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Characterizing pyrethroid and fipronil concentrations in biosolids

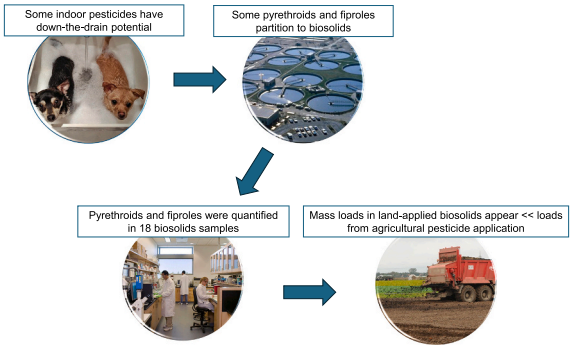
John Wheeler [a,*], Gabrielle P. Black [b], Michelle L. Hladik [b], Corey J. Sanders [b], Jennifer Teerlink [a], Luann Wong [c], Xuyang Zhang [a], Robert Budd [a], Thomas M. Young [c]

[a] California Department of Pesticide Regulation, California Environmental Protection Agency, 1001 I Street, Sacramento, CA 95812, USA
[b] U.S. Geological Survey, 6000 J Street, Placer Hall, Sacramento, CA 95819, USA
[c] University of California, Davis, 3113 Ghausi Hall, One Shields Avenue, Davis, CA 95616, USA

HIGHLIGHTS

- Biosolids were analyzed for pesticides with down-the-drain transport potential.
- Pyrethroids and fiproles were detected in all samples.
- Mass loads of pesticides in land-applied biosolids appear to be minimal.

GRAPHICAL ABSTRACT



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ABSTRACT

Pesticides are prevalent in wastewater, yet few studies have measured pesticides in biosolids and aqueous media from samples collected concurrently. Seventeen California wastewater treatment plants (WWTPs) were sampled in May 2020. Biosolids samples were analyzed for 27 analytes, and paired aqueous samples (influent and effluent) were analyzed for 23 analytes. Analytes included fipronil and its transformation products (fiproles), pyrethroids, novaluron, and several other pesticides with down-the-drain transport potential. Of the 27 compounds analyzed in biosolids samples, 16 were detected in at least one sample, and 10 had a detection frequency (DF) of at least 25 %. Fipronil sulfone, fipronil sulfide, and fipronil were the most frequently detected fiproles (DF = 100 %, 94 %, and 67 %, respectively); permethrin was the most frequently detected pyrethroid (DF = 100 %), followed by bifenthrin (DF = 94 %), cyhalothrin (DF = 89 %), and etofenprox (DF = 78 %). To elucidate fipronil transformation pathways within the treatment system, data from the three sample types were compared; findings were generally consistent with transformation pathways reported previously (e.g., some fiproles were rarely detected in influent or biosolids, but frequently detected in effluent, indicating their formation during the treatment process). No correlations were found between WWTP characteristics and pesticide concentrations in biosolids. The fraction of organic carbon (f_{OC}) of each biosolids sample was measured, and a statistically significant negative correlation was observed between f_{OC} and some fiproles, but not fipronil; possible explanations

* Corresponding author.
E-mail addresses: John.Wheeler@cdpr.ca.gov (J. Wheeler), gpblack@usgs.gov (G.P. Black), mhladik@usgs.gov (M.L. Hladik), csanders@usgs.gov (C.J. Sanders), Jennifer.Teerlink@cdpr.ca.gov (J. Teerlink), lhua@ucdavis.edu (L. Wong), Xuyang.Zhang@cdpr.ca.gov (X. Zhang), Robert.Budd@cdpr.ca.gov (R. Budd), tyoung@ucdavis.edu (T.M. Young).

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are discussed. Additional analysis for two major agricultural pesticides (bifenthrin and permethrin) indicated that estimated mass loads of these pesticides in biosolids applied to land as a soil amendment are minimal (approximately 2 to 3 orders of magnitude lower) compared to inputs from agricultural applications. This study provides insight on the magnitude of pesticides entering the environment via land-applied biosolids; existing regulations surrounding agricultural pesticide applications are expected to also be protective of the relatively low inputs from biosolids.

1. Introduction

Previous studies have presented a conceptual framework for how pesticides enter municipal waste streams (Sutton et al., 2019; Wells and Collins, 2022; Budd et al., 2023) and have assessed the down-the-drain transport potential of pesticide use patterns within California (Xie et al., 2021). The down-the-drain transport potential of pesticides has been established for certain types of products, including pet products (Teerlink et al., 2017) and indoor foggers (Dery et al., 2022). Specifically, down-the-drain transport of pesticides in topical pet products has been shown to occur due to pet bathing, owner handwashing, and laundering of pet bedding (Perkins et al., 2024). Previous research has documented pesticides in influent and effluent from wastewater treatment plants (WWTPs), often at concentrations that exceed aquatic life toxicity thresholds (Weston et al., 2013; Sadaria et al., 2016a; Sadaria et al., 2016b; Budd et al., 2023; Masoner et al., 2023). For example, Budd et al. (2023) measured fipronil concentrations in effluent as high as 95.9 ng/L, with 100 % of effluent samples exceeding the aquatic benchmark value of 11 ng/L. Although prior studies have detected fiproles (Supowit et al., 2016), permethrin (Rogers et al., 1989), and dichloro-diphenyl-trichloroethane (DDT) and several of its degradates (Dimitriou-Christidis et al., 2015) in biosolids samples, few studies have conducted a broad survey of current-use pesticides in biosolids or have examined pesticide concentrations in this matrix along with matched aqueous samples collected concurrently.

Due to their physicochemical properties, some pesticides introduced into the wastewater collection system may partition to solids, as opposed to remaining dissolved in the aqueous fraction. For example, chemicals with higher octanol-water partition coefficients (log K_{OW} values >4) can sorb to particles in wastewater and partition into biosolids during treatment (Sadaria et al., 2016a). Additionally, other properties, such as long degradation half-lives in anaerobic environments, may lead to accumulation in biosolids.

A total of 27 chemicals were chosen for analysis in biosolids samples in this study (Table SI-1): 15 pyrethroids and 2 pyrethroid degradates, fipronil and its 5 major degradates (collectively known as fiproles), an insect growth regulator (novaluron), and DDT and 2 of its major degradates. Note that the increased number of analytes compared to most prior studies is reflective of an increased interest in and awareness of available pesticide products with down-the-drain transport potential. Etofenprox is a pyrethroid derivative and was grouped with the pyrethroids for the purposes of this study. Analytes were selected based on their expected likelihood of being detected in biosolids by meeting one or more of the following general, equally weighted criteria: (1) high rate of reported use/sales of pesticide products with down-the-drain transport potential based on use site (e.g., pet products), (2) high likelihood of partitioning to solids before or during the wastewater treatment process, based on physicochemical properties (e.g., relatively high log K_{OW} and/or low water solubility; refer to Table SI-1), (3) high persistence in the environment (e.g., long degradation half-lives). The fraction of organic carbon (f_{OC}) of each biosolids sample was measured to determine whether any observed differences in pesticide concentrations in biosolids may be partially explained by differences in f_{OC}.

Some pesticides were not chosen for analysis in biosolids samples despite high rates of use and/or sales in products with down-the-drain transport potential. For example, imidacloprid is a neonicotinoid insecticide that is registered in the United States for use in pesticide products, including those with down-the-drain transport potential (e.g., topical pet products). However, imidacloprid has a very low log K_{OW} value of −0.41 (Sadaria et al., 2016a). Additionally, the percent removal due to sorption to sludge in a WWTP is estimated to be only 1.77 % (U.S. Environmental Protection Agency [EPA], 2012). This low removal is generally consistent with wastewater monitoring data (Campo et al., 2013; Sadaria et al., 2016a; Sadaria et al., 2016b). Although higher removal percentages have been observed in WWTPs using tertiary treatment (Budd et al., 2023), this increased removal is likely due to tertiary treatment processes (e.g., filtration) rather than sorption to sludge. Therefore, the partitioning of imidacloprid to sludge does not appear to be an important process, and biosolids samples were not analyzed for imidacloprid (or similarly hydrophilic compounds) in this study.

After sludge has undergone treatment processes, it is typically referred to as “biosolids” (Emmanouil et al., 2024). Biosolids are often used as soil amendments through direct application in various settings, commonly referred to as “land application.” In 2020, approximately 830,585 dry metric tons of biosolids were generated in California, of which approximately 535,448 metric tons (64 %) were applied to land (EPA, 2020). In the United States, Federal regulations define minimum requirements for the use of biosolids as fertilizers and soil amendments, as well as distinctions based on the extent of pathogen reduction (with Class A requiring further pathogen reduction compared to Class B; please refer to 40 CFR Part 503 for further information). These requirements include regulatory limits placed on nine pollutants (all metals), but there are currently no regulatory limits regarding specific pesticides or organic contaminants.

There is a concern that pollutants, including organic compounds, present in land-applied biosolids may pose an environmental risk (Harrison et al., 2006; Heidler et al., 2006; Singh and Agrawal, 2008; Petrie et al., 2015; Black et al., 2019; Black et al., 2021). Chemicals with high K_{OW} values are especially likely to sorb to solids before or during the wastewater treatment process, persist through digestion, and subsequently be applied to land. This study analyzed for 27 pesticides and related degradates in biosolids collected in California. Concentrations were compared to paired WWTP influent and effluent samples collected during a similar timeframe (because the biosolids and aqueous matrices were analyzed by different laboratories, the analyte lists for biosolids and aqueous samples are not identical). To assess the environmental importance of biosolids-borne pesticides, the median biosolids concentrations of bifenthrin and permethrin (chosen because of their high detection frequencies and high detected concentrations in biosolids in this study) were used to compare the mass of these pesticides, applied via the beneficial application of biosolids as a soil amendment, to mass loads applied during typical agricultural applications.

2. Materials and methods

2.1. WWTP sites and sampling

Seventeen California WWTPs volunteered to provide samples during one sampling event in May 2020. The WWTPs represent a range of

characteristics, including treatment methods, geographic region, capacity, and average dry weather flow rate (Table SI-2).

WWTPs provided samples of biosolids, influent, and effluent within a 2-week window (Table SI-2). Biosolids samples were collected in clean glass jars, shipped on ice, and frozen until analyzed at the U.S. Geological Survey Organic Chemistry Research Laboratory (Sacramento, CA). Aqueous samples were extracted within one day of receipt in the laboratory; extracts were frozen until analysis several months later in August 2020 at University of California, Davis (Davis, CA). Sixteen of the WWTPs use onsite anaerobic digestion (AD) as part of their biosolids treatment process; biosolids samples represented sludge which was anaerobically digested and met the standards put forth by 40 CFR Part 503 for safe land disposal (Table SI-2). WWTP "J" provided two samples (one was collected directly from an anaerobic digester, and the other was collected from a solids storage basin, which is used to store anaerobically digested biosolids). WWTP "H" contracts their biosolids processing to an offsite facility, so an undigested sample (dewatered, untreated sludge) was provided for this study. A method blank and matrix spikes were included for aqueous samples, and three matrix spikes, two method blanks, and a method duplicate were included for biosolids samples.

2.2. Materials

Pesticide standards were acquired from the EPA National Pesticide Standard Repository (Fort Meade, Maryland), Bayer Chemical (Whippany, New Jersey), Sigma Aldrich (Saint Louis, Missouri), HPC Standards (Atlanta, Georgia), Accustandard (New Haven, Connecticut), and ChemService (West Chester, Pennsylvania). Isotopically labeled surrogates (fipronil-$^{13}C_4$ $^{15}N_2$, p,p′-DDE-$^{13}C_{12}$, permethrin-$^{13}C_6$, and trifluralin-D_{14}) and internal standards (fipronil sulfone-$^{13}C_4$ $^{15}N_2$ and bifenthrin-d_5) were acquired from Cambridge Isotope Laboratories (Andover, Massachusetts). Mass spectrometry grade dichloromethane (DCM), hexane, acetone, acetonitrile (ACN), methanol, ethyl acetate, and anhydrous magnesium sulfate were acquired from Fisher Scientific (Waltham, Massachusetts). Ultra-pure water was acquired from an in-house water purification system (Milli-Q Millipore). Cellulose Q-disc® filters (0.25 micrometer [μm], C1) were acquired from CEM (Matthews, North Carolina). Graphitized carbon cartridges (Carboprep 90, 500 milligrams [mg], 6 cubic centimeters [cm^3]) and polytetrafluoroethylene (PTFE) filter vials (0.45 μm) were purchased from Restek (Bellefonte, Pennsylvania). Solid-phase cartridges (Oasis HLB, 500 mg, 6 cm^3) were purchased from Waters (Milford, Massachusetts). PALL Acrodisc syringe filters with PTFE membrane (0.2 μm) were acquired from VWR (Radnor, Pennsylvania).

2.3. Sample preparation and extraction

2.3.1. Biosolids

Sample extraction took place in October 2022. An Energized Dispersive Guided Extraction system (EDGE, CEM®) was used to extract 21 pyrethroid and pyrethroid transformation products, fipronil and 5 of its transformation products, and 4 isotopically labeled surrogate compounds published previously (Black et al., 2023). Briefly, 0.25 gram (g) of lyophilized biosolids were loaded into Q-Cups® prepared with cellulose disks and homogenized with ~1 g magnesium sulfate and fortified with 50 nanograms (ng) of surrogates (fipronil-$^{14}C_4$, $^{15}N_2$, p,p′DDE-$^{13}C_{12}$, permethrin $^{13}C_6$, and trifluralin-d_{14}) used to evaluate method performance. Samples were extracted with 30 milliliters (mL) hexane:acetone (1:1) at 100 °C then concentrated and exchanged into DCM. Graphitized carbon cartridges were preconditioned with 10 mL of DCM prior to loading the 1-mL extract, then eluted with 10 mL of DCM followed by 10 mL of ACN. The extract was solvent exchanged into ACN, concentrated to approximately 0.2 mL, filtered through a 0.45-μm vial filter then fortified with internal standards (bifenthrin-d_5 and fipronil-$^{13}C_4$ $^{15}N_2$) at a concentration of 250 ng mL^{-1}. In addition to the 18 samples analyzed, a replicate, three replicate matrix spikes, and two laboratory blanks were also included.

2.3.2. Influent and effluent

Wastewater treatment plant influent and effluent were processed and extracted following the method published by Budd et al. (2023) with the following minor modifications: a sub-sample of the influent (100 mL) and 1 liter (L) of treated effluent were filtered and extracted as previously described. Briefly, these samples were extracted after filtering through a 0.45-μm glass fiber filter, fortified with 40 ng of isotopically labeled surrogates, and extracted using an Oasis HLB solid phase extraction cartridge, dried and eluted with two volumes of 5 mL of ethyl acetate and combined with sample bottle rinses (three volumes of 4 mL of hexane:acetone (3:1, v/v) and concentrated to 1 mL). Solid phase extraction (SPE) cartridges were re-eluted with two volumes of 5 mL of methanol and concentrated to 1 mL. Filters were dried and extracted in two volumes of 20 mL of hexane:acetone (1:1, v/v) in a sonication bath, then concentrated to 1 mL Water and filter extracts were split evenly and recombined as a gas chromotography (GC) and liquid chromatography (LC) fraction. The GC fraction was concentrated to 0.2 mL and internal standard (10 ng DBOFB) was added, while the LC fraction was concentrated to 1 mL and a mixture of isotopically labeled internal standards (200 ng; imidacloprid-d_4, diuron-d_6, and pendimethalin-d_5) were added immediately prior to analysis.

2.4. Data acquisition & analysis

2.4.1. Biosolids

Fiproles and novaluron were analyzed on an Agilent 6430 triple quadrupole mass spectrometer coupled to an Agilent 1260 bio-inert high pressure liquid chromatograph (LC-MS/MS, Agilent Technologies Inc., Santa Clara, California). Pyrethroids and DDTs (p,p′-DDT, p,p′-DDD, and p,p′-DDE) were analyzed on a Thermo Fisher Scientific Trace 1310 gas chromatograph coupled to a TSQ 9000 triple quadrupole mass spectrometer (GC–MS/MS, Thermo Fisher Scientific, Waltham, Massachusetts). Further details on chromatographic conditions and instrument method details can be found in Gross et al. (2023). LC- and GC–MS/MS data were collected, and a list of transitions monitored is reported in Tables SI-3 and SI-4.

Two product ions were selected for each compound and integration required a signal-to-noise ratio of at least 10. A nine-point internal calibration curve was used (2.5–1000 ng mL^{-1}) with linear coefficient of determination (R^2) > 0.99. Matrix spike recoveries ranged from 79 to 115 % with a maximum standard deviation of 12 % among triplicates. Four isotopically labeled surrogate compounds were added to each sample prior to extraction. Surrogate recoveries ranged from 68 to 113 % (with a mean of 94 % for all four surrogates across all samples) with a maximum standard deviation among samples of 15 %. One sample was chosen as a replicate and observed concentrations varied by 7.1 %. Method detection limits (MDLs) were between 2.5 and 5 micrograms per kilogram (μg/kg). Sand was used as method blanks and processed in parallel to biosolids samples. No detections were observed.

2.4.2. Influent and effluent

Data acquisition followed the method of Budd et al. (2023), with minor modifications. All extracts were analyzed via an Agilent 6530 liquid chromatograph quadrupole time-of-flight (QTOF) mass spectrometer (Agilent Technologies, Santa Clara, California) operated in positive electrospray ionization and an Agilent 7890B gas chromatograph QTOF mass spectrometer, operated in negative chemical ionization (NCI). Further details on chromatographic and mass spectrometer conditions are reported in the supplemental information of Budd et al. (2023). A list of quantifier and qualifier ions are reported in Tables SI-5 and SI-6.

Quantifier ions and confirming fragment ions were used to quantitate each compound and required a signal-to-noise ratio of at least 10.

An 11-point internal calibration curve was used (0.1–250 ng mL^{-1}) with a linear $R^2 > 0.99$. Matrix spike recoveries of most compounds ranged from 60 to 140 % with a few exceptions.

In influent, MDLs ranged from 2 to 1000 ng/L, and reporting limits (RLs) ranged from 10 to 2000 ng/L; in effluent, MDLs ranged from 0.2 to 100 ng/L, and RLs ranged from 1 to 200 ng/L (Table SI-7). The differences in MDLs and RLs in influent compared to effluent are due to the different extraction volumes used for these two media and their corresponding matrix interferences. Observations of analytes in influent and effluent samples were qualified as either "non-detect," "trace," or "detection."

2.4.3. Fraction of organic carbon in biosolids

The f_{OC} was analyzed using a modified version of the EPA Method 440.0 for the Determination of Carbon and Nitrogen in Sediments and Particulates of Estuarine/Coastal Waters Using Elemental Analysis (Zimmermann et al., 1997). In summary, 5–10 mg of dry, homogenized biosolids were weighed into silver capsules and acidified via hydrochloric acid vapor in a desiccator for 14 h to remove inorganic carbon. Samples were dried in an oven at 105 °C for an hour to remove any remaining moisture before being pressed into sealed balls. Samples were analyzed by a Costech ECS 4010 CHNSO analyzer (Costech Analytical Technologies Inc., Valencia, California) in carbon nitrogen mode. The combustion furnace was 980 °C, the reduction furnace temperature was 650 °C, the gas chromatographic column temperature was 65 °C, and the carrier gas flow rate was 110 mL min^{-1}. Concentrations were calculated with a five-point calibration curve of acetanilide with a minimum $R^2 = 0.999$.

2.4.4. Statistical analysis

Statistical analyses were carried out using the *EnvStats*, *NADA*, and *NADA2* packages in R (version 4.2.1), using procedures appropriate for environmental datasets with censored observations (Helsel, 2012). For analytes with censored observations, some of the summary statistics (e.g., medians), were estimated with the Regression on Order Statistics (ROS) method, using the *censtats* function in the *NADA* package. To investigate correlation between pesticide concentrations in biosolids and explanatory variables, the *ATS* function in the *NADA2* package was used. This function estimates the slope of a linear trend between two variables by computing the median of pairwise slopes. It is a robust method resistant to outliers. The *ATS* function generates non-parametric Kendall's tau correlation coefficients and *p* values for the null hypothesis test that the slope of the Akritas-Theil-Sen line (ATS line) is equal to zero. To investigate whether certain pesticide concentrations in influent were a statistically significant predictor of the biosolids concentration in each paired sample, the *cen1way* function in the *NADA2* package was used. This function performs a Peto-Peto nonparametric test of differences in cumulative distribution functions between groups (Peto and Peto, 1972). For both the ATS and Peto-Peto analyses, a significance level (alpha) of 0.05 was used. Using this significance level, a p value of less than or equal to 0.05 would indicate a statistically significant result.

2.4.5. Mass loading analysis

Bifenthrin and permethrin were used to compare the mass loads of pesticides via agricultural application and via land-applied biosolids. Reliable data on biosolids application rates to crops are limited; however, corn was chosen as a representative crop because typical biosolids application rates to corn are available (EPA, 2000), and because bifenthrin and permethrin are applied to corn in California, thus allowing for comparisons to be made. Data on agricultural applications of bifenthrin and permethrin were obtained from CDPR's Pesticide Use Reporting (PUR) database (CDPR, 2020, Tables SI-8, SI-9). Statewide mass loads in 2020 were estimated for agricultural applications of the two pesticides, as well as the estimated statewide mass of each pesticide in biosolids applied to land as soil amendments. These statewide mass load estimates did not focus on any specific crop; instead, all agricultural applications of bifenthrin and permethrin were considered (regardless of crop).

Average application rates for corn were calculated as follows:

$$\text{Average Application Rate} = \frac{\text{Total Annual Mass of Pesticide Applied to Corn}}{\text{Total Annual Area Treated}}$$

where "Total Annual Mass of Pesticide Applied to Corn" is the estimated total mass of each pesticide applied to all applicable corn sub-types for which data were available in 2020. "Total Annual Area Treated" refers to the cumulative area treated, accounting for multiple pesticide applications in a year (e.g., if a 1-acre field were treated three times in a year, the cumulative acres treated would be 3 acres). However, because pesticides might be applied multiple times per year on the same piece of land, pesticide application rates in agricultural settings may be underestimated in this analysis.

To estimate the total statewide mass of each pesticide in land-applied biosolids, the median pesticide concentrations in biosolids measured in this study (expressed in units of micrograms pesticide per dry kilogram biosolids) were multiplied by the total estimated mass of biosolids that were applied to land in California in 2020 (535,448 dry metric tons; data obtained from the Biosolids Annual Report [EPA, 2020]), per the following equation:

$$\text{Total Applied Pesticide Mass} = \text{Median Pesticide Conc.} \times 535{,}448{,}000\,\text{kg}$$

To estimate typical application rates of pesticides in land-applied biosolids, biosolids application rate data were obtained for corn (EPA, 2000). Then, median pesticide concentrations in biosolids (measured in this study) were multiplied by the application rate of biosolids to corn, per the following equation (detailed calculations are provided in Tables SI-10, SI-11, and SI-12):

$$\text{Pesticide Appl.Rate} = \text{Median Conc.}\left(\frac{\mu\text{g Pesticide}}{\text{dry kg biosolids}}\right) \times \text{Biosolids Appl.Rate}\left(\frac{\text{dry kg}}{\text{acre}}\right)$$

In the equation above, "Biosolids Appl. Rate" is estimated as the annual mass of biosolids applied per acre to corn, using the median of values provided in the EPA's Biosolids Fact Sheet (EPA, 2000). The biosolids application rate to corn was provided as 5 to 10 dry tons per acre (EPA, 2000); therefore, the median value of 7.5 dry tons per acre was used in this analysis.

3. Results and discussion

Sixteen pesticides and degradates were detected in at least one biosolids sample (Fig. 1). Among the most prevalent were permethrin, bifenthrin, cyhalothrin, and etofenprox, which were detected in 100 %, 90 %, 85 %, and 75 % of biosolids samples, respectively. Fipronil sulfone and fipronil sulfide were detected at higher frequencies (100 % and 94 %, respectively) than the parent compound, fipronil (67 %), indicating that these transformation products may have formed during WWTP treatment, and ultimately partitioned into the solid fraction. Individual results for each WWTP are provided in Table SI-13.

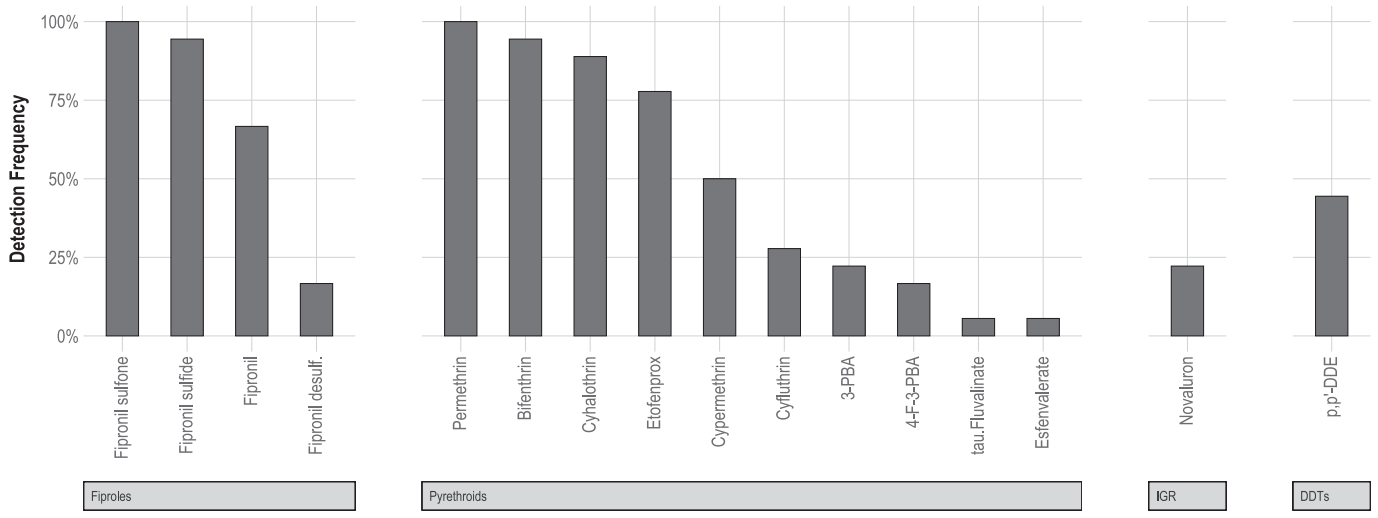

Fig. 1. Detection frequencies for analytes detected in biosolids samples (n = 18). In this study, the term "detection frequency" with regard to biosolids samples refers to the percentage of samples in which a given analyte had a measured concentration greater than or equal to the method detection limit (MDL). "3-PBA" = 3-Phenoxybenzoic acid (pyrethroid degradate). "4-F-3-PBA" = 4-Fluoro-3-phenoxybenzoic acid (cyfluthrin degradate). "IGR" = insect growth regulator. "DDTs" = p,p′-DDT and the following two degradates: p,p′-dichloro-diphenyl-dichloroethane (p,p′-DDD) and p,p′-dichloro-diphenyl-dichloroethylene (p,p′-DDE). Note: the following 11 analytes were not detected in any biosolids samples, and are therefore not plotted: fipronil amide, fipronil desulfinyl amide, allethrin, deltamethrin, fenpropathrin, phenothrin, resmethrin, tefluthrin, tetramethrin, p,p′-DDT, and p,p′-DDD.

The following 11 analytes were not detected in any biosolids samples: fipronil amide, fipronil desulfinyl amide, allethrin, deltamethrin, fenpropathrin, phenothrin, resmethrin, tefluthrin, tetramethrin, p,p′-DDT, and p,p′-DDD (dichloro-diphenyl-dichloroethane).

3.1. Fipronil and degradates

Fipronil was observed in trace concentrations (i.e., between the MDL and the RL) in 15 influent samples, and was quantified (i.e., greater than the RL) in two samples (Fig. SI-1, Table SI-7). Additionally, fipronil constituted the highest concentration of fiproles entering WWTPs in influent (Table SI-7); this is consistent with previous studies reporting that the degradation of pesticides within the collection system (e.g., sewer lines) is expected to be minimal (Xie et al., 2021). However, the median fipronil concentration in effluent (18.4 ng/L) is within the range of trace concentrations for fipronil in influent (i.e., between the MDL of 10 ng/L and the RL of 40 ng/L). The fipronil concentration in the undigested biosolids sample (WWTP "H") was on the higher end of the observed range (compared to fipronil concentrations in anaerobically digested biosolids samples), indicating that anaerobic processes occurring during the biosolids digestion process play an important role in fipronil degradation. This is further supported by data from a prior study, which found median fipronil concentrations of 70 μg/kg in anaerobically digested biosolids, 130 μg/kg in aerobically digested sludge, and 160 μg/kg in undigested sludge (Heidler and Halden, 2009).

In the anaerobically digested biosolids samples, fipronil sulfide concentrations were approximately 2–3 times greater than fipronil concentrations (Fig. 2). In addition, fipronil sulfide was not detected in

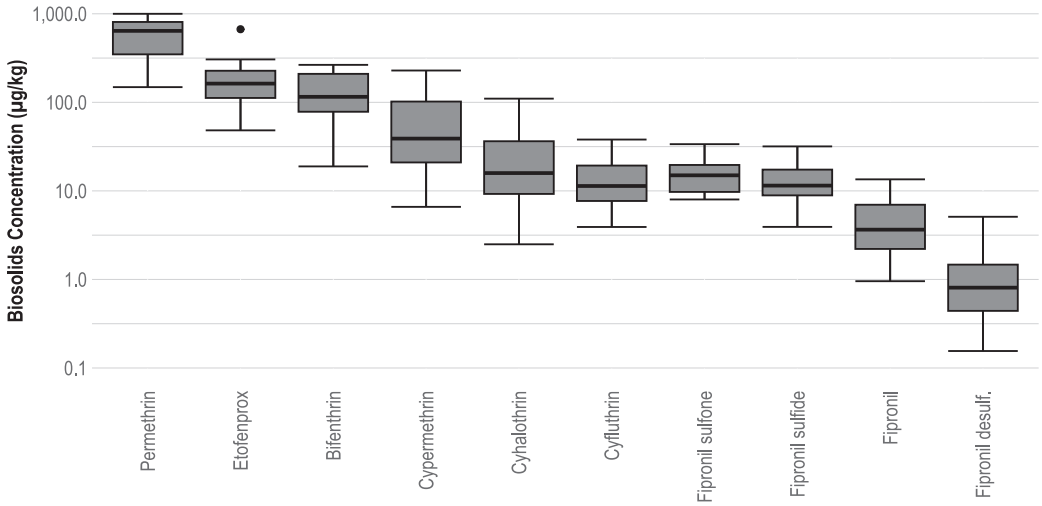

Fig. 2. Fiprole and pyrethroid concentrations in biosolids samples (n = 18), aggregated across all wastewater treatment plants (WWTPs) in the study. Estimated values for censored observations (non-detects) were imputed using the Regression on Order Statistics (ROS) method (using the NADA package in R) and are incorporated into the boxplots. For all analytes, boxes represent the interquartile range (IQR; 25th–75th percentile) and horizontal black lines represent the medians. For all analytes except etofenprox, whiskers represent minimum and maximum concentrations. For etofenprox: the lower whisker represents the minimum concentration, the upper whisker represents the second greatest concentration, and the black point indicates a measurement greater than the 75th percentile +1.5 x IQR, and is also the maximum concentration. Fipronil amide and fipronil desulfinyl amide concentrations were not plotted because there were no detections in biosolids.

the undigested biosolids sample from WWTP "H," further supporting that fipronil degradation to the sulfide degradate occurs under anaerobic conditions (Dyk et al., 2012). Fipronil sulfone was detected in 100 % of biosolids samples and was detected at concentrations as large as 33.7 μg/kg in biosolid samples, 20.0 ng/L in influent, and 8.8 ng/L in effluent samples (Fig. 2). Previous studies reported that fipronil sulfone is formed through oxidative processes (Budd et al., 2015) and is likely formed in either aeration basins or secondary clarifiers (Supowit et al., 2016), and is less likely to have formed during anaerobic digestion. This is further supported by the fact that fipronil sulfone was detected in the undigested biosolids sample. Fipronil sulfide and fipronil sulfone were frequently observed at trace concentrations (<RL) in influent (Table SI-7). In addition, these two compounds were ubiquitously detected in effluent samples (fipronil sulfide: 2.3–10.5 ng/L and fipronil sulfone: 2.4–8.8 ng/L).

Fipronil amide and fipronil desulfinyl were infrequently detected in biosolids and influent samples, but frequently observed at trace concentrations in effluent samples. This may indicate that these two compounds were formed in the aqueous fraction of wastewater during treatment via hydrolysis or photolysis (Gunasekara et al., 2007). However, these compounds were observed at concentrations less than their respective RLs in effluent, and the influent MDLs for each compound were greater than the RLs in effluent; therefore, these compounds may have been present in influent samples but undetected due to their concentrations being lower than the influent MDLs. Detailed comparisons between fiprole concentrations reported in prior studies and in this study are provided in Table SI-14.

Fipronil is used extensively in pet products (Xie et al., 2021), which likely represents the most substantial source of fiproles entering WWTPs sampled in this study. Substantial wash-off of fipronil from the use of topical products for dogs has been demonstrated to occur via pet bathing, owner handwashing, and laundering of pet bedding (Teerlink et al., 2017; Perkins et al., 2024). Fipronil is also found in gel bait products intended to control ants and cockroaches indoors; use of these products may constitute a source of fipronil entering WWTPs, either directly (if the product may be applied to floor drains, as is the case for some cockroach gel bait products) and/or indirectly (through cleaning activities such as mopping floors). In addition, fipronil is used outdoors in structural pest control applications; this use pattern may potentially lead to fipronil being indirectly transferred indoors and transported down-the-drain via cleaning and laundering activities.

3.2. Pyrethroids

At least two pyrethroids were detected in each biosolids sample, and six pyrethroids had a detection frequency of 25 % or greater in biosolids samples (Fig. 1). The sum of all pyrethroids and pyrethroid degradates ranged from 205 to 1788 μg/kg per sample, with a median concentration of 1151 μg/kg. Bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, and permethrin showed strong sorption to biosolids with few detections observed in effluent samples (Fig. SI-2).

Permethrin had the greatest detection frequency (100 %), median concentration (694 μg/kg), and maximum concentration (1048 μg/kg) of all pyrethroids detected in biosolids (Fig. 2; Table SI-17), and also constituted the highest overall concentrations of pyrethroids detected in influent samples (Fig. 3). Likely sources of permethrin entering the wastewater collection system include topical pet products, indoor sprays, and indoor foggers (Budd et al., 2023). Other possible sources may include products intended to treat lice in humans, such as shampoos (Turner et al., 2011), and indirect transfer from products used outdoors. There is considerable variability in permethrin concentrations in biosolids reported in the literature (Table SI-14).

Bifenthrin concentration in influent samples (classified as either greater than or <100 ng/L, the RL for this analyte in influent) was a statistically significant predictor of the bifenthrin concentration in the paired biosolids sample (Peto-Peto one-way test of difference between

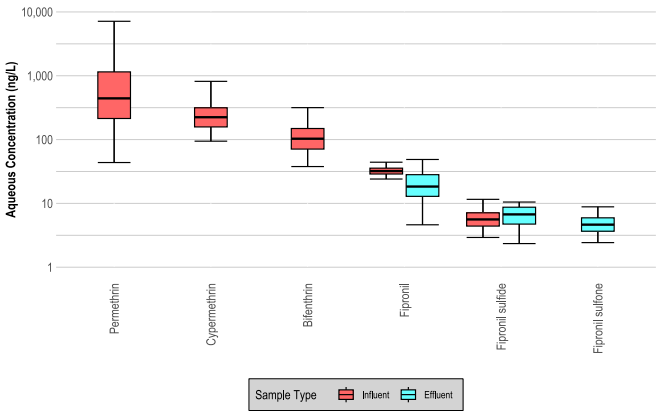

Fig. 3. Fiprole and pyrethroid concentrations in aqueous samples (influent and effluent; n = 17 for each sample type), aggregated across all wastewater treatment plants (WWTPs) in the study. Estimated values for censored observations (non-detects and unquantifiable trace observations) were imputed using the Regression on Order Statistics (ROS) method (using the NADA package in R), and are incorporated into the boxplots. Boxes represent the interquartile range (25th–75th percentile), whiskers represent minimum and maximum concentrations and horizontal black lines represent the medians. Effluent data for pyrethroids are not plotted because each pyrethroid shown in the figure had, at most, only one detection in effluent. Cyfluthrin and cyhalothrin were not detected in influent (although there were several trace observations); therefore, no influent boxplots were generated for these compounds. Etofenprox was not analyzed in aqueous samples; therefore, no influent boxplot was generated for this compound.

groups: p = 0.013). For WWTPs with an influent concentration of >100 ng/L, the median bifenthrin concentration in biosolids was 201 μg/kg, whereas the median was 89 μg/kg for WWTPs with an influent concentration of <100 ng/L. This trend was not observed for cypermethrin (p = 1) or permethrin (p = 0.598), using a 200 ng/L cutoff value (the MDL for these pesticides in influent). This analysis was not performed for cyfluthrin or cyhalothrin because these analytes were not detected in aqueous matrices although there were several "trace" observations, or for etofenprox because this analyte was not analyzed in aqueous samples.

For this study, bifenthrin MDLs were 40 ng/L in influent and 4 ng/L in effluent. Results indicate that bifenthrin was detected in influent in eight WWTPs, yet only detected in effluent in one WWTP (Fig. SI-2). Also, WWTPs with influent detections tend to have higher concentrations in biosolids in this study, as explained above. These details support the hypothesis that the majority of bifenthrin mass entering WWTPs is partitioning to biosolids during the treatment process, as would be predicted by its low water solubility (estimated to be 0.24 μg/L by EPI Suite (EPA, 2012)) and high log K_{OW} value of >6.0 (CDPR, 2023a). The other pyrethroids shown in Fig. SI-2 also appear to be mostly partitioning to biosolids, based on the relatively lower number of detections and trace observations in effluent, compared to influent and biosolids.

Two pyrethroid degradates, 3-phenoxybenzoic acid (3-PBA) and 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA) were quantified in biosolids samples (these compounds were not analyzed in the matched aqueous samples). 3-PBA was detected in 22 % of samples, with a median value of 14.7 μg/kg; 4-F-3-PBA was detected in 17 % of samples, with a median value of 3.1 μg/kg. The lower detection frequencies and observed concentrations (relative to parent pyrethroid compounds) could indicate that either (1) pyrethroids are not readily degraded during wastewater treatment, or (2) that these compounds are further transformed into other compounds, as suggested by Cycon and Piotrowska-Seget (2016). However, 3-PBA and 4-F-3-PBA are more hydrophilic than their parent compounds, as estimated by lower predicted partitioning coefficient (logP) values (Wishart et al., 2022). They would therefore be expected to preferentially partition into the aqueous phase as they form.

Some pyrethroids can be degraded into 3-PBA and/or 4-F-3-PBA by various species of microorganisms, some of which have been isolated from WWTP sludge (Galadima et al., 2021). Additionally, some pyrethroids are transformed to 3-PBA and/or 4-F-3-PBA in the human body and are detectable in the urine of exposed individuals (Biomonitoring California, 2013; Kim et al., 2021). Specifically, 3-PBA indicates exposure to cyhalothrin, cypermethrin, deltamethrin, fenpropathrin, permethrin, and/or tralomethrin; 4-F-3-PBA indicates exposure to cyfluthrin (Biomonitoring California, 2013). It is therefore possible that a portion of the mass of these degradates observed in biosolids is due to inputs from human urine entering the wastewater collection system, rather than from microbial degradation of the parent compounds during the treatment process (Table SI-15). Based on data from a biomonitoring study (Biomonitoring California, 2013), calculations indicate that a substantial proportion (>33 %) of the observed mass of these pyrethroid degradates in biosolids may be due to human input, rather than microbial degradation (refer to Table SI-14 for more details).

The following pyrethroids were not detected in any biosolids samples: allethrin, deltamethrin, fenpropathrin, phenothrin, resmethrin, tefluthrin, and tetramethrin. It is unclear why these compounds were not detected in biosolids samples. This observation may be explained by low rates of product use within the sewersheds sampled. It is also possible that these compounds were present below detection limits. In addition, the lack of detections of certain pesticides may be explained by physicochemical properties (e.g., high volatility, short degradation half-lives). Ongoing wastewater monitoring studies may provide additional information regarding the down-the-drain transport potential of pesticides used in California (CDPR, 2024).

3.3. DDT and degradates

Biosolids samples were analyzed for p,p′-DDT and two of its degradates (p,p′-DDD and p,p′-DDE). p,p′-DDE was detected in 44 % of biosolids samples, but neither p,p′-DDT nor p,p′-DDD were detected (Table SI-13). The lack of p,p′-DDT detections may be due to degradation to p,p′-DDE, because p,p′-DDE concentrations ranged from <MDL (2.5 μg/kg) to 9.7 μg/kg, with a median of 2.3 μg/kg. These findings agree somewhat with prior studies with respect to p,p′-DDE being more frequently detected than p,p′-DDT or p,p′-DDD (Table SI-14). Reported biosolids concentrations of p,p′-DDE vary widely in the literature but are generally higher than those reported in this study. Median values of 13 μg/kg (Stevens et al., 2003), 19 μg/kg (Katsoyiannis and Samara, 2004), and 6 μg/kg (Dimitriou-Christidis et al., 2015) have been reported.

Three of the sampled WWTPs utilize a combined sewer system (i.e., sanitary wastewater and stormwater/irrigation runoff are collected and treated together) in at least part of their sewersheds. Analysis of DDE concentrations in biosolids samples from these WWTPs, compared to WWTPs with separate sewer systems, did not reveal any clear differences. All of the WWTPs with combined systems had detectable amounts of DDE in their biosolids samples, indicating that DDE may be entering the treatment system via runoff, rather than sanitary wastewater. During the 30 days prior to sampling, a total of approximately 0.5 in. of precipitation had fallen in each of the sewersheds of the WWTPs with combined systems (National Weather Service, 2024), indicating influence from rainfall in addition to dry weather sources such as excess irrigation runoff. However, several other WWTPs with separate sewer systems also had detectable DDE in biosolids, indicating that there are other sources in addition to runoff.

Despite the fact that DDT was phased out in the United States approximately 50 years prior to sample collection, DDE was still detected in this study. Potential sources of DDT and/or DDE entering the sewershed are unknown; however, DDE is widely detected in environmental samples such as stormwater runoff (Masoner et al., 2019) and soil (Sudharshan et al., 2012), as well as biological samples such as human serum (CDC, 2016). These results speak to the ubiquity of persistent legacy pesticides such as DDT and are likely due to past use and sources of DDT (Stevens et al., 2003).

3.4. Insect growth regulators

Two insect growth regulators (IGRs) were examined in this study: novaluron (analyzed in aqueous and biosolids samples) and pyriproxyfen (analyzed in aqueous samples only). Neither of these analytes were detected in any of the aqueous samples. Novaluron was detected in 22 % of biosolids samples, with a median concentration of 4.1 μg/kg (Table SI-17).

As an IGR, novaluron is intended to control pests in the juvenile stages (e.g., larva, pupa), and is often formulated in products in conjunction with an adulticide (e.g., pyrethroids, fipronil). A search of the California Department of Pesticide Regulation (CDPR) product label database (CDPR, 2023b) for products containing novaluron returned six products that (1) had use patterns(s) associated with potential down-the-drain transport, and (2) were actively registered by CDPR either during the sampling period (May 2020), or several years prior (because products may still be sold and used some time after the product is no longer actively registered). Two of the products were indoor/outdoor sprays, and four were topical treatments for dogs. Both of these use patterns may have resulted in down-the-drain transport of novaluron. However, in California, available data indicate that sales of novaluron are relatively low (on a mass basis) compared to other pesticides such as fipronil and pyrethroids (Xie et al., 2021). This fact may help explain why the detection frequency and median concentration for novaluron were relatively low compared to other pesticides analyzed in this study.

3.5. Correlation with f_{oc} and other physical characteristics

The f_{OC} of sorbent particles (which will settle during treatment and become sludge) is known to influence the partitioning behavior of organic pollutants during the wastewater treatment process (Katsoyiannis and Samara, 2007). Therefore, the relationship between f_{OC} and pesticide concentrations was investigated.

Measured f_{OC} values from biosolids samples ranged from 0.248 to 0.458, with a median value of 0.325 (Table SI-2). No statistically significant correlation was observed between f_{OC} values and total pyrethroid concentrations (Table SI-16). However, a statistically significant negative correlation between f_{OC} and total fiproles (Fig. SI-3) was observed. Furthermore, a statistically significant negative correlation was observed between f_{OC} and fipronil sulfide, and between f_{OC} and fipronil sulfone, but not between f_{OC} and fipronil (Figs. SI-4, SI-5, and SI-6).

One hypothesis for the observed correlations is that higher f_{OC} interferes with the extraction process. However, this is not supported by the quality control data included in this dataset. Triplicate matrix spikes were performed on several biosolids samples, including one sample that had an f_{OC} value toward the higher end of the range (0.385). In this sample, recoveries of spiked fiproles ranged from 80 % to 104 %, indicating that high f_{OC} did not cause matrix extractability issues in this sample set (if high f_{OC} were interfering with the extraction, then the negative correlation would have been observed in the quality control samples and across all environmental samples).

Another hypothesis is that the dissolved organic carbon (DOC) concentrations in the influent varied across WWTPs and may have affected the partitioning of some pesticides to settleable particles. Prior research has found that increased DOC in the aqueous phase of wastewater can lead to a decrease in the distribution coefficient (K_d; the sorbed concentration divided by the dissolved concentration) of organic pollutants (Katsoyiannis and Samara, 2007). In other words, increased DOC amounts tend to shift pollutants away from the sorbed phase and into the dissolved phase. A similar tendency has also been noted for pyrethroids in surface waters (Delgado-Moreno et al., 2010). Decreased sorption of a pollutant to settleable particles would likely lead to a lower observed concentration of that pollutant in biosolids samples. Influent DOC

concentrations were not measured in this study; therefore, it is unknown whether DOC concentrations in influent tend to be positively correlated with f_{OC} in biosolids. However, if they were positively correlated, one might expect lower pollutant concentrations in biosolids samples that had higher f_{OC} values. High loads of organic matter in wastewater lead to an increased volume of sludge produced, which results in lower observed concentrations of studied compounds in biosolids (Plagellat et al., 2004). However, another major class of pesticides (i.e., pyrethroids) did not show a correlation with f_{OC} in the present study. This piece of evidence counters the hypothesis above because pyrethroids would be expected to be more strongly correlated with DOC than fiproles would be. Additionally, differences in upstream treatment processes make it difficult to compare across WWTPs (O'Keeffe and Akunna, 2022). The likeliest explanation may be that if fipronil were sorbed to a lesser extent in a given environmental sample, then the fipronil in that sample would have a greater chance of being transformed.

During anaerobic digestion, f_{OC} decreases as microorganisms degrade organic compounds to carbon dioxide and methane (McInerney and Bryant, 1981). Additionally, the evidence indicates that fipronil is degraded into fipronil sulfide as digestion proceeds. Therefore, one might expect a positive correlation between f_{OC} and fipronil concentrations in biosolids. However, the majority of fiprole mass in biosolids samples measured in this study is due to fipronil sulfide and fipronil sulfone (Fig. 2). Therefore, this may explain why fipronil was not correlated with f_{OC} even though it is degraded as treatment proceeds. The negative correlation between f_{OC} and fipronil sulfide is expected given that as anaerobic digestion proceeds, fipronil sulfide is formed and f_{OC} decreases. However, the reason for the correlation between fipronil sulfone and f_{OC} is unclear. Additionally, the retention time of biosolids in the anaerobic digesters from each WWTP was not recorded in this study. This factor may have affected the observed variation in f_{OC} values and could also affect the degradation of fipronil.

No other correlations were found between pesticide concentrations and physical characteristics (i.e., facility capacity, average dry weather flow, population served, and percentage of influent flow attributed to industry; refer to Table SI-2). In all cases, Kendall's tau values were $<$ 0.33 and all p values were $>$0.05 (Table SI-16). This agrees with prior research, which did not identify any correlation between volume of influent flow received and fiprole concentrations in biosolids (Sadaria et al., 2019), or between the presence or absence of industrial inputs into the sampled WWTPs and permethrin concentrations in biosolids (Plagellat et al., 2004).

3.6. Mass loadings of pesticides applied to land

Pesticide loadings to land were calculated for pesticides applied in agricultural settings, and pesticides applied via land-applied biosolids. Bifenthrin and permethrin were the focus of this analysis because of their high detection frequencies and median concentrations in biosolids measured in this study, and their extensive use in agriculture. For the application rate analysis, corn was chosen as a model crop because biosolids application rates were publicly available.

In the case of bifenthrin and permethrin application, agricultural application rates far exceed the application rates resultant from biosolids applied to land as soil amendment (Fig. 4). Considering the fact that agricultural application rates may be underestimated (Section 2.4.5), the true differences in rates between the two pathways are likely even greater than shown by Fig. 4. Detailed information on post-application bifenthrin and permethrin concentrations in soil is not available; therefore, it is not possible to compare land-applied biosolids concentrations to residual soil concentrations at this time.

Estimated total mass loads in 2020 due to all agricultural applications in California (regardless of crop type; refer to Section 2.4.5 for further details) are far greater than mass loads due to beneficial land application of biosolids for both bifenthrin and permethrin (Fig. 5). The estimated statewide mass load due to agricultural application of permethrin is approximately 2 orders of magnitude greater than the estimated contribution from biosolids applied to land as soil amendment. For bifenthrin, agricultural applications are $>$3 orders of magnitude greater than those due to land-applied biosolids.

Fipronil and its transformation products were among the most frequently detected pesticides in biosolids in this study. Because the agricultural application of fipronil is not allowed in California, any amount of fipronil in land-applied biosolids will be greater than agricultural applications (i.e., greater than zero); this is predicted to be the case for all non-agriculturally applied pesticides. However, in the case of fipronil, the median biosolids concentration was 3.65 μg/kg. This is approximately 32 times lower than the median bifenthrin concentration (116 μg/kg) and approximately 190 times lower than the median permethrin concentration (694 μg/kg); in addition, the estimated statewide mass load of fipronil in land-applied biosolids in 2020 was only 2 kg (Table SI-11).

4. Conclusions

This study provides data on many pesticides and transformation

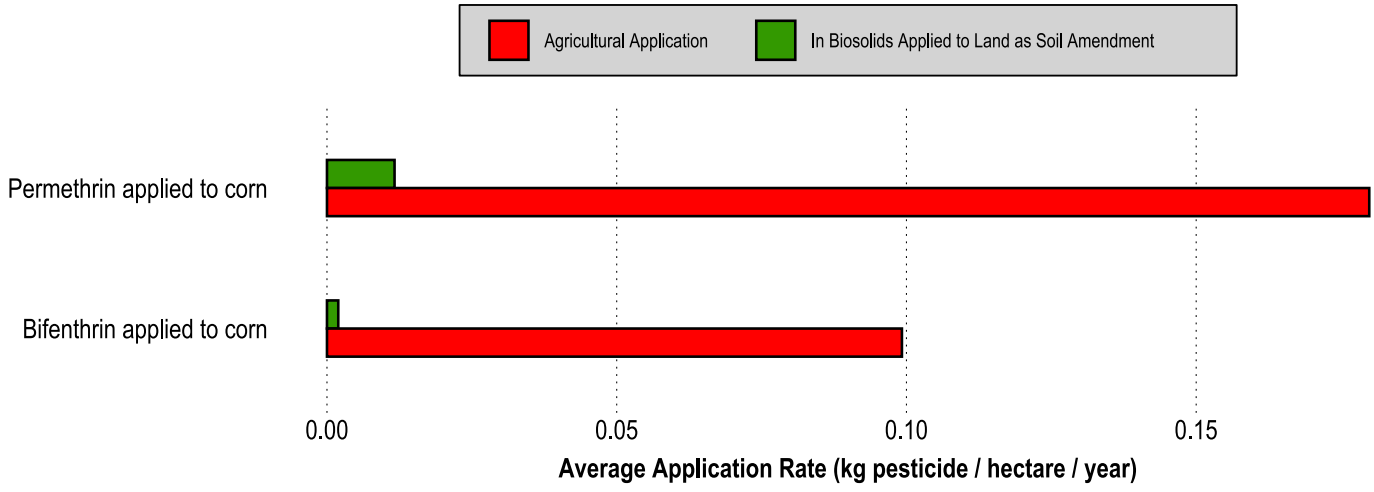

Fig. 4. Comparison of estimated average application rates of pesticides to agricultural land in California in 2020, via agricultural application of pesticide products and land-applied biosolids. The values shown in this figure are based on estimates using corn as a representative crop. Corn was chosen as a representative crop because typical biosolids application rates to corn are available, and because bifenthrin and permethrin are applied to corn in California.

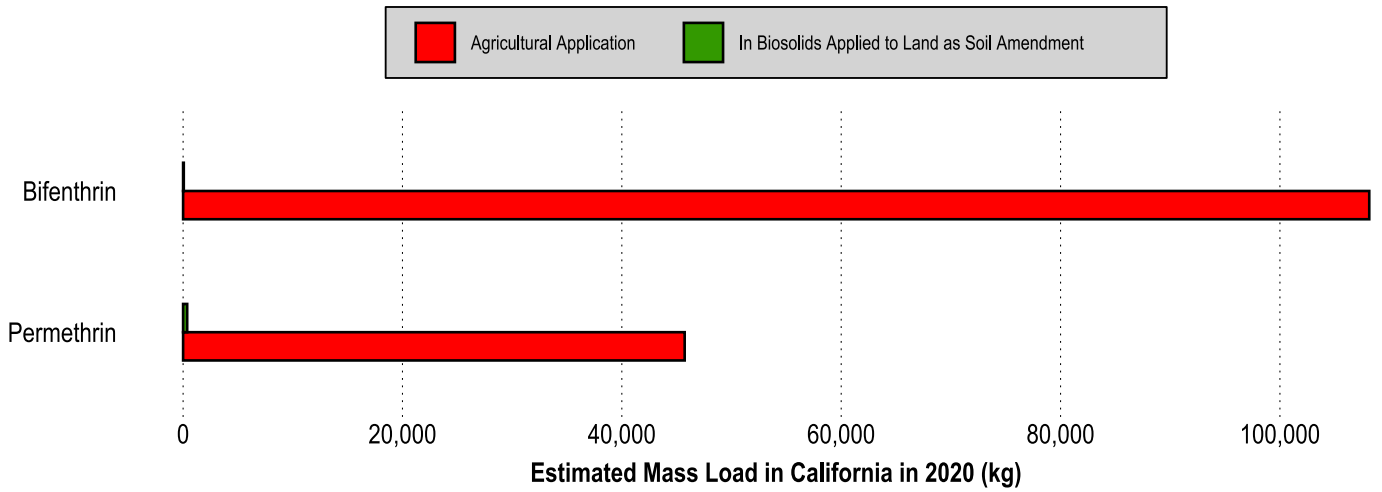

Fig. 5. Estimated mass loads of pesticides to agricultural land in California in 2020, via agricultural application of pesticide products and land-applied biosolids. The estimates of agricultural applications shown in this figure consider all reported agricultural applications of bifenthrin and permethrin in California, regardless of crop.

products in three distinct WWTP media (influent, biosolids, and effluent) from samples collected concurrently, and adds to the body of knowledge regarding pesticide transformation and removal processes within treatment systems. Little to no existing data quantifying pyrethroid concentrations in biosolids are available, and combined with the paired influent and effluent samples, makes this dataset one of the first of its kind. The pesticides that constituted the highest detection frequencies and concentrations in biosolids (bifenthrin and permethrin) were selected for mass loading analysis. Comparison of pesticide mass loads from land-applied biosolids and agricultural applications of pesticide products indicates that these pesticides entering the environment from the beneficial application of biosolids to land (as a soil amendment) occur at levels that are only a small fraction of what is applied in a typical agricultural setting. Agricultural applications of pesticides reflect a use that has already met a high regulatory standard and are subject to ongoing scientific assessment regarding any potential hazard of such applications. Existing agricultural pesticide applications are being considered for potential impacts to human health and the environment, by multiple regulatory mechanisms at both the federal and state levels. For example, the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) requires the EPA to review a pesticide product's registration every 15 years to ensure that the registration continues to satisfy FIFRA requirements (among other things, FIFRA requires that a pesticide generally will not cause unreasonable adverse effects on the environment); if a product fails to satisfy the FIFRA standard for registration, the product's registration may be subject to cancellation or other remedies under FIFRA (40 CFR Part 155). In addition, CDPR is required by state legislation to continuously evaluate pesticides after registration; this is accomplished by several programs, including environmental monitoring and risk assessment (CDPR, 2017). Given that pesticide inputs from land-applied biosolids are generally estimated to be much lower than typical agricultural applications, existing regulations surrounding agricultural pesticide applications are expected to also be protective of the relatively low inputs from biosolids. Further research investigating pesticide concentrations in various environmental media (e.g., biosolids, soil) may be warranted.

CRediT authorship contribution statement

John Wheeler: Writing – original draft, Visualization, Formal analysis, Data curation. **Gabrielle P. Black:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Michelle L. Hladik:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Formal analysis, Data curation. **Corey J. Sanders:** Methodology, Investigation, Formal analysis. **Jennifer Teerlink:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Luann Wong:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Xuyang Zhang:** Writing – review & editing. **Robert Budd:** Writing – review & editing, Data curation, Conceptualization. **Thomas M. Young:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.178954.

Data availability

Anonymized data are provided in the supplementary information file.

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