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RETROSPECTIVE NATIONWIDE OCCURRENCE OF FIPRONIL AND ITS DEGRADATES IN U.S. WASTEWATER AND SEWAGE SLUDGE FROM 2001-2016

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Abstract

The insecticide fipronil is under regulatory scrutiny worldwide for its toxicity to pollinators and aquatic invertebrates. We conducted the first U.S. nationwide, longitudinal study of sewage sludges for fiproles, i.e., the sum of fipronil and its major degradates (fipronil sulfone, sulfide, amide, and desulfinyl). Archived sludges ($n=109$) collected in three campaigns over 15 years were analyzed by isotope dilution liquid chromatography tandem mass spectrometry, revealing ubiquitous fiprole occurrence ($0.2 - 385.3 \mu\text{g/kg}$) since 2001 and a significant increase (2.4 ± 0.3 fold; $p < 0.005$) both from 2001 to 2006/7 and from 2001 to 2015/6, but not a significant increase from 2006/7 to 2015/6 ($p = 0.275$). A geospatial analysis showed fiprole levels in municipal sludges to be uncoupled from agricultural use of fipronil on cropland surrounding sampled municipalities, thus pointing to non-agricultural uses (i.e., spot-on treatment and urban pest control) as a major source of fiprole loading to wastewater. Whereas anaerobic digestion was correlated with increases in fipronil sulfide at the expense of parental fipronil ($p < 0.001$), total fiprole levels in sewage sludges were similar regardless of the solids treatment approach applied ($p = 0.519$). Treatment plant effluent available from 12 facilities in 2015/6 contained fiproles at $0.3\text{--}112.9 \text{ ng/L}$, exceeding the United States Environmental Protection Agency (USEPA) aquatic invertebrate life benchmark for chronic fipronil exposure (11 ng/L) in 67% of cases. Whereas the USEPA identified fipronil in sludge only recently (2015), retrospective analyses and modeling conducted here show contaminant ubiquity and nationwide increases of fiprole mass (compared to 2001 levels) in U.S. municipal sludge ($1140 \pm 230 \text{ kg}$ in 2015/6), and treated effluent nationwide ($1970 \pm 390 \text{ kg}$ in 2015/6) over the past 15 years.

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Declaration of interests

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Abstract

Keywords

Fipronil; United States; Sewage sludge; Treated effluent

1 INTRODUCTION

Fipronil, a phenylpyrazole insecticide having both agricultural and domestic uses, was first registered in the United States in 1985 (United States Environmental Protection Agency 2011). Fipronil has caught the attention of regulatory agencies worldwide (Hileman 2007, Italy Ministry of Health 2008, Office of the Ministry of Agriculture of People's Republic of China 2009, United States Environmental Protection Agency 2011) due to its unwanted toxicity to pollinators (particularly honeybees) (Bonmatin et al. 2015, Gunasekara and Troung 2007, Krupke et al. 2012, Simon-Delso et al. 2015, Vidau et al. 2011, Xiqing et al. 2010), various beneficial terrestrial insects (Bonmatin et al. 2015, Gunasekara and Troung 2007, Simon-Delso et al. 2015), and aquatic invertebrates (Bonmatin et al. 2015, Gunasekara and Troung 2007, Hapke et al. 2016, Weston and Lydy 2014). In the United States, some turf grass and all in-furrow corn product registrations were discontinued in 2010 due to concerns over potential ecosystem health impacts identified in more than 20 USEPA ecological risk assessments (United States Environmental Protection Agency 2011). Currently, registered U.S. agricultural applications are for use on potato, turnip, and rutabaga plants only, in-furrow treatment of wire worm in potatoes, and corn seed treatments for export only (BASF 2016, United States Environmental Protection Agency 2011). Registered non-agricultural and outdoor uses of fipronil include small area turf care, bait treatment for select species of ants and flies, and structural termite treatment (spraying, trenching, and soil injection) (United States Environmental Protection Agency 2011). Registered residential uses for fipronil include spot-on treatment for flea and tick control on domestic animals, indoor cockroach traps, and outdoor use on residential properties (United States Environmental Protection Agency 2011). These non-agricultural applications contribute to fipronil release to the environment, as evidenced by fipronil detections on urban paved surfaces (Jiang et al. 2016, Weiying et al. 2014), in indoor and outdoor dust (Mahler et al. 2009, Starr et al. 2014, Stout II et al. 2009), stormwater (Carpenter et al. 2016, Weston et al. 2015), residential runoff (Gan et al. 2012), and in rinsate from dogs treated with spot-on formulations (Dyk et al. 2012, Teerlink et al. 2017).

Fipronil occurs at ng/L levels in the U. S. wastewater, persists during conventional sewage treatment, and is inadvertently discharged into the environment via reclamation of treated wastewater and of sewage sludge deemed fit for application on U.S. land (biosolids) (Foster et al. 2012, Heidler and Halden 2009, McMahan et al. 2016, Morace 2012, Sadaria et al. 2017, Supowit et al. 2016, Weston and Lydy 2014). In wastewater systems fipronil usually occurs along with its major degradates formed during aerobic transformation (sulfone), anaerobic transformation (sulfide), and to a lesser degree, degradates from hydrolysis (amide) and photodegradation (desulfinyl) (McMahan et al. 2016, Sadaria et al. 2017, Supowit et al. 2016). Fipronil and its degradates have unit-less octanol-water partitioning coefficient (K_{OW}) values of 10^4 or greater, indicating their tendency to associate with organic particulates contained in wastewater and to accumulate in sewage sludge (Sadaria et al. 2017). A study of eight California wastewater treatment plants (WWTPs) established that about $65 \pm 11\%$ of the mass of fipronil and its degradates (total fiproles) entering WWTPs is discharged with effluent, with the balance persisting and accumulating in sludge (Sadaria et al. 2017). Similarly, the modeling tool used by the USEPA, EPISuite, predicts that approximately 30% of fipronil will be removed during the course of treatment, with almost all of that adsorbing to sludge and less than 0.5% being biodegraded (United States Environmental Protection Agency 2017b). Since the partitioning of the compounds during wastewater treatment is known, the analysis of sewage sludge can provide insights not only into the extent of secondary fiprole releases from sewage sludge application on land, but also on the minimum pesticide loading to the treatment facility and approximate concentrations of these pollutants in discharged effluent.

Fiproles in the environment raise concerns over inadvertent toxic exposure to aquatic and terrestrial biota and thus are of regulatory interest. In a recent U.S. surface water monitoring study, fipronil and degradates were detected at a frequency of 21-84% in 38 streams studied, with median and maximum fipronil concentrations of 23.8 and 153 ng/L, respectively (Bradley et al. 2017). Fipronil occurrences in fish tissue and in sediments of rivers, estuaries, and coastal embayments receiving discharged wastewater in California (Lao et al. 2010, Maruya et al. 2016), Todos Santos Bay, Mexico (Hernández-Guzmán et al. 2017) and Guangzhou, China (Yi X et al. 2015) also have been established. Fipronil and its degradates have endocrine disrupting properties (Goff et al. 2017) and have been shown to impart toxicity toward sensitive non-target aquatic invertebrates (Gunasekara and Troung 2007, Weston and Lydy 2014) and pollinators at low parts-per-trillion levels (Gunasekara and Troung 2007, Kairo et al. 2017, Vidau et al. 2011, Xiqing et al. 2010). Fipronil degradates further have been shown to exhibit long environmental half-lives (Brennan et al. 2009, Kunde et al. 2009) and can exceed parental fipronil in toxic potency and bioaccumulation potential in many species (Konwick et al. 2006, Schlenk et al. 2001, Weston and Lydy 2014). Fipronil sulfone is observed to have three times longer half-life in fish than fipronil (Konwick et al. 2006). Fipronil degradates are twice or more toxic to certain invertebrates than parental fipronil; for example, *Chironomus dilutus* has a mean 96-h EC_{50} of 7-10 ng/L for its sulfone & -sulfide degradates and 32.5 ng/L for fipronil (Weston and Lydy 2014). USEPA's Office of Pesticide Programs (OPP) has determined freshwater aquatic life benchmarks for fipronil and its degradates. Fipronil sulfone & -desulfinyl OPP benchmarks

for fish (acute and chronic) are three to four times higher than fipronil (United States Environmental Protection Agency 2017a).

At present, no studies exist assessing the nationwide longitudinal occurrence of fipronil and its degradates in U.S. wastewater systems. Therefore, we conducted a nationwide longitudinal analysis of fiproles using 109 sludges collected across the contiguous United States between 2001 and 2016 as well as contemporary wastewater from 12 U.S. WWTPs sampled during 2015/6 to determine current levels of fiproles in raw and treated wastewater prior to discharge into U.S. surface waters.

2 MATERIALS AND METHODS

2.1 Sample Characteristics and Selection

Archived sludge samples collected nationwide in 2001 and 2006/7 by the USEPA, and were procured our research team to be maintained in frozen storage as a part of the National Sewage Sludge Repository and Human Health Observatory housed in the Biodesign Center for Environmental Security at Arizona State University (Venkatesan et al. 2015). Briefly, activated sludge samples were collected by trained personnel from multiple areas of large piles or multiple grabs from a continuous process such as a belt press (United States Environmental Protection Agency 2009). Several kilograms were collected into amber glass jars with no addition of preservatives. Samples were then placed on ice and stored at -11°C upon arrival at the EPA (United States Environmental Protection Agency 2009). Upon procurement by Arizona State University, samples were stored at -20°C and temperature monitored by a Rees Scientific Centron System (Trenton, NJ, USA). Wastewater samples (24-hour flow weighted composites of influent and effluent) were provided by 12 WWTPs voluntarily in 2015/6. Samples were made available under the condition of de-identifying participating WWTPs. Samples were stored at -20°C upon arrival at our laboratory. Samples represented a spectrum of flow volumes treated, and wastewater and sludge treatment processes employed. Additional sample information can be elsewhere (United States Environmental Protection Agency 2009, Venkatesan et al. 2015).

Thirty-five randomly selected samples originated from the 2001 National Sewage Sludge Survey from WWTPs representing a spectrum of flow volumes treated and sludge processes employed (11 performing aerobic sludge digestion and 24 performing anaerobic sludge digestion). Three WWTPs received greater than 378 million liters per day (MLD; 100 million gallons per day or MGD), 10 WWTPs received 37.8-378 MLD (10-100 MGD), and 22 WWTPs received less than 37.8 MLD or 10 MGD. Fifty sludge samples collected in the 2006/7 survey represented 18 WWTPs performing anaerobic digestion and 32 WWTPs performing other/no treatment (no sludge digestion, chemical treatment by lime, ferric chloride and polymer addition, and/or aerobic sludge digestion), with treatment capacity ranging from greater than 378 MLD ($n=7$) to 37.8-378 MLD ($n=12$) to less than 37.8 MLD ($n=31$). Twenty-four samples collected by our team in 2015/6 included 17 WWTPs performing anaerobic digestion and the rest performing other or no sludge treatment, with treatment capacity ranging from >378 MLD ($n=8$) to 37.8-378 MLD ($n=10$) to less than 37.8 MLD ($n=6$). Twelve WWTPs providing composite influent and effluent samples in 2015/6 featured – only activated sludge treatment ($n=7$), activated sludge treatment with nutrient

removal ($n=5$), and filtration (total, $n=4$; granular-media effluent filtration, $n=3$; sand filtration, $n=1$).

2.2 Standards and Reagents

High performance liquid chromatography (HPLC) grade methanol, acetonitrile, acetone, hexane, and water were purchased from Sigma-Aldrich Corp., (St. Louis, MO, USA). Analytical standards of fipronil, and fipronil-desulfinyl were obtained from Sigma-Aldrich Corp., (St. Louis, MO, USA). Analytical standards of fipronil sulfide, –sulfone, and –amide were obtained from Bayer (Leverkusen, Germany) and BASF (Ludwigshafen, Germany). Labeled $^{13}\text{C}_2$ $^{15}\text{N}_2$ fipronil, $^{13}\text{C}_4$ $^{15}\text{N}_2$ fipronil sulfone, and $^{13}\text{C}_4$ $^{15}\text{N}_2$ fipronil sulfide were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada) and Cambridge Isotope Laboratories, Inc., (Tewksbury, MA, USA), respectively. Stock solutions of analytical standards were prepared in acetonitrile and stored at -20°C . Working calibration standards were prepared in water and methanol (50/50 v/v) with 0.1% formic acid. For those compounds without isotopically labeled standards available, quantitation was performed using the method of standard addition with 4 spike standard levels.

2.3 Sample Extraction and LC-MS/MS Analysis

Sludge samples were dried using a stream of nitrogen and then spiked with 40 ng of $^{13}\text{C}_4$ $^{15}\text{N}_2$ fipronil, $^{13}\text{C}_4$ $^{15}\text{N}_2$ fipronil sulfone, and $^{13}\text{C}_4$ $^{15}\text{N}_2$ fipronil sulfide isotopes per gram dry weight sludge, and extracted as previously described (Sadaria et al. 2017, Supowit et al. 2016). Briefly, one gram of solids was extracted with 10 mL methanol by shaking for 24 hours followed by 60 minutes of sonication. The samples were centrifuged and the eluent collected. The sample was extracted again with 10 mL acetone and 90 minutes of sonication. Obtained extracts were mixed, dried, and reconstituted with 4 mL hexane:acetone mixture (98/2, v/v) and subjected to solid phase extraction (SPE) on 1g/6 mL Sep-Pak (Waters Corporation, Milford, MA) cartridges containing Florisil. Cartridges were conditioned with 6 mL dichloromethane, followed by 6 mL acetone, and finally 6 mL of hexane. Samples were loaded, and then eluted with 4 mL of dichloromethane and acetone (50/50 v/v). SPE eluent was dried down and reconstituted in 1 mL 50/50 water/methanol (v/v) for LC-MS/MS analysis (Sadaria et al. 2017, Supowit et al. 2016)

Raw (non-filtered) 500 mL wastewater samples were spiked with 40 ng of $^{13}\text{C}_4$ $^{15}\text{N}_2$ fipronil, $^{13}\text{C}_4$ $^{15}\text{N}_2$ fipronil sulfone, and $^{13}\text{C}_4$ $^{15}\text{N}_2$ fipronil sulfide isotopes. Later samples were loaded on a Strata XL cartridge (Strata XL 500 mg/3 mL, Phenomenex, Torrance, CA) using an Autotrace 280 (Thermo Scientific Dionex, Sunnydale, CA). Further details about the extraction can be found elsewhere (Sadaria et al. 2017, Supowit et al. 2016). Briefly, the cartridges were conditioned with 3 mL of methanol followed by 3 mL of water. 500 mL of wastewater were loaded onto the cartridge with a flow rate of 2 mL/min. Cartridges were washed with water and allowed to dry under a gentle stream of nitrogen for 5 minutes. Analytes of interest were eluted with 2 successive 4 mL aliquots of methanol and formic acid (95/5, v/v). The eluents were combined, dried down, and reconstituted to one mL solution of water and methanol (50/50, v/v) for LC-MS/MS analysis.

The resulting sludge and wastewater extractions were analyzed by tandem mass spectrometry using a Shimadzu Prominence UHPLC system (Shimadzu Corporation, Kyoto, Japan) connected to an ABSciex API 4000 (Sciex, Concord, ON, Canada) triple quadrupole MS. Fipronils were separated on a Waters XBridge BEH C8-column (3.5 μm , 2.1 $\times$ 100 mm; Waters Corporation, Milford, MA) using methanol and water. The column temperature was set at 40°C, the flow rate was 0.2 mL/min, and 50 μL of sample was injected. The gradient started at 50% methanol and was held for 1 minute. The gradient then followed a linear change to 90% methanol over 3 minutes and was held for 6 minutes. The gradient was returned linearly to the starting conditions over 2 minutes and allowed to re-equilibrate for 2 minutes. The MS was operated in negative electrospray mode with multiple reaction monitoring (MRM) using two characteristic ion transitions for each target. Mass spectrometric parameters can be found in Table S1 (Sadaria et al. 2017, Supowit et al. 2016). Sludge and wastewater concentrations are reported in units of $\mu\text{g/kg}$ dry weight and ng/L , respectively. Degradates amide and desulfinyl, lacking isotope-labeled standards, were quantified using the nearest eluting isotope-labeled fiprole standard.

2.4 Statistical Analysis

Statistical analysis was performed with IBM SPSS Statistics software (version 25, IBM Corporation, Armonk, NY, USA). Two-tailed *t*-tests were performed to compare two datasets, and differences were determined at the $\alpha = 0.05$ significance level. A non-parametric Mann-Whitney *U* test was also performed and corresponding *p* values can be found in Table S2.

2.5 Quality Assurance and Quality Control

Random replicate analyses were performed on 22% of samples analyzed yielding relative percent deviation (RPD) values averaging $20\% \pm 18\%$. Method detection limits (MDLs; sludge, 0.02-0.30 $\mu\text{g/kg}$ dry weight; wastewater, 0.02-2 ng/L) were established using EPA methodology involving seven replicates (United States Environmental Protection Agency 1991). See Table 1 for calculated MDLs values. Method and instrument blanks included in each analytical batch showed no background peaks or analyte carry-over from prior runs. An evaluation of analyte loss due to sample storage involved repeat analysis of 8 sludge samples over one year, showing no significant analyte concentration differences ($p = 0.680$).

2.6 Geospatial and Nationwide Emissions Analyses

Geospatial data on the agricultural application of fipronil were obtained from the United States Geological Survey (USGS) online database (United States Geological Survey 2018). Agricultural usage was calculated by averaging the EPest-high and EPest-low estimates; minimum and maximum values of agricultural usage are represented in the Results section in the form of error values (Thelin and Stone 2013, United States Geological Survey 2018). Nationwide annual emissions from U.S. wastewater infrastructure were computed by multiplying corresponding detected mean concentrations with annual U.S. sludge generation and effluent discharged (Seiple et al. 2017), respectively (see SI Nationwide Annual Environmental Emission Calculations for details). Average RPD established in the method validation were used as the emission error values.

3 RESULTS

3.1 Longitudinal Nationwide Fiprole Detection in Sludge

Analysis of fiprole congeners (Figure 1A) in 109 sludges collected between 2001 and 2016 showed ubiquitous presence of fipronil (0.04–191.8 µg/kg) and its major aerobic degradate, fipronil sulfone (0.1–208.6 µg/kg). The anaerobic degradate, fipronil sulfide, also was detected in 90% of the samples (0.9–149.2 µg/kg). Fipronil amide and fipronil desulfinyl occurred less frequently (22% and 50%, respectively), and at relatively lower concentrations (0.2–18.7 µg/kg; refer to Table 1 for additional data on analyte concentrations and detection frequencies). Total fiproles were dominated by fipronil (22±19%; mean ± standard deviation) and its major degradates, fipronil sulfone (45±11%) and fipronil sulfide (30±19%), whereas fipronil amide (1±3%) and fipronil desulfinyl (1±2%) were only minor contributors to the overall fiprole mass.

Detected total concentrations of fipronil and its degradates expressed as fipronil equivalents ranged between 2.9 and 177.8 µg/kg in 2001, 0.2 and 385.3 µg/kg in 2006/7, and 4.9 and 240.5 µg/kg in 2015/6 (Figure 1B). 2006/7 and 2015/6 concentrations were 2.1 and 2.7 times higher, respectively than 2001 levels and were found to be significantly different ($p < 0.05$; Figure 1B; See Table S2 for individual p values). Total fiprole concentrations in U.S. sludge from 2006/7 and 2015/6 were not statistically different ($p = 0.275$).

Data available from the USGS was used to understand the change in agriculture fipronil usage between 2001 and 2015 (United States Geological Survey 2018). Figure 2A shows a shift from more agricultural application of fipronil in the Corn Belt states in 2001 and 2006 to other regions in the U.S. in 2015, all the while total application of fipronil is decreasing (United States Geological Survey 2018). WWTP facilities studied from 2001–2016 are shown jointly in Figure 2B to preserve the identity of participating WWTPs in the 2015/6 campaign. Average sludge concentrations between 2001 and 2016 were 1.5 to 2 times higher in USEPA Regions 1, 4, 5, and 7 than in others ($p < 0.05$). It is unclear if this is a result of the number of samples obtained per region or if these regions truly do have a higher frequency of non-agricultural fipronil use. Further investigation is warranted.

3.2 Fate of Fipronil During Sludge Treatment

In Figure 3A, the molar distribution of fiproles is shown along with total concentrations detected in anaerobically digested sludges ($n=58$) and in sludges subjected to no or to other treatments ($n=51$; Figure 3B). The latter category included no digestion, chemical treatment, and aerobic digestion. The molar distribution of fiproles in anaerobically digested sludge (average ± standard deviation; 16 ± 14% fipronil, 45 ± 11% sulfone, 38 ± 17% sulfide, 1 ± 3% amide, and 1 ± 1% desulfinyl) differed from the group of sludges subjected to other or no treatment (29 ± 21% fipronil, 46 ± 11% sulfone, 22 ± 19% sulfide, 2 ± 3% amide, and 1 ± 2% desulfinyl) (Figure 3A). The co-occurrence of most compound pairs was found to be moderately correlated whether samples were treated by anaerobic digestion or by some other/no treatment (Figure S2). The co-occurrence of fipronil and fipronil sulfide and the co-occurrence of fipronil and fipronil desulfinyl were found to be poorly correlated (Table S3).

3.3 Fiprole Occurrence in Sludge as a Function of WWTP Size

The relationship of wastewater flow received by the treatment facility and fiprole concentrations in sludge was investigated (Figure S2). Detected total fiprole levels showed no conclusive statistical differences when grouped either by sampling time or when analyzed by size or volume of flow only (Figure S4; $p > 0.05$; see Table S2 for individual p values) indicating that fiproles concentrations are not proportional to the total volume of wastewater influent.

3.4 Total Fiproles in Raw Sewage and Effluent

Analysis of 12 WWTPs showed raw sewage (influent) and discharged effluent concentrations of total fiproles to range between 1.7-132.5 ng/L (mean: 62.5 ± 45.9 ng/L, median: 67.1 ng/L) and 0.3-112.9 ng/L (mean: 41.4 ± 36.0 ng/L, median: 38.5 ng/L), respectively (Figure 4). Fate during wastewater treatment was driven by the filtration performed (during tertiary treatment) rather than by the biological treatment step likely due to the hydrophobic nature of fiproles compounds. Effluent of WWTPs performing anthracite and sand bed filtration showed 52.8 to 99.7% total fiprole removal ($n=3$); without such treatments, removal was insignificant ($p=0.480$; $n=9$). See Table S4 for concentrations of fipronil and its degradates in WWTPs studied.

4 Discussion

4.1 Usage Practices and Detection in Sludge

As demonstrated by this study, fipronil and fiproles have been ubiquitous contaminants in U.S. wastewater for at least the past 15 years. However, during this 15-year observational period, important changes occurred in U.S. agricultural use of fipronil. Data abstracted from the USGS database show a decline of nationwide agricultural usage of fipronil from ~139,700 kg/y in 2001 down to ~3,100 kg/y in 2015 (Figure 2A) due to a suspension of some fipronil applications and replacement mostly with two neonicotinoids: clothianidin and thiamethoxam (United States Geological Survey 2018). Yet, levels of total fiproles in U.S. municipal sludge, and by extension wastewater, actually increased during this time by a factor of 2.1 in 2006/7 and 2.7 in 2015/6 compared to 2001 data (Figure 1B). These data suggest fipronil use has shifted from more agriculture use to non-agricultural use, but non-agriculture use of fipronil has remained relatively steady over the last decade. To examine this further, we selected sewage sludge samples for which matching information on regional fipronil applications in agriculture were available and performed a statistical analysis. Available data pairs included samples collected between 2006-2016. Of 74 sludge samples suitable for entering this analysis, 17 originated from areas having experienced agricultural fipronil usage and 57 from areas lacking such usage. As seen in Figure 1C, total fiprole concentrations in sludge were not statistically different between two groups of sample originating WWTPs, one in close proximity to agriculturally applied fipronil and the other one not ($p = 0.215$). Thus, results support the conclusion that non-agricultural usage is dominating fipronil levels in municipal sewage sludge and by extension municipal wastewater, and that regional agricultural fipronil usages exert no discernable change.

Major non-agricultural applications of fipronil for structural termite control, for subterranean pest control and for domestic pet flea treatment have been established in prior works (McMahan et al. 2016, Sadaria et al. 2017, Teerlink et al. 2017), but no corresponding nationwide data on urban usage are available to probe for the relative contribution of specific fipronil products to fiprole levels in municipal wastewater. The State of California collects data on the sale of fipronil, but the data are self-reported to the state and may not accurately reflect actual sales (California Department of Pesticide Regulation 2018). Despite this, a general increasing sales trend is observed between 2001 and 2015 (Figure S3) (California Department of Pesticide Regulation 2018). With California currently having the 5th largest economy in the world, one can gather that sales data, even self-reported, is representative U.S. fipronil sales trends.

4.2 Fate of Fipronil During Sludge Treatment

Previous studies have shown that wastewater treatment causes fipronil to undergo limited biotransformation to major degradates (Sadaria et al. 2017, Supowit et al. 2016), and results consistent with these observations are reported here for different sludge treatments. Before thickening and dewatering, sludge was either subjected to no digestion, chemical treatment (lime, ferric chloride, polymer addition), digestion by microorganisms in the presence of oxygen (aerobic digestion) or in the absence of oxygen (anaerobic digestion). In Figure 3A, the anaerobically treated sludge and the other treated sludges were found to have statistically distinct speciation of fipronil and sulfide ($p < 0.05$), with anaerobically digested sludge featuring relatively higher amounts of fipronil sulfide, echoing prior studies identifying fipronil sulfide as a characteristic degrade arising during anaerobic biodegradation (Sadaria et al. 2017, Supowit et al. 2016). However, anaerobic digestion was not capable of lowering total fiprole concentrations (Figure 3B, $p = 0.519$). Additional data and results from statistical analyses are presented in Figure S1 and Tables S2 and S3 in the SI.

4.3 Environmental Emission through Sludge Application and Treated Effluent

The amount of sludge generated in the U.S. annually is about 12.56 million metric tons (Seiple et al. 2017). At a mean concentration of total fiproles of 91.1 $\mu\text{g/kg}$ dry weight, the amount of fiproles accumulated in sewage sludge is estimated to have been around 1140 ± 230 kg/year in 2015/6 (see SI for calculations). 47 percent of the generated U.S. biosolids are applied on the land with the rest being subjected to incineration (15%), surface disposal (6%), and other uses - deep well injection, cement kiln, gas production and landfill cover (32%) (United States Environmental Protection Agency, 2018). Considering the long half-lives of fipronil and its degradates in soil (up to several hundred days) (Brennan et al. 2009, Gunasekara and Troung 2007, Kunde et al. 2009), uptake of fiproles by plants, leaching into ground water, and water run-off into waterways should be considered for risk assessment of applied biosolids.

One hundred thirty billion liters of treated wastewater effluent are discharged into U.S. surface waters daily (Seiple et al. 2017), making fiprole congeners in effluent relevant for risk assessment. Aquatic invertebrates and pollinators are highly susceptible to fipronil and its degradates at ng/L levels (United States Environmental Protection Agency 2007, Vidau et al. 2011, Weston and Lydy 2014, Xiqing et al. 2010). Discharged effluent concentrations

from the 12 WWTPs studied here (0.3–112.9 ng/L; median: 38.5 ng/L) exceed the USEPA established aquatic life benchmark for chronic fipronil exposure (11 ng/L) in 67% of cases (Figure 4) (United States Environmental Protection Agency 2017a). Furthermore, the southern parts of the U.S. contain a high number of effluent-dominated ecosystems, where toxic effects of effluent-borne fiproles could be significant.

Fipronil was identified in the 2015-released 2011 Biennial Review of 40 CFR Part 503 under the Clean Water Act section 405(d)(2)(C) in U.S. sewage sludge as a pollutant for regulation purposes (United States Environmental Protection Agency 2015). Fipronil and its degradates have been detected nationwide in 84% surface water bodies at a combined concentration of 0.02–190.1 ng/L (mean: 32.8 ng/L; median: 17.6 ng/L), exceeding the USEPA established 21-day chronic benchmark of fipronil for invertebrates in 37% of cases (Figure 4) (Bradley et al. 2017). The present study adds key information on the occurrence of fipronil by identifying WWTPs nationwide as major emitters of fiproles back into the environment and by emphasizing the importance of non-agricultural sources (McMahan et al. 2016, Sadaria et al. 2017, Teerlink et al. 2017). These WWTP releases are distinct from agricultural usage and currently occur without having programs in place to track them geospatially nationwide, as is done for agricultural use (United States Geological Survey 2018).

5 CONCLUSIONS

It is estimated here that in the last decade, assuming steady loading during the time period of 2006–2016, about 19.7 ± 3.9 metric tons of fipronil and its degradates have been discharged from U.S. WWTPs in treated effluent and that an additional 11.4 ± 2.3 metric tons were accumulated in biosolids of which about 3.2 ± 0.6 metric tons were applied on land. Considering the persistence, toxicity, and ubiquitous discharge of fipronil and its degradates from U.S. WWTPs, the data presented here should be of notable importance to U.S. regulatory agencies warranting further investigation and fipronil regulation in non-agriculture uses, particularly spot-on treatment for flea and tick control on domestic animals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- First U.S. nationwide, longitudinal study of sewage sludges for fipronil
- Ubiquitous fipronil occurrence in sewage sludges since 2001
- Significant increase in levels from 2001 to 2015/6
- Fipronil in effluent exceeds aquatic invertebrate chronic exposure in 67% of cases

Figure 1.

Chemical structures of fipronil and its major degradates, jointly referred to as “fiproles” (A); longitudinal, nationwide concentrations of total fiproles in U.S. sewage sludge (B); fiprole abundance in sludge in regions featuring or lacking agricultural (ag) use of fipronil (C); Statistically significant differences are denoted by asterisks ($p < 0.05$); # denotes data from samples collected in 2006/7 and 2015/6.

Figure 2.

Fipronil agricultural application (A; data extracted from (United States Geological Survey 2018)), studied WWTPs in 2001-2016 (black circles), and detected mean sludge concentrations in USEPA regions from 2006-2016 (B). MT = metric tons.

Figure 3. Molar distribution of fipronil and its degradates in U.S sewage sludge (A); and concentrations of total fiproles detected in sewage sludge subjected to different treatments (B). Statistically significant differences are denoted by asterisks ($p < 0.05$).

Figure 4. Total fiproles concentrations detected in 12 U.S. wastewater treatment plants during 2015/6 (the present study), contrasted with observed concentrations in U.S. surface water during 2012/4 (reported by Bradley et al. 2017).

Table 1.

Method detection limit (MDL), detection frequency (*df*) and detected concentrations of fipronil and its degradates in municipal sewage sludge from the United States.

		Concentration, µg/kg dry weight					
		Fipronil	-sulfone	-sulfide	-amide	-desulfinyl	Total
		MDL	0.02	0.1	0.1	0.3	0.1
							-
2001 (<i>n</i> = 35)	min	0.04	1.3	1.2	0.9	0.2	2.9
	mean	9.2	18.3	16.4	2.3	0.5	43.1
	median	6.6	11.6	10.2	2.6	0.5	26.8
	max	44.3	56.0	113.4	3.8	1.3	177.8
	<i>df</i>	100%	100%	91%	23%	46%	100%
2006/7 (<i>n</i> = 50)	min	0.1	0.1	1.5	0.4	0.3	0.2
	mean	31.4	53.4	35.6	4.6	1.5	116.4
	median	12.7	37.5	27.5	4.0	1.0	81.7
	max	191.8	208.6	149.2	18.7	3.7	385.3
	<i>df</i>	100%	100%	84%	22%	46%	100%
2015/6 (<i>n</i> = 24)	min	0.3	2.2	0.9	1.1	0.4	4.9
	mean	17.0	45.0	27.5	3.7	2.0	91.1
	median	10.2	40.0	21.0	2.2	1.7	75.1
	max	62.7	110.1	114.7	7.9	6.6	240.5
	<i>df</i>	100%	100%	100%	21%	63%	100%