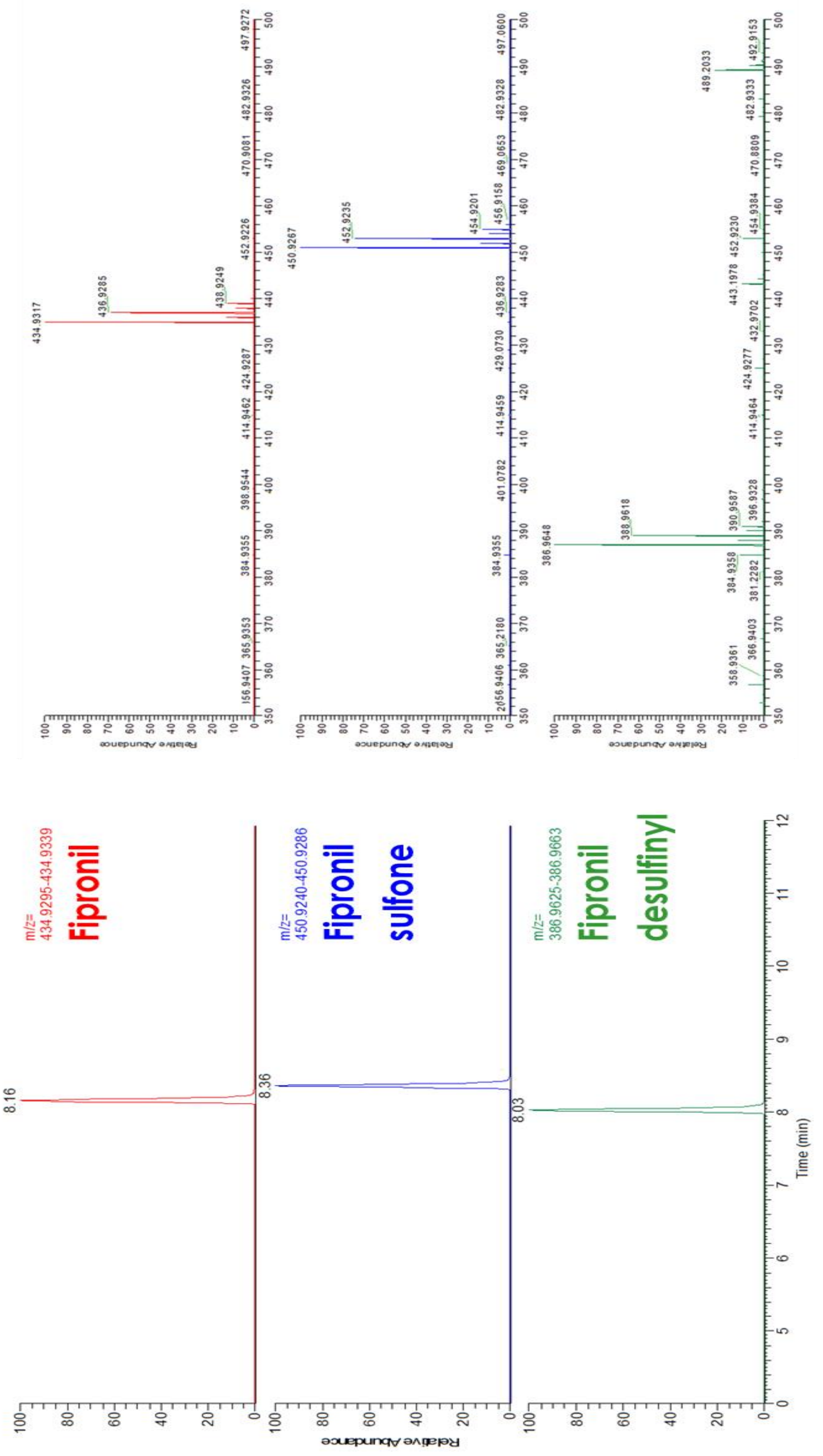

Figure 1S. Chromatograms and High-resolution mass spectra for Fipronil, Sulfone, and Desulfinyl.

Table 1S. Molecular, retention time, accurate mass, mass errors of Fipronil and its products.

Compound	Molecular formula	RT (min)	Precursor ion		Mass error
			Theoretical	Experimental	(ppm)
Fipronil	$C_{12}H_4Cl_2F_6N_4OS$	8.16	434.9314	434.9317	0.3
Fipronil-sulfone	$C_{12}H_4Cl_2F_6N_4O_2S$	8.36	450.9263	450.9267	0.4
Fipronil desulfinyl	$C_{12}H_4Cl_2F_6N_4$	8.03	386.9639	386.9648	0.9

Table 2S. Computed use of Fipronil as veterinary drug per year in cats and dogs.

Population dogs (N)	Treated %	Treated (N)	kg bw mean	Posology mg/kg per month	Treatments per year (N)	F use mg per year
	62	6,612,685			8	6,273,586,080
10,717,479			17.07	6.7		
	82	8,756,180			8	8,307,163,415
Population cats (N)						
	62	4,298,639			8	1,018,399,163
6,967,000			4.42	6.7		
	82	5,692,039			8	1,348,512,344

Table 3S. Descriptors of total Fipronil presence in house dust in the presence of pets (pets+), accounting for the availability or not (g+/g-) of a private garden in the household. Test statistic = 0.430356; P value = 0.511814.

	Count	*Average*	*Median*	*Standard deviation*	*MAD*	*Minimum*	*Maximum*	*Lower quartile*
pet+ g-	22	3298.77	832.61	5756.04	772.96	15.0	22605.9	155.91
pet+ g+	38	7499.18	442.48	15672.6	424.7	15.0	67798.6	54.48
Total	60	6032.37	466.7	13182.1	448.92	15.0	67798.6	75.68

	Upper quartile	*Stnd. skewness*	*Stnd. kurtosis*
pet+ g-	3775.49	4.92505	6.21046
pet+ g+	6090.57	6.7734	8.26557
Total	4284.5	10.0411	16.1735