SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 36, No. 6, pp. 1473–1482, 2017

Printed in the USA

PASSAGE OF FIPROLES AND IMIDACLOPRID FROM URBAN PEST CONTROL USES THROUGH WASTEWATER TREATMENT PLANTS IN NORTHERN CALIFORNIA, USA

Akash M. Sadaria,† Rebecca Sutton,‡ Kelly D. Moran,§ Jennifer Teerlink,‖ Jackson Vanfleet Brown,‡ and Rolf U. Halden*†

†Biodesign Center for Environmental Security, Biodesign Institute, School of Sustainable Engineering and the Built Environment, and Global Security Initiative, Arizona State University, Tempe, Arizona, USA
‡San Francisco Estuary Institute, Richmond, California, USA
§TDC Environmental, LLC, San Mateo, California, USA
‖California Department of Pesticide Regulation, Sacramento, California, USA

(Submitted 19 May 2016; Returned for Revision 22 July 2016; Accepted 1 November 2016)

Abstract: Urban pest control insecticides—specifically fipronil and its 4 major degradates (fipronil sulfone, sulfide, desulfinyl, and amide), as well as imidacloprid—were monitored during drought conditions in 8 San Francisco Bay (San Francisco, CA, USA) wastewater treatment plants (WWTPs). In influent and effluent, ubiquitous detections were obtained in units of ng/L for fipronil (13–88 ng/L), fipronil sulfone (1–28 ng/L), fipronil sulfide (1–5 ng/L), and imidacloprid (58–306 ng/L). Partitioning was also investigated; in influent, 100% of imidacloprid and $62 \pm 9\%$ of total fiproles (fipronil and degradates) were present in the dissolved state, with the balance being bound to filter-removable particulates. Targeted insecticides persisted during wastewater treatment, regardless of treatment technology utilized (imidacloprid: $93 \pm 17\%$; total fiproles: $65 \pm 11\%$ remaining), with partitioning into sludge (3.7–151.1 μg/kg dry wt as fipronil) accounting for minor losses of total fiproles entering WWTPs. The load of total fiproles was fairly consistent across the facilities but fiprole speciation varied. This first regional study on fiprole and imidacloprid occurrences in raw and treated California sewage revealed ubiquity and marked persistence to conventional treatment of both phenylpyrazole and neonicotinoid compounds. Flea and tick control agents for pets are identified as potential sources of pesticides in sewage meriting further investigation and inclusion in chemical-specific risk assessments. *Environ Toxicol Chem* 2017;36:1473–1482. © 2016 SETAC

Keywords: Insecticide Water quality Persistant organic pollutants (POPs) Fate and transport Pesticides

INTRODUCTION

Over the last decade, 2 newer insecticides, fipronil and imidacloprid, have gradually replaced older active ingredients in common urban pest control applications, such as pet flea treatments and professional insect control products [1,2]. The phase-out of most organophosphate insecticides for urban uses in the early 2000s opened markets that soon were filled by fipronil and imidacloprid formulations. Continued growth of urban uses is likely in the present decade in large part because of the replacement of pyrethroids, an older class of insecticides that are widely detected in urban streams and have come under scrutiny for adverse impacts on the health of aquatic invertebrates [3–6], findings that triggered federal and state regulatory responses [7,8]. Fipronil, a phenylpyrazole insecticide, has multiple urban uses including sprays for the outdoor perimeter of buildings to control ants and other insects, underground injections to control termites, pet treatments for fleas and ticks, gels for crack and crevice treatment, insect control baits, and, except in California, landscape maintenance [1,9,10]. Imidacloprid, a neonicotinoid insecticide, has urban applications in lawn and landscape maintenance, outdoor structural pest control, indoor bedbug and nuisance insect control, underground injections to control termites, and pet treatments for fleas and ticks [1,11]. Imidacloprid is also used as an insecticidal component of manufactured materials such as polystyrene insulation, vinyl siding, adhesives, sealants, textiles for outdoor uses, and pressure-treated wood decking [11–13].

Both pesticides are toxic to sensitive aquatic invertebrates at low parts-per-trillion concentrations (<100 ng/L) [14,15]. In 2007, the US Environmental Protection Agency (USEPA) established aquatic life benchmarks for fipronil (11 ng/L), as well as its degradates fipronil sulfone (37 ng/L), fipronil sulfide (110 ng/L), and fipronil desulfinyl (590 ng/L) based on chronic exposure studies of multiple freshwater invertebrates [16]. Recently published invertebrate toxicity data [15] show chronic effects to aquatic invertebrates at concentrations of 7 ng/L to 8 ng/L for fipronil sulfone and 9 ng/L to 11 ng/L for fipronil sulfide, lower than the USEPA's 2007 benchmarks. Fish appear to be less sensitive to fipronil and its degradates; USEPA chronic aquatic life benchmarks for freshwater fish range from 6600 ng/L for fipronil to 590 ng/L for fipronil desulfinyl [16]. In 2008, the USEPA established an aquatic life benchmark of 1050 ng/L for imidacloprid based on chronic exposure studies of *Daphnia magna* [11]. However, a recent summary of chronic toxicity data indicates that mayflies can experience effects such as immobilization after long-term exposure at concentrations of less than 100 ng/L and that the majority of other invertebrates studied are 100 to 1000 times more sensitive to imidacloprid than *D. magna* [14]. A more recent evaluation by the European Union of imidacloprid toxicity data [17] has established a predicted no-effect concentration (PNEC) of 4.8 ng/L; this was based on species sensitivity distribution information incorporating recent toxicity data, such as the mayfly nymph immobilization effective concentration, 10% (EC10) value of approximately 30 ng/L [18]. Fish are less sensitive to

This article includes online-only Supplemental Data.

* Address correspondence to halden@asu.edu

Published online 3 November 2016 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.3673

imidacloprid, as evidenced by the USEPA fish chronic benchmark of 1.2 mg/L [11].

Both fipronil and imidacloprid are commonly detected in urban streams [6,19,20]. For example, a survey of storm drains in California found median levels of fipronil to be 33 ng/L in northern California and 76 ng/L in southern California; fipronil sulfone (medians of 26 ng/L and 77 ng/L for northern and southern California, respectively) and fipronil desulfinyl (medians of 15 ng/L and 41 ng/L for northern and southern California, respectively) were also frequently detected [6,19]. Another California survey of urban surface waters measured maximum imidacloprid levels of 160 ng/L during the dry season and 670 ng/L during the wet season [6].

For both of these pesticides, relatively few data are available concerning levels in urban wastewater before and after treatment. This data gap also exists for treated and untreated wastewater sludge, despite ubiquitous urban application of these pesticides, as well as the demonstrated presence of another group of popular urban insecticides, the pyrethroids, in treated wastewater and biosolids [21]. Fipronil has been detected in treated wastewater discharged by 9 of 25 US wastewater treatment plants (WWTPs; <10–70 ng/L) [22]; 6 Florida WWTPs (16–110 ng/L) [23]; 7 of 9 Oregon and Washington municipal WWTPs (27–130 ng/L) [24]; 2 California WWTPs (<1–57 ng/L) [15]; and 1 southwestern US WWTP (13–21 ng/L) [25]. Some of the facilities studied thus far treat a mixture of wastewater and urban runoff (California, 1; Florida, 6). Past measurements of influent and effluent suggested little if any removal of fipronil during typical wastewater treatment [15,22,23,25]. However, prior studies were sometimes limited by featuring approximately 2- to 50-fold higher method detection limits, failing to monitor all major fipronil transformation products, or omitting analysis of suspended particulates that were removed by filtration or other treatment prior to analysis [15,22,23,25]. Presently available and still limited data on fipronil degradates suggest sporadic, low-level occurrence of fipronil desulfinyl [15,23,24], as well as fipronil sulfone, sulfide, and amide [25], in wastewater treatment flows. Fipronil and its degradates were also detected in 2 effluent-dominated rivers in southern California during low flow conditions [26]. Available data suggest that concentrations of fipronil in treated effluent frequently approach or exceed USEPA chronic invertebrate aquatic life benchmark [25,26].

Fipronil and its degradates, jointly referred to as total fiproles, feature logarithmic octanol–water partitioning coefficient ($\log K_{OW}$) values > 4. This characteristic enables them to sorb to particles in wastewater that settle during treatment, ultimately leading to a sequestration of fipronil-related compounds in sewage sludge and treated sludge deemed fit for application on land (biosolids). Two studies have reported measurable concentrations of fipronil and degradates in this matrix [22,25].

Likewise, few studies have examined imidacloprid in municipal wastewater. Imidacloprid was detected in <10% of treated effluent samples from 52 Oregon municipal WWTPs (202–387 ng/L), using a higher method detection limit of 200 ng/L; influent and biosolids were not sampled [27]. To date, there are no published studies reporting on measured imidacloprid levels in biosolids, possibly because the low log K_{OW} value of imidacloprid (<1) does not suggest partitioning into sludge as an important process. Studies of imidacloprid in wastewater in China and Spain (where allowable uses may differ from those in the United States) suggest that typical treatment technologies may result in low removal of imidacloprid from the liquid phase prior to discharge into receiving waters [28,29]. A study of an effluent-dominated waterway in Iowa indicated that treated wastewater can introduce imidacloprid to receiving waters [20].

In the present study, we explored the presence of fipronil, its 4 major degradates, and imidacloprid in urban wastewater before and after treatment, providing the first regional set of data for WWTPs across varying treatment technologies. Furthermore, to add to still limited literature data, we also analyzed sludges from the sampled plants for insecticide occurrence. Finally, we assessed sources related to urban uses of these pesticides.

MATERIALS AND METHODS

Standards and reagents

High-performance liquid chromatography (HPLC)–grade organic solvents (methanol, acetone, methylene chloride, and hexane) and water were purchased from Sigma-Aldrich and Thermo Fisher Scientific, respectively. Analytical standards of imidacloprid, fipronil, fipronil desulfinyl, and deuterated labeled standard [d_4] imidacloprid were obtained from Sigma-Aldrich. Analytical standards of fipronil sulfide, sulfone, and amide were obtained from Bayer and BASF. Labeled [$^{13}C_2{}^{15}N_2$] fipronil and [$^{13}C_4{}^{15}N_2$] fipronil sulfone were bought from Toronto Research Chemicals and Cambridge Isotope Laboratories, respectively. Stock solutions of analytical standards were prepared in acetonitrile and stored at –20 °C.

Sample collection

Single 24-h composite samples of influent and effluent were collected from each of 8 facilities that discharge to San Francisco Bay (San Francisco, CA, USA). Facilities that provided samples were selected based on multiple factors, including higher discharge levels, geographic diversity, and range of treatment technologies (secondary only vs tertiary filtration; Table 1). As a consequence of drought-related water use restrictions, facilities were operating well below capacities (Table 1). One facility sampled serves only a large airport and the associated operations. The remaining 7 locations, representative of more typical municipal WWTPs, had per capita daily influent flows of 235 L/person/d to 302 L/person/d. Autosamplers at all facilities provided flow-weighted composite samples, with the exception being the San José–Santa Clara influent compositer, which provides a flow-weighted composite of 6 subsamples collected regularly throughout the 24-h period. Wastewater recycling, including reverse osmosis treatment of <10% of the San José–Santa Clara facility secondary effluent, reduces effluent flow. Reverse osmosis recycling returns a concentrate that is mixed with effluent prior to discharge. The sampling location includes the returned concentrate volume and represents roughly 2% of the total effluent volume.

Influent, effluent, and dewatered/treated sludge samples were collected simultaneously during mid-week of September 2015. The San Francisco Bay region is subject to a mild, Mediterranean climate; September is within the dry season and was selected specifically as an appropriate period of study to avoid rainfall-related inflow and infiltration. Inflow of urban runoff would include fiproles and imidacloprid; by excluding runoff as a potential source, the study design allows specific insight regarding indoor sources. Of note, none of the facilities typically treat storm water. The mild climate in this coastal region also allows fleas to flourish year-round [30], motivating continued residential use of flea control pesticides. Wastewater

Table 1. Characteristics of wastewater treatment plants and processes monitored in the present study

WWTP	Population served (thousands)	Plant capacity (MGD)	Wastewater treatment				Sludge treatment	Influent flow (MGD)	HRT (h)	TSS (mg/L)	
			Primary	Secondary	Disinfection	Advanced				Inf	Eff
SFTP	[a]	2.2	PS	SBR	Cl2	—	AD	0.45	5.75	1004	20
PARP	220	39	PS	FFR, AS	UV	F	NT	16.86	22	322	<1
SJSC	1400	167	PS	AS	Cl2	F	AD	92.76	9	315	1
SLWP	55	7.6	PS	FFR, AS	Cl2	—	AD	4.15	10	517	9
SMWP	140	15	PS	AS	Cl2	F	AD	9.02	14.6	414	9
EBMUD	650	120	PS	AS	Cl2	—	AD	45.00	15	340	11
FSSD	139	23.7	PS	AS	UV	F	AD	11.21	24	237	<1
CCSD	471	53.8	PA, PS	AS	UV	—	NT	29.27	6.5	312	8

[a]Annually, 56 million people pass through airport facilities.
WWTP = wastewater treatment plant; MGD = million gallons per day; HRT = hydraulic retention time; TSS = total suspended solids; Inf = influent; Eff = effluent; SFTP = San Francisco International Airport Commission Mel Leong Treatment Plant; PARP = City of Palo Alto Regional Water Quality Control Plant; SJSC = San Jose-Santa Clara Regional Wastewater Facility; SLWP = San Leandro Water Pollution Control Plant; SMWP = City of San Mateo Waste Water Treatment Plant; EBMUD = East Bay Municipal Utility District Wastewater Treatment Plant; FSSD = Fairfield-Suisun Sewer District Wastewater Treatment Plant; CCSD = Central Contra Costa County Sanitary District Treatment Plant; PS = primary sedimentation; PA = pre-aeration; SBR = sequential batch reactor; FFR = fixed film reactor; AS = activated sludge; Cl2 = chlorine disinfection; UV = ultraviolet disinfection; F = filtration; AD = anaerobic digestion; NT = no treatment;

samples were collected in 2-L amber glass jars to which the biocide Kathon CG/ICP (for more information see the Supplemental Data) and sodium thiosulfate were added for disinfection and preservation. Sludge samples were collected in 0.5-L amber glass jars. Wastewater samples were refrigerated at 4 °C and analyzed within 10 d of collection, and sludge samples were stored at –20 °C until extraction.

Extraction of influent solid and biosolids samples.

Wastewater influent was separated into aqueous phase and particulates and analyzed separately to determine the distribution and total mass loading of pesticides entering the WWTPs. For this purpose, influent samples were centrifuged at 3000 *g* for 5 min, and settled particulates were dried under a gentle stream of nitrogen. Analyte extraction of solids from influent and of biosolids was performed using established protocols [25,31]. One gram of nitrogen-dried solid sample was spiked with 20 ng of labeled [$^{13}C_2{}^{15}N_2$] fipronil, [$^{13}C_4{}^{15}N_2$] fipronil sulfone, and 200 ng of labeled [d_4] imidacloprid, extracted with 10 mL acetone, twice, by 24 h of shaking, followed by 1 h of sonication. Later, extracts were centrifuged, supernatants were nitrogen-dried and reconstituted to 2 mL hexane, and Florisil cleanup (solid-phase extraction with a sorbent bed containing mixture of magnesium oxide and silica gel) was performed. Analytes were eluted successively from a Florisil cartridge (Sep-Pak Vac Florisil Cartridge 6 cc containing 1 g of sorbent; Waters) with 4 mL methylene chloride and 4 mL acetone. Later, 1 mL of each extract was mixed, evaporated with nitrogen, and reconstituted to 1 mL of water and methanol solution (50:50, v/v) for fipronil and its degradates (sulfone, sulfide, and amide). Similarly, extracts were mixed, dried, and reconstituted to 1 mL of hexane for fipronil desulfinyl, and 1 mL of water, methanol, and formic acid solution (80:20:0.1, v/v/v) for imidacloprid analysis.

Extraction of wastewater samples

The wastewater extraction protocol was similar to that described in previous studies [25,31]. First, 20 ng of labeled [$^{13}C_2{}^{15}N_2$] fipronil and [$^{13}C_4{}^{15}N_2$] fipronil sulfone, and 200 ng of labeled [d_4] imidacloprid were spiked to a 500-mL wastewater sample. Later, samples were loaded on a cartridge having reverse-phase functionalized polymeric styrene divinylbenzene sorbent (Strata X & XL, 500 mg/3 mL; Phenomenex) using an automatic solid-phase extraction instrument (Dionex AutoTrace 280; Thermo Scientific) at a constant flow rate of 2 mL/min. Cartridges were eluted with 8 mL of methanol and formic acid mixture (95:5, v/v). Extracts were dried and reconstituted similarly to solid samples and prepared for analysis by chromatography separation and tandem mass spectrometry (MS/MS).

Chromatography separation and MS/MS

Imidacloprid, fipronil, and degradates, except for fipronil desulfinyl, were separated by liquid chromatography (LC) and detected and quantified by electrospray ionization−MS/MS. Liquid chromatography mass spectrometric analyses were performed using a Shimadzu Prominence HPLC (Shimadzu Scientific) coupled to an ABSciex API-4000 MS/MS (Applied Biosystems). Liquid chromatographic separation was achieved by an XBridge C8-column (3.5-μm particle size, 2.1 mm × 100 mm; Waters). The injection volume was 50 μL. For fipronil and its degradates, the mobile phase consisted of water and methanol at a total flow rate of 0.2 mL/min with a total runtime of 10 min. The binary gradient consisted of 40% methanol with a 5-min ramp of 10% solvent content increase per minute to 95% methanol, where it was held for 3.5 min. For imidacloprid, the mobile phase consisted of 0.1% formic acid in water and methanol at a total flow rate of 0.2 mL/min with a total run time of 12 min. The binary gradient consisted of 20% methanol with a 6-min ramp of 16.7% solvent content increase per minute to 95% methanol, where it was held for 3.5 min. The electrospray ionization probe was operated in negative mode for fipronil and its degradates, and in positive mode for imidacloprid. Multiple reaction monitoring was used for qualitative analysis. Fipronil desulfinyl was analyzed using gas chromatography–electron impact–MS/MS because it exhibited a considerably lower detection limit than LC−MS/MS (see the Supplemental Data).

Quality assurance and quality control

For every 5 samples analyzed, 1 method blank was included in the analytical batch. Matrix spike and matrix spike duplicates were performed at a frequency of 1-in-4 and 1-in-6 for wastewater and solids, respectively. Replicate analyses were

performed at a frequency of 1-in-3 and 1-in-5 for wastewater and solids, respectively, to determine relative percentage deviation. Field duplicates (blind samples) were also collected and analyzed for all analytes for quality assurance. Every shipment of samples included 1 field/trip blank to judge the integrity of sample handling and shipping.

Method performance

The MS/MS method targeted analytes by monitoring 2 ion transitions. Mass spectrometry parameters optimized for multiple reaction monitoring are provided in Supplemental Data, Table S1. Method detection limits of analytes in wastewater ranged from 0.1 ng/L to 0.8 ng/L and in sewage particulates from 0.1 μg/kg to 1.1 μg/kg dry weight (Supplemental Data, Table S2) [25,31]. Relative percentage difference values determined for the studied analytes in samples and in the corresponding duplicates (laboratory and field duplicates) averaged 11 ± 12%. Absolute recoveries (average ± standard deviation) of analytes in all matrix spike and matrix spike duplicate samples were 58 ± 30%, and relative recoveries (isotope-corrected) were 98 ± 10%. Field blanks and method blanks (included to monitor for postsample collection contamination) showed no detectable levels of analytes.

RESULTS AND DISCUSSION

Detection of fipronil and its degradates in wastewater treatment streams

Fipronil, fipronil sulfone, and fipronil sulfide were detected with 100% detection frequency in all influent and effluent samples of 8 WWTPs analyzed (Figure 1; Supplemental Data, Tables S3 and S4). Fipronil amide, a product of fipronil hydrolysis, was absent in all influent samples (<0.3 ng/L), but was detected in effluent samples of 7 of 8 WWTPs, suggesting that hydrolysis took place primarily during biological treatment. The photolysis degradate, fipronil desulfinyl, was detected only in a single WWTP, in both influent and effluent. In this and 2 additional WWTPs, ultraviolet disinfection was performed but it did not lead to increase in the photolysis degradate. In all WWTPs examined, fipronil and fipronil sulfone were the most prevalent fiproles by concentration. In the aqueous phase of

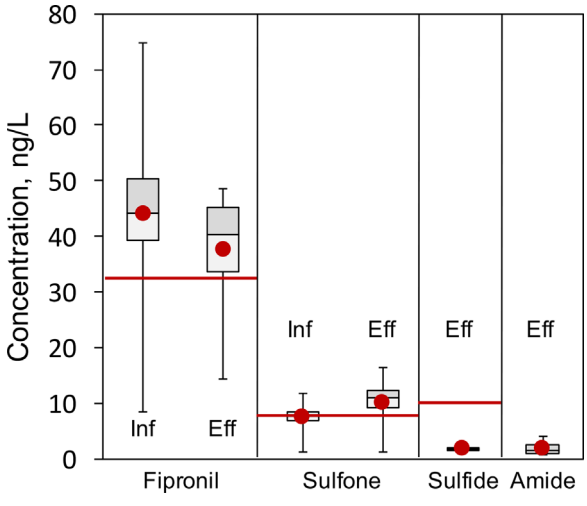

Figure 1. Detected concentrations of fipronil and its degradates (ng/L) in the dissolved phase from 8 wastewater treatment plants in northern California. Red horizontal lines indicate published chronic toxicity values for *Chironomus dilutus*, a freshwater invertebrate [15]. Inf = influent; Eff = effluent.

influent and effluent samples, fipronil concentrations ranged between 8.6 ng/L and 74.9 ng/L and between 14.3 ng/L and 48.6 ng/L, respectively, and fipronil sulfone concentrations ranged between 1.1 ng/L and 11.9 ng/L and between 1.1 ng/L and 16.3 ng/L, respectively. For 6 of the 8 WWTPs studied, sulfone concentration in the effluent was greater than the aqueous phase influent concentration (Figure 1). Fipronil sulfide, amide, and desulfinyl concentrations were less than 5 ng/L. Although the WWTPs studied performed a variety of treatment processes (Table 1), fipronil persistence was roughly comparable across all treatment regimes. Paired *t* test revealed that the total molar concentration of all fipronil-related compounds in aqueous phase influent and effluent at all 8 WWTPs was statistically indistinguishable ($p = 0.95$); however, it should be noted that the sampling strategy was not designed to account for hydraulic retention time (HRT) of treatment trains and instead was meant to yield an average concentration over a 24-h time period.

Distribution of fipronil and its degradates in wastewater

Previous studies have analyzed wastewater samples by filtering [15,23] or by analyzing supernatants [22,25]. As fipronil and its degradates have log K_{OW} values >4 (Supplemental Data, Table S2), there may be a considerable mass bound to the particulate fraction, unassessed by previous studies of influent. Among all 8 WWTPs studied, the majority of fipronil (76 ± 8% by mass) was present in the aqueous phase (Supplemental Data, Figure S1). For fipronil sulfone, however, 66 ± 7% of the mass was particulate bound. Fipronil sulfide, the anaerobic degradate, was present in the particulate fractions of all influent samples but was not detected in the aqueous phase (method detection limit = 0.2 ng/L). Of note, the molar distribution of fiproles in the influent phases likely reflects biotransformation in the sewer as well as physical partitioning and potential other, nonhydrophobic interactions. Individual mass distributions of fipronil and its degradates in all influent samples is provided in the Supplemental Data, Table S5. Of the total molar mass of fiproles, 62 ± 9% was present in the dissolved phase, and a considerable fraction (38 ± 9%) was particulate bound, which reflects the intermediate log K_{OW} values of fipronil and its degradates. Measured concentrations in different phases of analytes are provided in Supplemental Data, Table S3 and S4. As effluent samples featured low total suspended solids values between <1 mg/L and 20 mg/L, extraction and analysis of particulates from effluent was not feasible; however, considering the low amounts of particulates in treated effluent, calculations suggest that the sorbed mass of fipronil-related compound on effluent particulates was less than 0.75% of the total.

Among all 8 treatment facilities studied, the molar distribution of fipronil and its degradates differed by treatment stream and matrix, but some general trends were consistently seen across all WWTPs investigated (Figure 2). In influent, significant differences in the molar distribution of fipronil and its degradates were evident within the aqueous versus particulate phases. Aqueous phase influent was composed of 86 ± 3% fipronil and 14 ± 3% sulfone. In particulates, the molar distributions of fipronil, sulfone, and sulfide were 44 ± 4%, 46 ± 8%, and 9 ± 8%, respectively. Total influent was comprised of 70 ± 3% fipronil, 26 ± 4% sulfone, and 4 ± 4% sulfide. Individual molar distributions for each influent sample are provided in Supplemental Data, Table S5. Discharged effluent, on average, carried fiproles distributed in the following way: 74 ± 6% fipronil, 18 ± 6% sulfone, 4 ± 1% sulfide, 3 ± 2%

Total fiprole loading at 8 wastewater treatment plants in pico-mol/L

	Fipronil	Sulfone	Sulfide	Amide	Desulfinyl	Total fiproles
Influent - aq phase	806	131	0	0	3	940
Influent - particulates	234	251	44	0	0	528
Total influent	1040	382	44	0	3	1468
Effluent	690	180	34	28	6	938

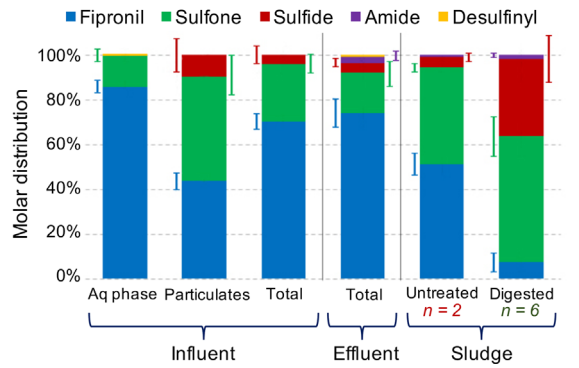

Figure 2. Molar distribution of fipronil and its degradates in treatment streams of 8 wastewater treatment plants. Error bars indicate standard deviation or min/max values when only 2 measurements were available (i.e., for untreated sludge).

amide, and $1 \pm 1\%$ desulfinyl. The small variability observed in the molar distribution in effluent from different treatment plants also suggests that the proportion of the fipronil and its degradates is not strongly influenced by factors such as unit operations, HRT, and sludge age.

Fate of fipronil and its degradates in wastewater and comparison with previous studies

On a molar concentration basis, $65 \pm 11\%$ of the sum of fipronil and its degradates entering each facility (considering both aqueous and particulate phases of influent) was measured in effluent. As mentioned earlier, aqueous phase influent contained $62 \pm 9\%$ of the total fiprole loading, also suggesting no significant removal from the aqueous phase during treatment, with reductions largely attributable to fiprole removal via partitioning to settleable particulates from the waste stream.

Detected total concentration (aqueous phase + sorbed phase) of the present study, termed "California 2015," are compared with previous studies in Figure 3. Influent and effluent of the same 8 WWTPs were analyzed by the California Department of Fish and Wildlife's Water Pollution Control Laboratory in fall 2014 at the behest of the Regional Monitoring Program for Water Quality in San Francisco Bay; however, the method of isotope dilution was not employed. Furthermore, neither sludge samples nor imidacloprid were analyzed, and samples were filtered prior to analysis. Therefore, data obtained in the 2014 study do not account for fipronil mass sorbed to wastewater particulates. The corresponding results are listed in Figure 3 as "California 2014," and concentrations detected are provided in Supplemental Data, Table S6. A comparison of concentrations and detection frequency of other studies shows the northern California data to be mostly consistent with those of prior work in different geographic regions (Figure 3). A study in the southwestern United States [25] is excluded from the comparison in Figure 3, as it studied fipronil and its degradates in only a single facility.

Accumulation of fipronil and its degradates in solids

Six of 8 treatment facilities performed anaerobic digestion of excess solids to produce treated sludge, whereas the remaining 2 facilities incinerated wastewater sludge after dewatering. The molar distribution of fipronil and its degradates in solids was consistent among WWTPs, but differed between anaerobically digested (biosolids) and untreated sludge (Figure 2). Raw excess sludge had $51 \pm 5\%$ fipronil, $43 \pm 2\%$ sulfone, $5 \pm 2\%$ sulfide, and $1 \pm 1\%$ amide, a molar distribution resembling that observed for influent-borne particulates (Figure 2). In anaerobically digested sludge, the molar distribution was different, with the anaerobic degradate fipronil sulfide accounting for $35 \pm 11\%$ and fipronil for only $8 \pm 4\%$ of all fiproles, indicating biotransformation of fipronil as a result of the treatment. The molar distribution of fipronil sulfone ($56 \pm 9\%$) and amide ($2 \pm 1\%$) was somewhat similar to that of untreated sludge. Individual molar distributions for solids from each WWTP are provided in Supplemental Data, Table S5.

Fipronil (0.2–44.1 μg/kg) and the sulfone (1.6–91 μg/kg) and sulfide (0.7–60.3 μg/kg) degradates were detected with 100% detection frequency, and fipronil amide was detected with 88% detection frequency (Figure 4). In the digested sludge produced by 6 of the 8 WWTPs, concentrations of the fipronil degradates sulfone and sulfide were considerably higher than those of the parent compound; this stands in sharp contrast to the composition of the (undigested) sludges produced in 2 facilities utilizing dewatering and incineration. Fipronil desulfinyl was not detected in any of the sludges. Only 2 prior studies have detected fipronil in sludge or biosolids. One of these studied fipronil only in sludge samples of 25 facilities nationwide [22], and another studied fipronil and its degradates in a single facility performing anaerobic digestion for solids treatment [25]. Detected total fipronil concentrations in these studies ranged between 3 μg/kg and 180 μg/kg, which is comparable to the levels observed in the present study (3.7–151.1 μg/kg as fipronil).

Detection of imidacloprid in wastewater treatment streams

Imidacloprid was detected with 100% detection frequency in all influent (58.1–306.1 ng/L) and effluent (83.8–305.2 ng/L) samples and was never detected in any of the sludge samples from the 8 WWTPs examined (Figure 5; Supplemental Data, Table S7). In influent, imidacloprid was only detected in the aqueous phase and was not detected on sewage particulates. Although the WWTPs studied employed different treatment processes (Table 1), the occurrence post-treatment of imidacloprid was a phenomenon extant at all facilities. Although sampling did not account for HRT, effluent concentrations accounted for $93 \pm 17\%$ of the loading arriving at the WWTPs on a concentration basis. Thus, none of the diverse treatment processes sampled was effective at imidacloprid removal.

At the San Francisco Airport WWTP, imidacloprid concentrations in effluent were approximately 3 times higher than influent levels, suggesting inconsistent loading into this facility that provides sanitary services to a major US airport. Alternate explanations could not be supported with available evidence [32]. Higher effluent than influent concentrations were not suggested to result from signal suppression because of matrix effects during the LC–MS/MS detection, as an isotope dilution method was used. Furthermore, proper sample preservation measures were taken, and no rainfall events occurred during the sampling event. Thus, the most likely reason for the observation was inconsistent loading at the treatment facility, particularly given that the sampling strategy was not designed to account for the HRT of the treatment train.

When this facility was excluded from the analysis, a 2-tailed paired *t* test for the remaining 7 plants revealed that influent and

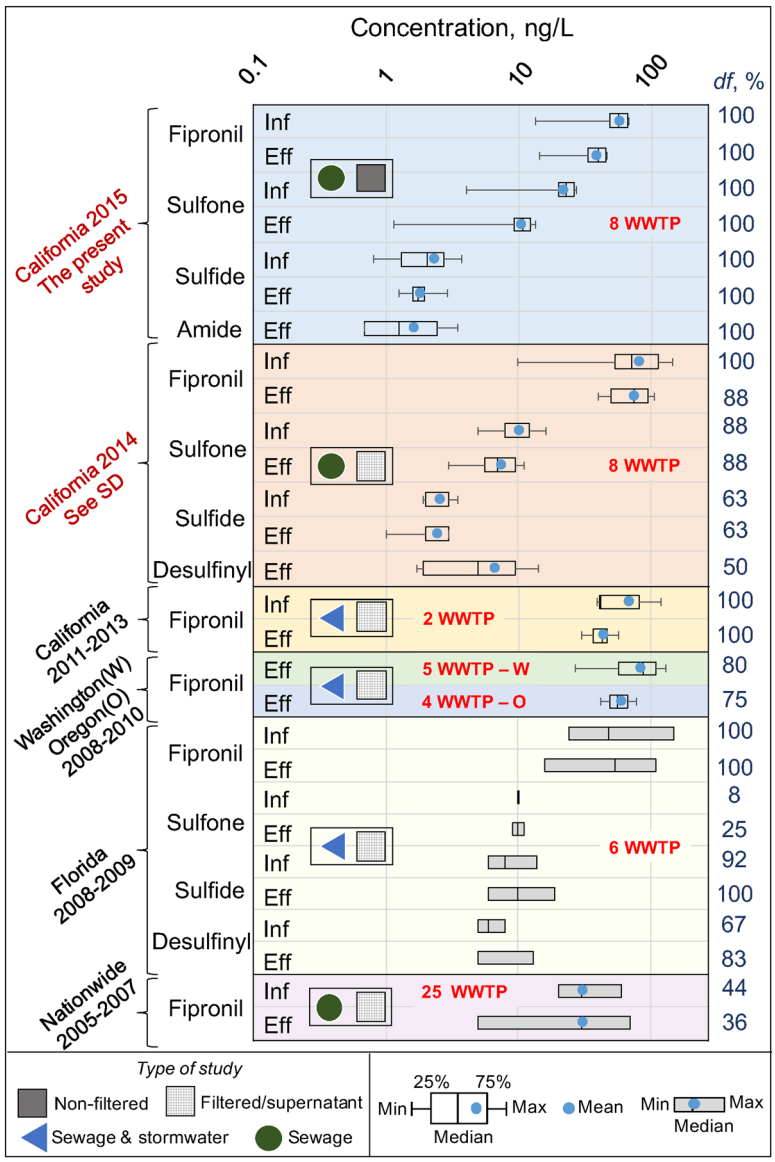

Figure 3. Concentrations of fipronil and its degradates in wastewater samples from 8 California wastewater treatment plants (present study) contrasted with data from past studies [15,22–24]. Years correspond to sampling period. df = detection frequency of compound in process flow; inf = influent; eff = effluent; SD = Supplemental Data.

effluent concentrations were statistically indistinguishable ($p = 0.49$; 95% confidence level), supporting the conclusion of pass-through of imidacloprid.

Levels of imidacloprid in effluent of northern California facilities determined in the present study are generally higher than those observed in a recent assessment of 12 WWTPs from across the United States, which reported a concentration range of 18.5 ng/L to 146.4 ng/L, a dataset included in Figure 5 [31]. An earlier study of effluent from 52 Oregon WWTPs found a relatively low level of detection (9.8% detection frequency);

WWTP	Fipronil	Sulfone	Sulfide	Amide
SFTP	0.2	1.6	1.8	0.1
PARP	*11.4*	*9.0*	*0.7*	*<0.1*
SJSC	0.5	9.1	4.3	0.4
SLWP	8.8	85.0	32.3	1.0
SMWP	17.7	91.0	43.0	1.4
EBMUD	5.6	71.0	60.3	1.4
FSSD	13.9	65.5	24.8	1.4
CCSD	*44.1*	*42.2*	*5.5*	*1.4*

Desulfinyl was not detected at detection limit of 0.3 µg/kg dry weight

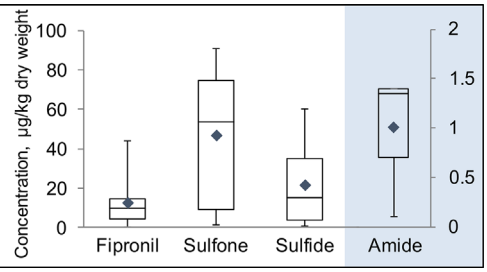

Figure 4. Concentrations of fipronil and its degradates detected in sludge samples obtained from 8 wastewater treatment plants in northern California in 2015. Highlighted in red italics are facilities not performing anaerobic treatment. In the plot, amide concentrations (highlighted blue) correspond to the secondary *y*-axis. See Table 1 for definition of site abbreviations.

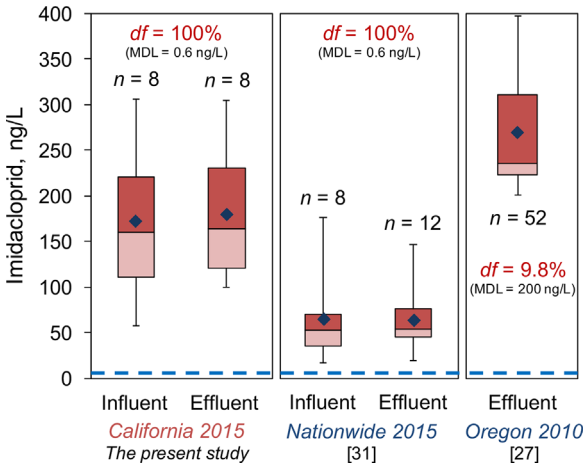

Figure 5. Detected concentrations of imidacloprid (ng/L) in 8 wastewater treatment plants in northern California and summary of data from previous studies [27,31]. Dashed blue horizontal line indicates European Union freshwater predicted no-effect concentration value [17]. df = detection frequency; MDL = method detection limit.

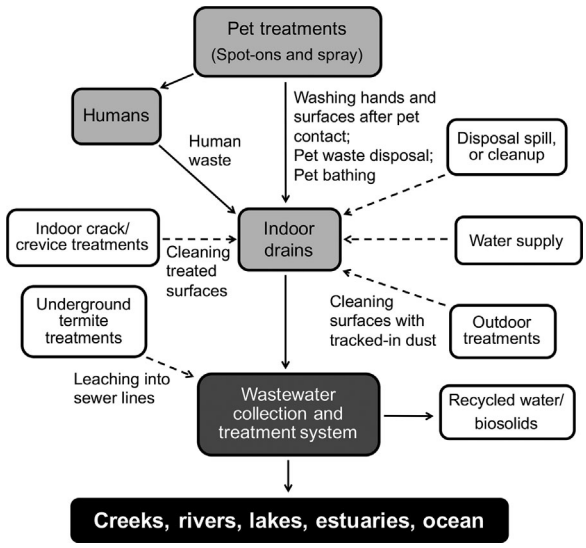

Figure 6. Conceptual model for sources of fipronil and imidacloprid in municipal wastewater. Dashed lines denote pathways believed to be relatively small in the present study. Uses without a clear pathway (e.g., containerized baits) and with unlikely pathways (e.g., air transport and deposition) [50] are excluded from the figure.

effluents with detectable imidacloprid had levels in the range of 202 ng/L to 387 ng/L (Figure 5) [27]. A limit of quantification of 200 ng/L [27], significantly higher than the method detection limit of the present study (0.6 ng/L), may account to some extent for the difference in results observed. Higher overall concentrations and detection frequencies in effluent from northern California may reflect regional, seasonal, and/or climate-related differences from other sampled facilities, such as lower dilution caused by drought-related water use reductions, presence of pests during all seasons because of the mild coastal climate, and pesticide use responding to regional pest pressures (e.g., high flea populations in California coastal areas) [30], suggesting the value of understanding regional and seasonal factors to inform estimates of the potential loading of imidacloprid in wastewater.

Fipronil and imidacloprid sources

Examination of the per capita influent load of wastewater pollutants can be instructive, as it eliminates effects of flow differences among WWTPs and provides a reference discharge quantity for comparison with various potential sources. For the 7 typical municipal WWTPs in the study, the measured per capita influent loads expressed in nanomoles per person per day, for fiproles (54 ± 9 nmol/person/d, mean ± standard deviation) and imidacloprid (190 ± 80 nmol/person/d) were relatively consistent. The concentration of contaminants in wastewater influent can vary by several orders of magnitude over the course of a single day for a single analyte, so a low variability in daily per capita load suggests a larger number of ubiquitous sources rather than episodic concentrated sources [33–35]. Although episodic discharges from spills, cleanup, or improper disposal of a pesticide are possible, such an event was not likely captured during this sampling event, as evidenced by similar per capita influent loads at all WWTPs.

As regulated pesticides, fipronil and imidacloprid have limited indoor uses in California: pet flea control, crack and crevice treatments intended for out of the way locations, and containerized bait stations [1,9]. All uses are considered unlikely to entail discharges to the sewer system [10,11].

A simple conceptual model (Figure 6) clarifies potential pathways between fipronil and imidacloprid use and the sewer system and facilitates examination of the potential importance of each discharge source. Although no fipronil and imidacloprid products, for either indoor or outdoor use, are designed to be directly discharged to indoor (sewer) drains, actions after use—such as bathing pets treated with flea control products, washing hands and other surfaces that come in contact with treated areas or pets, or wet-mopping treated indoor areas—provide indirect pathways for introduction of both pesticides to the sewer. Outdoor-use pesticides can enter sewer systems through cleaning of surfaces containing pesticides tracked indoors by pets and humans after outdoor applications. Leaching into sewer lines (which are not water tight) during underground building treatments is another possible pathway. However, leaking sewer laterals as a pathway would vary as a function of age of building sewer infrastructure. Drinking water supply may potentially be a source for contaminants. Although imidacloprid and fipronil concentrations have not been reported in any of the diverse water systems serving the 7 WWTPs, there is no or very limited agricultural and urban influence on drinking water sources for all but 2 of the WWTPs (Supplemental Data, Table S8). The low variability of per capita influent loads in the 7 municipal WWTPs, despite differing building sewer infrastructure ages and differing water sources, renders tap water an unlikely or minor source that nevertheless deserves future investigation. A third indirect source—human waste—has been verified for imidacloprid, which is known to be present in human urine [36], but is only suspected for fipronil based on rat oral exposure studies where most fiprole mass was excreted in feces [37]. As noted, episodic discharges from spills, cleanup, and improper disposal are likely small pathways, given the low data variability.

An examination of potential pathways suggests that pet flea treatments may be the primary source of both pesticides in WWTP influent. Pet flea treatments have typical concentrations of 9.8% fipronil and 9.1% imidacloprid; single pet applications involve 0.07 g to 0.4 g fipronil or 0.04 to 0.4 g imidacloprid. In contrast, the only other type of uncontainerized indoor treatments—crack and crevice applications—entails pesticide concentrations of 0.05% or less. Even the highest concentration (0.05%), professional-sized (33-g) fipronil crack and crevice

product on the market contains <0.02 g fipronil; 40 to 1200 of these crack and crevice products would need to be emptied directly into the sewer daily to achieve the influent fipronil load at the 7 typical municipal WWTPs sampled (see the Supplemental Data for calculations).

The transport of pesticides indoors from outdoor applications has been well documented [38], and fipronil is nearly omnipresent in indoor residential dust [39]. Reported concentrations were >20 times higher in households owning a dog treated with fipronil-containing spot-on products than those without treated pets [39], suggesting that residues associated with flea treatments for pets are more significant than residues tracked indoors from outdoor applications.

Dog and cat ownership in the United States is 0.24 and 0.27 per capita, respectively [40,41], and survey data indicate that 75% of dog and cat households use a flea/tick product [42]. The prevalent use of flea and tick treatment is consistent with ubiquitous rather than episodic source. Residues associated with pet products may be transferred to companions or indoor spaces [43] or may be transported directly down the drain through bathing. Washing surfaces and materials that have come in contact with and accumulated pesticides, such as companion hands, pet bedding, and companion clothing, represents indirect pathways of pesticides to wastewater.

A 2012 study [43] that quantified the mass of fipronil transferred to cotton gloves worn while owners petted their dogs for 2 min reported levels of 5600 μg 24 h postapplication, declining to 220 μg at 2 wk, and 76 μg at 4 wk, which coincides with recommended retreatment. To evaluate flea and tick treatments as a potential indirect source to wastewater, the daily influent loads measured at the WWTP are converted to mass per dog per day. Assuming fipronil has a 30% market share, each fipronil-treated dog would provide 300 μg/d, suggesting (by comparison with the hand transfer quantities) that hand washing and other indirect transfer could be a large source (see the Supplemental Data for calculations). Because flea treatments remain on pet fur for weeks after treatment [43], dog washing may result in an even greater proportion of applied pesticide discharging to the sewer system. Although comparable studies are not available for imidacloprid, the similarity of use patterns suggests comparable pathways. Imidacloprid's higher solubility may result in a larger portion washing off during bathing.

The results for the San Francisco airport WWTP, which has no on-site residential or animal populations, were the lowest reported influent concentrations for both analytes, with a midrange effluent concentration for imidacloprid compared with the other WWTPs evaluated. Airport facilities managers report no professional applicator use of imidacloprid, and fipronil is only applied via containerized baits and gels. This suggests that indirect pathways from off-site use are the major source, but does not eliminate the potential for discharges associated with nonprofessional use of retail products. Transport of pesticides through hand washing, introduction of human waste of the airport's transient population, and discharges associated with retail product use could contribute the relatively small influent loads (fiproles, 79 μmol/d; imidacloprid, 400 μmol/d) received at this unique WWTP. Available retail products contain similar mass as the total daily load (fiproles, 38 μmol/container; imidacloprid, 878 μmol/container).

Environmental implications

Several studies have demonstrated that organic micropollutants (such as pharmaceuticals, personal care products, and household pesticides) and their degradates persist through conventional wastewater treatment [32,44–46]. Wastewater effluents flow continuously into diverse freshwater and saltwater aquatic environments, creating continuous ecosystem exposure to entrained pollutants. The potential for pesticides in wastewater effluents to cause adverse effects on aquatic species depends not only on their concentrations, but also on site-specific factors at the discharge point such as dilution (if any), presence of substances that may alter bioavailability or toxicity (e.g., dissolved organic carbon), and presence of other toxicants with cumulative toxic effects. Water available to dilute effluents may already contain both fipronil-related compounds and imidacloprid from upstream sources [20]. Partitioning and fate in the receiving water can have long-term implications not revealed solely by effluent pesticide concentrations, a possibility for fipronil and its degradates, which are likely to partition into sediment based on log K_{OW} values > 4 (Supplemental Data, Table S2).

A direct comparison of fiprole and imidacloprid concentrations in these effluents with established chronic toxicity reference values [15,17] suggests a potential for harm to aquatic species, meriting further investigation. Prior work has shown that for the majority of freshwater macroinvertebrates, fipronil degradates are more toxic than fipronil [15]; these findings were not available when the USEPA established its aquatic life benchmarks in 2007 [16]. A comparison of detected concentrations with 96-h EC50 values for *Chironomus dilutus* is shown in Figure 1. It can be seen that degradate (fipronil sulfone, sulfide, and amide) concentrations in effluent were increased relative to influent as a result of the treatment. Therefore, change in fiprole distribution did not result in a marked decrease in toxicity and potentially may have increased toxicity for 7 of the 8 WWTPs (see Supplemental Data, Table S9, for calculation). A similar conclusion was reached in a prior study on a WWTP discharging into a freshwater environment [25]. However, these toxicity thresholds are derived from data for freshwater organisms in laboratory conditions, and thus may not accurately reflect potential risks in an estuarine environment such as San Francisco Bay. The present study did not include measurement of the toxicity or bioavailability of the effluent-borne insecticides to downstream biota. At present, there is a lack of toxicity data on susceptible receptor organisms in these saltwater settings. As a result, appropriately protective thresholds such as PNECs have not been established for saltwater environments, and thus further investigation is called for.

Other factors specific to San Francisco Bay may inform an evaluation of the potential impacts of effluent discharges containing these pesticides, particularly as findings from the present study suggest that existing treatment technology appears to be unable to significantly remove these pesticides. For example, effluents discharged in the southernmost regions of the Bay experience less dilution and oceanic exchange than effluents discharged in more central locations. Effluents are not the only pathway for these pesticides to enter San Francisco Bay; other studies have detected fipronil and imidacloprid in the region's urban creeks and storm water discharges [6,15,19,47]. As predicted, fipronil and its degradates have partitioned to Bay sediment (data publicly available via cd3.sfei.org), with levels of fipronil sulfone approaching a toxicity threshold for freshwater invertebrates [48]. As a result, the parent compound has been classified as an emerging contaminant of moderate concern for San Francisco Bay [49]. Imidacloprid has not yet been evaluated by local authorities relative to the region's tiered risk and management action framework for emerging

contaminants [49]. Results from the present study may inform ongoing regional monitoring and management efforts as well as broader state and federal actions to limit the potential for environmental contamination with these pesticides and to develop modeling approaches to better predict pesticide wastewater discharge and fate in municipal WWTPs and in receiving waters.

These findings must be considered in light of other important considerations. A one-time sampling event, as conducted in the present study and other similar studies [25,29,31,45], cannot assess the effects of temporal variations in pesticide use and discharge, particularly as it relates to seasonality. Although the San Francisco Bay region is less likely to display large shifts in urban flea control pesticide use, with its mild climate and relatively uniform flea pest pressures [30], seasonality is likely to be a major influence in other urban areas with marked seasonal temperature shifts. Another consideration is the potential for pesticide contamination of the water sources supplying tap water to urban residents. Although most of the source waters for San Francisco Bay urban water supplies related to the present study are essentially free of agricultural, urban, and treated wastewater influences (Supplemental Data, Table S8), the same cannot be said for the water supplies of many other regions. Source or tap water testing for relevant pesticides is likely to be an important element of studies conducted elsewhere. A third consideration concerns the wastewater treatment technology used. Although the treatment trains employed by WWTPs participating in the present study were diverse, they do not cover all available technologies. Alternate technologies, such as reverse osmosis, may have different impacts on pesticide levels, and could be explored in future studies.

CONCLUSIONS

The levels of fiproles and imidacloprid measured in wastewater influent and treated wastewater effluent suggest that conventional treatment has little promise for reducing the release of fiproles or imidacloprid into the environment once discharged to the sewer system. An investigation of potential sources suggests that pet flea and tick products are the primary source of fiprole and imidacloprid to WWTP influent. Additional work is needed to quantify the relative contribution of suggested sources and pathways (e.g., pet products, human waste, underground termite treatments). The findings of the present study, particularly identification of pet products as a likely primary source, can inform upcoming USEPA risk assessments for fipronil and imidacloprid, which for the first time will evaluate the aquatic risks associated with urban use of these pesticides [10,11]. Available toxicity thresholds have been established only for freshwater environments, highlighting the need for saltwater toxicity studies to evaluate the risks of these pesticides to the ecological health of estuarine and ocean environments in addition to freshwater systems.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3673.

Acknowledgment—The present study was funded by the Regional Monitoring Program for Water Quality in San Francisco Bay (Contribution 783) and in part by Award Numbers R01ES015445 and R01ES020889, and their supplements from the National Institute of Environmental Health Sciences (NIEHS). The authors acknowledge participants from the following wastewater treatment plants in our study: Palo Alto Regional Water Quality Control Plant, Fairfield Suisun Sewer District, Central Contra Costa Sanitary District, East Bay Municipal Utility District, East Bay Dischargers Authority, City of San Mateo Wastewater Treatment Plant, San Jose/Santa Clara Regional Wastewater Facility, and San Francisco International Airport Sanitary Waste Treatment Plant. The authors also thank E.F. Willis-Norton for assistance with the earlier 2014 sampling campaign and P.L. Tenbrook for assistance with development of the conceptual model. Reviews by D. Muir, W. Arnold, H. Stapleton, wastewater participants, and anonymous reviewers led to significant improvements.

Disclaimer—The views expressed herein are those of the authors and do not necessarily reflect those of the California Department of Pesticide Regulation, the NIEHS, or the National Institutes of Health.

Data Availability—Most of the data are available in the Supplemental Data. Additional data requests should be directed to the corresponding author (halden@asu.edu).

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